ΕΝΕΡΓΕΙΑΚΗ ΑΞΙΟΠΟΙΗΣΗ ΑΓΡΟΤΟ-ΒΙΟΜΗΧΑΝΙΚΩΝ ΑΠΟΒΛΗΤΩΝ ΚΑΙ ΓΛΥΚΟΥ ΣΩΡΓΟΥ ΓΙΑ ΤΗΝ ΠΑΡΑΓΩΓΗ ΑΕΡΙΩΝ ΒΙΟΚΑΥΣΙΜΩΝ ΜΕΣΩ ΤΗΣ ΑΝΑΕΡΟΒΙΑΣ ΧΩΝΕΥΣΗΣ

Υπό

Μαργαρίτα Α. Δαρειώτη

Εργαστήριο Βιοχημικής Μηχανικής και Τεχνολογίας Περιβάλλοντος
Τομέας Μηχανικής Διεργασιών και Περιβάλλοντος
Τμήμα Χημικών Μηχανικών Πανεπιστημίου Πατρών

ΕΠΙΤΑΜΕΛΗΣ ΕΞΕΤΑΣΤΙΚΗ ΕΠΙΤΡΟΠΗ

Μ. ΚΟΡΝΑΡΟΣ, Αναπληρωτής Καθηγητής (Επιβλέπων)
Τμήμα Χημικών Μηχανικών, Παν/μιο Πατρών
Πρόεδρος Εξεταστικής Επιτροπής

Γ. ΛΥΜΠΕΡΑΤΟΣ, Καθηγητής
Τμήμα Χημικών Μηχανικών, Ε.Μ.Π.

Σ. ΠΑΥΛΟΥ, Καθηγητής
Τμήμα Χημικών Μηχανικών, Παν/μιο Πατρών

Δ. ΜΑΝΤΖΑΒΙΝΟΣ, Καθηγητής
Τμήμα Χημικών Μηχανικών, Παν/μιο Πατρών

Ι. ΚΟΥΚΟΣ, Αναπληρωτής Καθηγητής
Τμήμα Χημικών Μηχανικών, Παν/μιο Πατρών

Χ. ΠΑΡΑΣΚΕΥΑ, Αναπληρωτής Καθηγητής
Τμήμα Χημικών Μηχανικών, Παν/μιο Πατρών

Α. ΣΤΑΜΑΤΕΛΑΤΟΥ, Επίκουρη Καθηγήτρια
Τμήμα Μηχανικών Περιβάλλοντος, Παν/μιο Θράκης

Πανεπιστημιούπολη, 265 04 Ρίο
Dedicated to my parents, Andreas & Anastasia

my brother, Tasos

& my Nikos!
Acknowledgments

First, I would like to express the special appreciation to my advisor, Michael Kornaros, for his excellent guidance, caring, patience and providing me with an excellent atmosphere for doing research. I would like to thank him for encouraging my research, for his brilliant comments and suggestions and for allowing me to grow as a research scientist. I will be always thankful for everything he has offered me and very fortunate having such a wonderful person as an advisor. I would also like to thank my committee members, Professor G. Lyberatos, Professor S. Pavlou, Professor D. Mantzavinos, Associate Professor I. Kookos, Associate Professor C. Paraskeva, and Assistant Professor A. Stamatelatou.

I would also like to thank all the members of Kornaros group, and the undergraduate students with whom I have cooperated during their diploma thesis. Especially, Thodoris Vgenis, Dimitris Poupakis and my special friend Kostas Stavropoulos for scientific and intimate support with whom I have enjoyed working together and sharing great moments in the same laboratory. I would also like to acknowledge Dr. Aikaterini Vavouraki for the creative collaboration and our scientific discussions and also Dr. Spyros Dokianakakis who shared with me his knowledge and give me invaluable help during my early stages of my Ph.D research.

I dedicate this thesis to my loving family; my parents Andreas, Anastasia and my brother Tasos as they were always there for me, giving me unconditional love and supporting me throughout my life. I would also like to thank all of my friends, who supported me in writing and incented me to strive towards my goal. Last but not the least important, I would also like to dedicate this thesis to a special person, my fiance Nikos Vrachatis, who was always standing by me during both the good times and those when things haven’t gone so well. I give my deepest expression of love and appreciation for the encouragement that he gave me all these years.

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.
Abstract

It is clear that renewable resources have received great interest from the international community during the last decades and play a crucial role in the current CO$_2$-mitigation policy. In this regard, energy from biomass and waste is seen as one of the most dominant future renewable energy sources, especially since that a continuous power generation from these sources can be guaranteed, unlike other types such as solar energy and wind energy. Thus, organic waste i.e. animal wastes, wastewaters, energy crops, agricultural and agro-industrial residues are of specific importance since these sources do not compete with food crops in agricultural land usage. The various technologies that are available for power generation from biomass and waste can be subdivided into thermochemical, biochemical and physicochemical conversion processes. Anaerobic digestion (AD), classified within the biochemical conversion processes, is a robust process and is widely applied. Various types of biomass and waste, can be anaerobically co-digested to generate a homogeneous mixture increasing both process and equipment performance. This technology is an attractive option to improve the yields of the anaerobic digestion of substrates due to the positive synergisms established in the digestion medium; a fact that increases the economic viability of the biogas plants.

This study focused on the valorization of agro-industrial wastes (such as olive mill wastewater (OMW), cheese whey (CW) and liquid cow manure (LCM)) and sweet sorghum stalks. Olive mills, cheese factories and cow farms are agro-industries that represent a considerable share of the worldwide economy with particular interest focused in the Mediterranean region. These industries generate millions of tons of wastewaters and large amounts of by-products, which are in many cases totally unexploited and thus dangerous for the environment. On the other hand, sweet sorghum as a lignocellulosic material represents an interesting substrate for biofuels production due to its structure and composition.

Anaerobic co-digestion experiments using different substrates were performed in a two-stage system consisting of two continuously stirred tank reactors (CSTRs) under mesophilic conditions (37°C) with a hydraulic retention time (HRT) of 19 days (3 d of acidogenesis and 16 d of methanogenesis). The maximum methane production rate (1.35 L CH$_4$/L$_R$·d) was obtained using the mixture of 55% OMW, 40% CW and 5% LCM with methane yield of 467.53 mL CH$_4$/g VS added, whereas equally high methane production rate of 1.33 L CH$_4$/L$_R$·d was obtained using the mixture of 90% CW and 10% LCM with 79% removal of total COD. Although the two-stage anaerobic treatment process has several advantages over the conventional single-stage process, experiments were conducted using CW and LCM as mono-substrates in order to investigate the role of each step in their treatment. In particular, negligible difference between single and two-stage process was observed treating the LCM, whereas using
the CW, an easily-degradable substrate, the two-stage process was considered as a better treatment system than single one.

Taking into account the aforementioned results, the mixture 55% OMW, 40% CW and 5% LCM was selected for further study and optimization. Subsequently, two more mixtures were studied, where sweet sorghum was added, in order to simulate the operation of a centralized AD plant fed with regional agro-wastes which lacks OMW or/and CW due to seasonal unavailability. The sorghum used in this dissertation was either fresh or ensiled. The ensiled sorghum (ES) was firstly pretreated using an alkaline hydrolysis. The aim of this study was to hydrolyze the ES targeting to carbohydrates’ solubilization and removal of lignin that hinders the access of enzymes to cellulose, thus facilitating its subsequent biochemical conversion to fermentable sugars.

For the optimization of these mixtures, two operational parameters were examined including pH and HRT. Batch experiments were performed in order to investigate the impact of controlled pH on the production of bio-hydrogen and volatile fatty acids, whereas continuous experiments (CSTRs) were conducted for the evaluation of HRT effect on hydrogen and methane production. Firstly, using the mixture of 55% OMW, 40% CW and 5% LCM, the highest hydrogen production yield was observed at pH 6.0 (0.642 mol H₂/mol equiv. glucose consumed), whereas lactic acid was identified as a major metabolite which presented an intense accumulation before its further bioconversion to butyric acid and hydrogen. Moreover, in continuous mode, the maximum hydrogen production rate of 1.72 L/LR·d was achieved at HRT 0.75 d, whereas at HRT 25 d the methanogenic reactor showed good stability with methane production 0.33 L CH₄/LR·d. Secondly, using the mixture of 55% sorghum, 40% CW and 5% LCM, the optimum pH value of 5.5 was obtained (0.52 mol H₂/mol equiv. glucose), whereas in continuous mode the maximum hydrogen and methane productivities (2.14 L/LR·d and 0.90 L/LR·d, respectively) were observed at HRTs 0.5 d and 16 d, respectively. Finally, using higher percentage of sorghum in the mixture (95% sorghum and 5% LCM), a lower optimum pH was obtained equal to 5.0 (0.92 mol H₂/mol equiv. glucose). Moreover, higher HRT (5 d) than previous experiments gave maximum hydrogen yield may be due to lignocellulosic material, whereas the highest CH₄ production rate of 0.44 L/LR·d was achieved at the HRT of 25 d.

Moreover, further exploitation of digestate from an anaerobic methanogenic reactor was studied using a combined ultrafiltration/nanofiltration system and further COD reduction was obtained. On the other hand, vermicomposting was conducted in order to evaluate the sludge transformation to compost and as a result, good results in terms of increased N-P-K concentration values were obtained. Furthermore, simulation of mesophilic anaerobic (co)-digestion of different substrates was applied, using the ADM1 modified model, where the results indicated that the modified ADM1 was able to predict reasonably well the steady-state experimental data. Finally, technical and economic evaluation was performed for an integrated biogas plant (1.0 MW) in terms of the amount of investment required, the operating costs, the income from electricity production and the sale of the produced compost.
Περίληψη

Είναι φανερό ότι οι ανανεώσιμες πηγές έχουν λάβει μεγάλο ενδιαφέρον από τη διεθνή κοινότητα τις τελευταίες δεκαετίες και διαδραματίζουν καθοριστικό ρόλο στην μείωση του CO2. Η ενέργεια από βιομάζα και απόβλητα θεωρείται ως μία από τις πλέον κυριαρχικές ανανεώσιμες πηγές ενέργειας του μέλλοντος. Έτσι, τα οργανικά απόβλητα όπως κτηνοτροφικά, λύματα, ενεργειακές καλλιέργειες, γεωργικά και αγροτο-βιομηχανικά υπολείμματα έχουν ιδιαίτερη σημασία, δεδομένου ότι οι πηγές αυτές δεν ανταγωνίζονται με τις καλλιέργειες τροφίμων της γεωργικής γης. Διάφορες τεχνολογίες είναι διαθέσιμες για την παραγωγή ηλεκτρικής ενέργειας από βιομάζα: θερμοχημικές, βιοχημικές και φυσικοχημικές διεργασίες. Η αναερόβια χώνευση, που υπάγεται στις βιοχημικές διεργασίες, είναι μια διεργασία που εφαρμόζεται ευρέως. Διάφορα είδη βιομάζας μπορούν να συν-χωνευτούν αναερόβια δημιουργώντας ένα ομοιογενές μίγμα και αυξάνοντας την απόδοση. Αυτή η τεχνολογία αποτελεί μια ελκυστική επιλογή για τη βελτίωση των αποδόσεων της αναερόβιας χώνευσης των υποστρωμάτων λόγω των θετικών συνεργιών που λαμβάνουν χώρα, γεγονός που αυξάνει την οικονομική βιωσιμότητα των μονάδων παραγωγής βιοαερίου.

Η παρούσα διατριβή επικεντρώθηκε στην αξιοποίηση των αγροτο-βιομηχανικών αποβλήτων (π.χ. ελαιοτριβείου, τυροκομείου, βουστασίου) και του γλυκού σόργου. Ελαιουργεία, τυροκομεία και αγροκτήματα αγελάδων είναι αγροτο-βιομηχανίες που αντιπροσωπεύουν ένα σημαντικό μερίδιο της παγκόσμιας οικονομίας με ιδιαίτερο ενδιαφέρον στην περιοχή της Μεσογείου. Αυτές οι βιομηχανίες παράγουν εκατομμύρια τόνους λυμάτων και μεγάλες ποσότητες υπο-προϊόντων, τα οποία σε πολλές περιπτώσεις είναι ανεκμετάλλευτα και επικίνδυνα για το περιβάλλον. Από την άλλη πλευρά, το γλυκό σόργο ως λιγνοκυτταρινούχο υλικό αντιπροσωπεύει ένα ενδιαφέρον υπόστρωμα για την παραγωγή βιοκαυσίμων λόγω της σύνθεσής του.

Πειράματα αναερόβιας χώνευσης πραγματοποιήθηκαν με χρήση διαφορετικών υποστρωμάτων, τα οποία διεξήχθησαν σε διβάθμιο σύστημα αντιδραστήρων συνεχούς λειτουργίας (CSTRs), υπό μεσοφιλής συνθήκης (37°C) και με υδραυλικό χρόνο παραμονής (HRT) 19 ημέρες (3 d και 16 d, αντίστοιχα). Ο μέγιστος ρυθμός παραγωγής μεθανίου (1.35 L CH4/LR·d) λήφθηκε χρησιμοποιώντας το μίγμα 55% ελαιοτριβείο, 40% τυροκομείο και 5% βουστάσιο με απόδοση μεθανίου 467.53 mL CH4/g VS, ενώ εξίσου υψηλός ρυθμός παραγωγής μεθανίου καιίσος με 1.33 L CH4/LR·d παρατηρήθηκε χρησιμοποιώντας το μίγμα 90% τυροκομείο και 10% βουστάσιο με 79% απομάκρυνση ολικού COD. Παρόλο που η διαδικασία αναερόβιας χώνευσης σε δύο στάδια υπερτερεί, εν γένει, σε σχέση με τη συμβατική διεργασία ενός σταδίου, πειράματα διεξήχθηκαν χρησιμοποιώντας απόβλητα τυροκομείου και βουστασίου. Πραγματικά, στην επεξεργασία βουστασίου δεν παρατηρήθηκε διαφορά μεταξύ ενός και δύο σταδίων. Αντιθέτως, στην επεξεργασία τυροκομείου, η διεργασία δύο σταδίων έδωσε καλύτερα αποτελέσματα.
Λαμβάνοντας υπόψη τα προαναφερθέντα, το μίγμα 55% ελαιοτριβείο, 40% τυροκομείο και 5% βουστάσιο επιλέχτηκε για περαιτέρω μελέτη και βελτιστοποίηση. Εν συνεχεία, μελετήθηκαν δύο επιπλέον μίγματα όπου γλυκό σόργο προστέθηκε με σκοπό την προσομοίωση λειτουργίας μίας κεντρικής μονάδας αναεροβικής χώνευσης η οποία τροφοδοτείται με τοπικά απόβλητα σε περίοδο μη εποχικής διαθεσιμότητας. Το γλυκό σόργο που χρησιμοποιήθηκε ήταν είτε φρέσκο είτε ενσιρωμένο, το οποίο χρησιμοποιήθηκε έπειτα από επεξεργασία αυτού. Η μέθοδος προεπεξεργασίας του ενσιρωμένου σόργου που χρησιμοποιήθηκε ήταν θερμο-αλκαλική υδρόλυση με σκοπό την διαλυτοποίηση των υδατανθράκων και την απομάκρυνση της λιγνίνης που λειτουργεί σαν παρεμποδιστή στην πρόσβαση των ενζύμων στην κυτταρίνη.

Για την βελτιστοποίηση αυτών των μιγμάτων, δύο λειτουργικές παράμετροι (pH και HRT) εξετάστηκαν. Πειράματα διαλείποντος έργου έγιναν προκειμένου να διερευνηθεί η επίδραση του pH στην παραγωγή υδρογόνου, ενώ πειράματα συνεχούς λειτουργίας διεξήχθησαν για την επίδραση του HRT την παραγωγή υδρογόνου και μεθανίου. Χρησιμοποιώντας το μίγμα 55% ελαιοτριβείο, 40% τυροκομείο και 5% βουστάσιο, η μέγιστη απόδοση παραγωγής υδρογόνου επιτεύχθηκε σε pH 6.0 (0.64 mol H2/mol καταν. υδαταν.), ενώ η κύρια μεταβολή που παρουσίασε ήταν η μετατροπή του γλυκοσίδου σε βιταμινές και υδρογόνο. Σε συνεχή λειτουργία, ο μέγιστος ρυθμός παραγωγής υδρογόνου (1.72 L/LR·d) επιτεύχθηκε σε HRT 0.5d, ενώ ο μεθανογόνος αντιδραστήρας παρουσίασε σταθερότητα με παραγωγή CH4 0.33 L CH4/LR·d σε HRT 25d. Κατά την επεξεργασία του μίγματος 55% σόργο, 40% τυροκομείο και 5% βουστάσιο, η βέλτιστη τιμή του pH βρέθηκε να είναι ίση με 5.5 με απόδοση υδρογόνου 0.52 mol H2/mol καταν. υδαταν., ενώ η μέγιστη παραγωγή CH4 επιτεύχθηκε σε διβάθμιο σύστημα συνεχούς λειτουργίας σε HRTs 0.5d και 16d και ήταν ίση με 2.14 L H2/LR·d και 0.90 L CH4/LR·d, αντίστοιχα. Τέλος, αυξάνοντας το ποσοστό του σόργου στο μίγμα (95%σόργο-5%βουστάσιο), το μέγιστο pH βρέθηκε να είναι ίσο με 5.0 (0.92 mol H2/mol καταν.), μετά την προσθήκη σε διαβάθμιο σύστημα συνεχούς λειτουργίας σε HRT 0.5d, κατά την επεξεργασία του μίγματος 55% σόργο, 40% τυροκομείο και 5% βουστάσιο, η βέλτιστη τιμή του pH βρέθηκε να είναι ίση με 5.5 με απόδοση υδρογόνου 0.52 mol H2/mol καταν. υδαταν., ενώ η μέγιστη παραγωγή CH4 επιτεύχθηκε σε HRT 5d, το οποίο μπορεί να οφείλεται στην ύπαρξη λιγνοκυτταρικού υλικού, ενώ η μέγιστη παραγωγή CH4 επιτεύχθηκε σε HRT 25d. Περαιτέρω αξιοποίηση του χωνευμένου υπολείμματος μελετήθηκε με χρήση συνδυασμένου συστήματος υπερδιήθησης/νανοδιήθησης επιτυγχάνοντας επιπρόσθετη μείωση του οργανικού φορτίου στο διήθημα. Η μετατροπή της αναερόβιας χωνευμένης δύσης σε λίπασμα αξιολογήθηκε μέσω κεντρικής μονάδας αναεροβικής χώνευσης ADM1 με στόχο την προσομοίωση της αναερόβιας διαμορφίκης υποστρωμάτων. Τα αποτελέσματα που προέκυψαν έδειξαν ότι το μοντέλο ήταν σε θέση να προβλέψει σε ικανοποιητικό επίπεδο την πορεία των πειραματικών δεδομένων.
# Table of Contents

ACKNOWLEDGMENTS III

ABSTRACT V

ΠΕΡΙΛΗΨΗ VII

TABLE OF CONTENTS IX

LIST OF TABLES XV

LIST OF FIGURES XIX

LIST OF PICTURES XXIX

CHAPTER 1. INTRODUCTION 1

1.1 ENERGY 1

1.2 RENEWABLE ENERGY SOURCES 2

1.3 BIOMASS 3

1.4 AGRO-INDUSTRIAL WASTES 6

1.4.1 Olive Mill Wastewater 6

1.4.2 Cheese Whey 7

1.4.3 Liquid Cow Manure 9

1.5 ENERGY CROPS 10

1.5.1 Sorghum 11

1.6 LIGNOCELLULOSIC BIOMASS 13

1.6.1 Goals of pretreatment 15

1.6.2 Pretreatment technologies for lignocellulosic biomass 17

1.7 ANAEROBIC DIGESTION 26

1.7.1 Background 26

1.7.2 The benefits of anaerobic digestion 27

1.7.3 Biochemical process 28

1.7.4 Types of anaerobic digesters 29

1.7.5 Process parameters 31

1.7.6 Co-digestion 34

1.8 REFERENCES 36

CHAPTER 2. MATERIALS AND METHODS 45

2.1 MATERIALS 45

2.1.1 Agro-industrial wastes 45

2.1.2 Sweet Sorghum 45

2.2 EXPERIMENTAL SET-UP 48

2.2.1 Continuous anaerobic digestion experiments 48

2.2.2 Batch experiments 50

2.2.3 Biochemical Methane Potential assay 51

2.3 ANALYTICAL METHODS 53

2.3.1 pH 53
CHAPTER 3. QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF THE MAIN AGRO-WASTES IN WESTERN GREECE

3.1 QUANTITATIVE CHARACTERISTICS

3.2 QUALITATIVE CHARACTERISTICS

3.3 BIOCHEMICAL METHANE POTENTIAL

3.4 BACTERIAL GROWTH MODEL

3.5 REFERENCES

CHAPTER 4. PRETREATMENT OF SWEET SORGHUM STALKS FOR ENHANCING BIOFUELS PRODUCTION

4.1 ABSTRACT

4.2 INTRODUCTION

4.3 CHEMICAL PRETREATMENT OF SWEET SORGHUM STALKS

4.3.1 Materials

4.3.2 Chemical pretreatment procedure

4.3.3 Results and Discussion

4.4 ENZYMATIC HYDROLYSIS OF SWEET SORGHUM STALKS

4.4.1 Materials

4.4.2 Enzymatic hydrolysis procedure

4.4.3 Results and Discussion

4.5 THERMAL-ALKALINE PRETREATMENT OF ENSILED SORGHUM STALKS FOR BIOGAS PRODUCTION

4.5.1 Materials
4.5.2 Pretreatment procedure 92
4.5.3 Results and Discussion 93
4.6 EXPERIMENTAL ENSILING PROCEDURE 97
4.6.1 Materials 97
4.6.2 Laboratory ensiling procedure 98
4.6.3 Results and Discussion 98
4.7 BIOCHEMICAL METHANE POTENTIAL 100
4.8 CONCLUSIONS 104
4.9 REFERENCES 105

CHAPTER 5. BIOGAS PRODUCTION FROM AGRO-INDUSTRIAL WASTES THROUGH ANAEROBIC CO-DIGESTION IN A TWO-STAGE CSTR SYSTEM. 109
5.1 ABSTRACT 109
5.2 INTRODUCTION 110
5.3 MATERIALS 112
5.4 ANAEROBIC MESOPHILIC CO-DIGESTION OF OLIVE MILL WASTEWATER, CHEESE WHEY AND LIQUID COW MANURE. 113
5.4.1 Acidogenic reactor 113
5.4.2 Methanogenic reactor 115
5.5 ANAEROBIC MESOPHILIC CO-DIGESTION OF CHEESE WHEY AND LIQUID COW MANURE. 118
5.5.1 Acidogenic reactor 118
5.5.2 Methanogenic reactor 120
5.6 ANAEROBIC MESOPHILIC CO-DIGESTION OF OLIVE MILL WASTEWATER AND CHEESE WHEY. 123
5.6.1 Acidogenic reactor 123
5.6.2 Methanogenic reactor 125
5.7 ANAEROBIC MESOPHILIC CO-DIGESTION OF OLIVE MILL WASTEWATER AND LIQUID COW MANURE. 128
5.7.1 Acidogenic reactor 128
5.7.2 Methanogenic reactor 130
5.8 CONCLUSIONS 134
5.9 REFERENCES 135

CHAPTER 6. ASSESSMENT OF SINGLE VS. TWO-STAGE ANAEROBIC DIGESTION USING LIQUID COW MANURE OR CHEESE WHEY. 139
6.1 ABSTRACT 139
6.2 INTRODUCTION 140
6.3 MATERIALS 142
6.4 RESULTS AND DISCUSSION 142
6.4.1 Liquid cow manure treatment 144
6.4.2 Cheese whey treatment 147
6.5 CONCLUSIONS 153
6.6 REFERENCES 154
### CHAPTER 7. ANAEROBIC CO-DIGESTION OF AGRO-INDUSTRIAL WASTEWATERS IN A TWO-STAGE SYSTEM. 157

#### 7.1 ABSTRACT 157

#### 7.2 INTRODUCTION 158

#### 7.3 MATERIALS AND METHODS 163

- **7.3.1 Agro-industrial wastewaters** 163
- **7.3.2 Bacterial growth model** 163

#### 7.4 EFFECT OF pH ON THE ANAEROBIC ACIDOGENESIS OF AGRO-INDUSTRIAL WASTEWATERS FOR MAXIMIZATION OF BIO-HYDROGEN PRODUCTION: A LAB-SCALE EVALUATION USING BATCH TESTS. 165

- **7.4.1 Effect of pH** 165
- **7.4.2 Kinetic analysis** 170

#### 7.5 EFFECT OF HYDRAULIC RETENTION TIME (HRT) ON THE ANAEROBIC CO-DIGESTION OF AGRO-INDUSTRIAL WASTES IN A TWO-STAGE CSTR SYSTEM 177

- **7.5.1 Effect of HRT in the acidogenic reactor** 178
- **7.5.2 Effect of HRT in the methanogenic reactor** 182

#### 7.6 BIOCHEMICAL METHANE POTENTIAL 186

#### 7.7 CONCLUSIONS 188

#### 7.8 REFERENCES 189

### CHAPTER 8. ANAEROBIC CO-DIGESTION OF AGRO-INDUSTRIAL WASTEWATERS AND SWEET SORGHUM IN A TWO-STAGE SYSTEM. 195

#### 8.1 ABSTRACT 195

#### 8.2 INTRODUCTION 196

#### 8.3 THE INFLUENCE OF pH AND RELATIVE FACTORS ON ACIDOGENIC HYDROGEN PRODUCTION FROM A MIXTURE OF SWEET SORGHUM STALKS, CHEESE WHEY AND COW MANURE 200

- **8.3.1 Materials** 200
- **8.3.2 Bacterial growth model** 202
- **8.3.3 Effect of pH** 203
- **8.3.4 Heat-treated inoculum** 211
- **8.3.5 Substrate concentration** 214
- **8.3.6 Effect of ensiled sorghum** 215

#### 8.4 ANAEROBIC MESOPHILIC CO-DIGESTION OF ENSILED SORGHUM, CHEESE WHEY AND LIQUID COW MANURE IN A TWO-STAGE CSTR SYSTEM: EFFECT OF HYDRAULIC RETENTION TIME 216

- **8.4.1 Materials** 217
- **8.4.2 Effect of HRT in the acidogenic reactor** 219
- **8.4.3 Effect of HRT in the methanogenic reactor** 223

#### 8.5 BIOCHEMICAL METHANE POTENTIAL 229

#### 8.6 CONCLUSIONS 230

#### 8.7 REFERENCES 231

### CHAPTER 9. IMPLICATIONS OF THE OMW USE IN CO-DIGESTION MIXTURES IN A TWO-STAGE SYSTEM. 237

#### 9.1 ABSTRACT 237
9.2 INTRODUCTION 238
9.3 MATERIALS AND EXPERIMENTAL SET-UP 239
9.4 FERMENTATIVE HYDROGEN PRODUCTION IN BATCH EXPERIMENTS USING AGRO-
WASTES: EFFECT OF CO-DIGESTION UNDER CONTROLLED pH. 240
9.5 A COMPARATIVE STUDY ON METHANOGENESIS OF AGRO-WASTES MIXTURES: EFFECT
OF SUBSTRATE TYPE AND INITIAL CONCENTRATION. 251
9.6 CONCLUSIONS 255
9.7 REFERENCES 256

CHAPTER 10. ANAEROBIC CO-DIGESTION OF COW MANURE AND SWEET
SORGHUM IN A TWO-STAGE SYSTEM. 259
10.1 ABSTRACT 259
10.2 INTRODUCTION 260
10.3 MATERIALS 262
10.4 EFFECT OF PH 264
10.5 EFFECT OF HRT 270
10.5.1 Effect of HRT in the acidogenic reactor 271
10.5.2 Effect of HRT in the methanogenic reactor 273
10.6 BIOCHEMICAL METHANE POTENTIAL 275
10.7 CONCLUSIONS 276
10.8 REFERENCES 277

CHAPTER 11. ADM1-BASED MODELING OF METHANE PRODUCTION IN A
TWO-STAGE SYSTEM 281
11.1 ABSTRACT 281
11.2 INTRODUCTION 282
11.3 KINETIC MODEL 283
11.4 MODELING OF METHANE PRODUCTION OF RAW LCM 290
11.5 MODELING OF METHANE PRODUCTION FROM DIFFERENT ACIDIFIED EFFLUENTS 291
11.5.1 Acidified mixture of 55%OMW, 40%CW and 5%LCM 291
11.5.2 Acidified mixture of 90%CW and 10%LCM 293
11.5.3 Acidified mixture of 20%OMW and 80%LCM 296
11.5.4 Acidified mixture of 55%ES3 stalks, 40%CW and 5%LCM 298
11.6 CONCLUSIONS 301
11.7 REFERENCES 301

CHAPTER 12. POST-TREATMENT OF ANAEROBIC DIGESTATE 303
12.1 ABSTRACT 303
12.2 INTRODUCTION 304
12.3 A COMBINED COAGULATION/FLOCCULATION AND MEMBRANE FILTRATION PROCESS
FOR THE TREATMENT OF ANAEROBIC DIGESTION EFFLUENT. 305
12.3.1 Materials 305
12.3.2 Experimental design 306
12.3.3 Results and Discussion 307
12.3.4 Conclusions 311
12.4 VERMI-CONVERSION OF ANAEROBIC SLUDGES BY EISENIA FOETIDA EARTHWORMS 312
CHAPTER 13. TECHNO-ECONOMIC EVALUATION OF A FULL-SCALE CENTRALIZED ANAEROBIC DIGESTION PLANT

13.1 INTRODUCTION

13.2 BIOGAS PLANT COMPONENTS

13.2.1 Feedstock storage

13.2.2 Feeding systems for wet and dry substrates

13.2.3 Types of full-scale digesters

13.2.4 Digester components

13.2.5 Biogas Conditioning

13.2.6 Energy recovery from biogas

13.2.7 Pipes and fittings

13.3 COMPOSTING OF DIGESTATE

13.3.1 Aerobic composting

13.3.2 Vermicomposting

13.4 PROCESS MONITORING

13.5 TROUBLESHOOTING

13.6 SAFETY PRACTICES FOR ANAEROBIC DIGESTION SYSTEMS

13.7 ECONOMIC ASSESSMENT OF ANAEROBIC DIGESTION PLANT

13.8 REFERENCES

CHAPTER 14. MAIN CONCLUSIONS AND FUTURE RECOMMENDATIONS

14.1 MAIN CONCLUSIONS

14.2 FUTURE RECOMMENDATIONS

APPENDIX A

APPENDIX B ABBREVIATIONS

APPENDIX C ΣΥΝΟΨΗ ΔΙΔΑΚΤΟΡΙΚΗΣ ΔΙΑΤΡΙΒΗΣ

CURRICULUM VITAE
List of Tables

Table 1-1: Sorghum types, conventional uses and possible applications (Zegada-Lizarazu and Monti, 2012). .................................................................12
Table 1-2: Physical pretreatment processes of lignocellulosic materials (Taherzadeh and Karimi, 2008). ........................................................................................................17
Table 1-3: Chemical, physicochemical and biological pretreatment processes of lignocellulosic materials (Taherzadeh and Karimi, 2008). .............................................18
Table 1-4: Effects of the different pretreatments on the physical/ chemical composition or structure of lignocellulose (Hendriks and Zeeman, 2009) .......25
Table 2-1: Different sorghum used with their main characteristics. .........................46
Table 3-1: Prefecture of Achaia ........................................................................59
Table 3-2: Prefecture of Ionian Islands. ...............................................................60
Table 3-3: Prefecture of Preveza. .....................................................................60
Table 3-4: Prefecture of Lefkada. .................................................................60
Table 3-5: Prefecture of Centro Ricerche Bonomo (Italian province of Bari) ...........61
Table 3-6: Chemical composition of different types of agro-wastes from different regions in Western Greece. .................................................................62
Table 3-7: Chemical composition of agro-wastes in Italian province of Bari .........63
Table 3-8: Chemical composition of each agro-industrial wastewater used in BMP assay. ......................................................................................................................64
Table 3-9: Methane yield, as obtained from the BMP assay of each agro-industrial waste and co-digested mixtures. .................................................................64
Table 3-10: Methane production potential and biodegradability for each agro-industrial waste and co-digested mixtures. .......................................................70
Table 3-11: Kinetic parameters of methane production estimated using the modified Gompertz equation .................................................................74
Table 4-1: Chemical composition of fresh (FS1) and ensiled (ES1) sweet sorghum. ....80
Table 4-2: Chemical composition of fresh (FS1) sweet sorghum. .......................85
Table 4-3: Enzymatic hydrolysis of FS1 (Proposed tests by GAME). ....................88
Table 4-4: Continue .......................................................................................89
Table 4-5: Chemical composition of ensiled (ES3) sweet sorghum. ......................92
Table 4-6: Thermal treatment of ES3 (control experiments). ...............................94
Table 4-7: Thermal-alkaline pretreatment of ES3 using 0.5%NaOH-0.5%KOH as alkaline solution. .......................................................................................94
Table 4-8: Thermal-alkaline pretreatment of ES3 using 1%NaOH-1%KOH as alkaline solution. .......................................................................................95
Table 4-9: Chemical composition of two fresh sorghums (FS2 and FS3). Values correspond to mean ± standard deviation of measurement performed in duplicate ........................................................................................................98
Table 9-2: Kinetic parameters of hydrogen production estimated using the modified Gompertz equation. 249
Table 9-3: Initial characteristics of acidified mixtures used in the experiments. 251
Table 9-4: Kinetic parameters of methane production of two different mixtures (A and B) estimated using the modified Gompertz equation. 253
Table 9-5: Removal efficiencies and methane yields during methanogenesis of mixtures A and B. 254
Table 10-1: Chemical composition of fresh and ensiled sweet sorghum (FS1 and ES3). 262
Table 10-2: Chemical composition of liquid cow manure (LCM). 263
Table 10-3: Kinetic parameters of hydrogen production estimated using the modified Gompertz equation. 268
Table 10-4: Operating conditions in the acidogenic and methanogenic CSTR. 270
Table 10-5: Fermentation performance under steady-state conditions for each HRT. 272
Table 11-1: Biochemical rate coefficients (v_{i,j}) and kinetic rate equations (p_j) for particulate components (i=16-28; j=1-22). 285
Table 11-2: Individual inhibition terms (Batstone et al., 2002). 286
Table 11-3: Stoichiometric coefficients from monosaccharide uptake. 287
Table 11-4: Stoichiometric coefficients from lactate uptake. 288
Table 11-5: Estimated coefficients for sugars and lactate degradation treating the acidified mixture of 55%OMW, 40%CW and 5%LCM. 292
Table 11-6: Estimated coefficients for sugars and lactate degradation treating the acidified mixture of 90%CW and 10%LCM. 294
Table 11-7: Estimated coefficients for sugars and lactate degradation treating the acidified mixture of 20%OMW and 80%LCM. 296
Table 11-8: Selected coefficients for sugars and lactate degradation treating the acidified mixture of 55%ES3, 40%CW and 5%LCM. 298
Table 11-9: Estimated maximum specific uptake rates treating the acidified mixture of 55%ES3, 40%CW and 5%LCM. 299
Table 12-1: Characteristics of methanogenic effluent. 305
Table 12-2: Main characteristics of methanogenic effluent after coagulation/flocculation at different polyelectrolyte concentrations. 308
Table 12-3: Physicochemical characterization of fractions of the two membrane modules (UF and NF). 309
Table 12-4: Flux and fouling results of the membrane filtration of anaerobic effluent. 311
Table 12-5: Main characteristics of cow dung (CD) and anaerobic sludges (AS1 and AS2). 312
Table 12-6: Compositions of the different feed mixtures tested of cow dung (CD) and anaerobic sludges of co-digested methanogenic agro-industrial mixture sludge (AS1) and municipal sewage sludge (AS2). 314
Table 12-7: Physicochemical analysis of the initial feeds of experiments in different feed mixtures of cow dung with anaerobic sludges. .................................................................315
Table 12-8: Physicochemical analysis of the vermicompost obtained at the termination of experiments in different feed mixtures of cow dung with anaerobic sludges. The duration (t) of earthworms’ survival ........................................317
Table 13-1: Typical Biogas Composition ...................................................................332
Table 13-2: The basic equipment of anaerobic digestion plant ................................350
Table 13-3: Basic infrastructures for the composting plant. .......................................350
Table 13-4: Basic infrastructures for the artificial wetland (disposal of liquid effluent) .........................................................................................................................351
Table 13-5: Primary and secondary composting plant equipment and general operating support of plant. ........................................................................................................351
Table 13-6: Maintenance and operating costs of building infrastructure and plant equipment ........................................................................................................................................352
Table 13-7: Staff costs on an annual basis (include insurance fees) ........................352
Table 13-8: Income from an integrated plant for waste management in case of investment with subsidy. .................................................................353
Table 13-9: Income from an integrated plant for waste management with self-financing investment. ........................................................................................................353
Table 13-10: Financial information for the investment with subsidy. .................353
Table 13-11: Financial information for the investment without subsidy. ............354
List of Figures

Figure 1-1: Schematic presentation of a lignocellulose structure. Cellulose microfibrils with crystalline and paracrystalline (amorphous) regions are embedded into a matrix consisting of lignin and hemicellulose in the cell wall (Kallioinen, 2014). ............................................................................................... 14

Figure 1-2: Pretreatment of lignocellulosic material prior to bioethanol and biogas production. ........................................................................................................... 15

Figure 1-3: Effect of pretreatment on accessibility of degrading enzymes (Taherzadeh and Karimi, 2008). ......................................................................... 16

Figure 1-4: Outline of anaerobic digestion process for biogas production. .................28

Figure 2-1: Schematic diagram of the two-stage system used in this study for combined hydrogen and methane production. ..................................................... 49

Figure 3-1: Cumulative biogas and methane production during the BMP assay from a) Olive Mill Wastewater (OMW), b) Cheese Whey (CW), c) Liquid cow Manure (LCM) and d) blank sample. Errors bars represent the standard deviation for the replicates.................................................................................. 65

Figure 3-2: Cumulative biogas and methane production during the BMP assay from mixtures with ratio a) S_{OMW}:S_{LCM}-75:25, b) S_{OMW}:S_{LCM}-50:50 and c) S_{OMW}:S_{LCM}-25:75. Errors bars represent the standard deviation for the replicates.................................................................................. 66

Figure 3-3: Cumulative biogas and methane production during the BMP assay from mixtures with ratio a) S_{CW}:S_{LCM}-75:25, b) S_{CW}:S_{LCM}-50:50 and c) S_{CW}:S_{LCM}-25:75. Errors bars represent the standard deviation for the replicates. ............................................................................................................. 67

Figure 3-4: Cumulative biogas and methane production during the BMP assay from mixtures with ratio a) S_{OMW}:S_{CW}-75:25, b) S_{OMW}:S_{CW}-50:50 and c) S_{OMW}:S_{CW}-25:75. Errors bars represent the standard deviation for the replicates. ............................................................................................................. 68

Figure 3-5: Cumulative biogas and methane production during the BMP assay from mixtures with ratio a) S_{OMW}:S_{CW}:S_{LCM}-50:25:25, b) S_{OMW}:S_{CW}:S_{LCM}-25:50:25 and c) S_{OMW}:S_{CW}:S_{LCM}-25:25:50. Errors bars represent the standard deviation for the replicates.................................................................................. 69

Figure 3-6: Methane yields at STP, as obtained from the BMP assay of three mono- and twelve co-digested substrates................................................................. 71

Figure 4-1: The effect of acid and alkaline pretreatment of fresh sweet sorghum biomass on soluble carbohydrates concentration expressed in glucose
equivalents at (a) 25°C, (b) 37°C, (c) 50°C and (d) 80°C. Dotted lines depict the initial amount of soluble sugars in fresh sweet sorghum (FS1).

Figure 4-2: The effect of acid and alkaline pretreatment of ensiled sweet sorghum biomass on soluble carbohydrates concentration expressed in glucose equivalents. Dotted lines depict the initial amount of soluble sugars in ensiled sweet sorghum (ES1).

Figure 4-3: Soluble sugar determination from untreated and pretreated (a) fresh sweet sorghum (FS1) and (b) ensiled sweet sorghum (ES1) with 0.5% H₂SO₄, HCl and NaOH at 50 and 80°C for 120 min.

Figure 4-4: Correlation between estimated total cost/profit and produced sugars (in equivalent glucose).

Figure 4-5: Soluble carbohydrate determination after thermal-alkaline treatment of ES3 with (a) 0.5%NaOH-0.5%KOH and (b) 1%NaOH-1%KOH at different temperatures and residence times.

Figure 4-6: Lignin removal after thermal-alkaline treatment of ES3 with (a) 0.5%NaOH-0.5%KOH and (b) 1%NaOH-1%KOH at different temperatures and residence times.

Figure 4-7: Evolution of (a) total, soluble and insoluble sugars, (b) ethanol, lactic and volatile fatty acids (mg/g dry FS2) and (c) biogas (H₂/CO₂) during ensiling of FS2 under ambient conditions.

Figure 4-8: Evolution of (a) total, soluble and insoluble sugars and (b) lactic acid (mg/g dry FS3) during ensiling of FS3 under ambient conditions.

Figure 4-9: Cumulative methane production during the BMP assay from (a) fresh sorghum (FS3) and (b) ensiled sorghum (ES3). Errors bars represent the standard deviation for the replicates.

Figure 4-10: Cumulative methane production during the BMP assay from ensiled sorghum (ES3) pretreated with (a) 0.5%NaOH-0.5%KOH and (b) 1%NaOH-1%KOH solution. Errors bars represent the standard deviation for the replicates.

Figure 4-11: Cumulative methane production (experimental data and modified Gompertz model simulation) during the BMP assay from (a) fresh sorghum (FS3) and (b) ensiled sorghum (ES3).

Figure 4-12: Cumulative methane production (experimental data and modified Gompertz model simulation) during the BMP assay from (a) fresh sorghum (FS3) and (b) ensiled sorghum (ES3).

Figure 5-1: Concentration of (a) soluble COD (SCOD) and (b) total (TS) and volatile solids (VS) during acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v).

Figure 5-2: Variation of volatile fatty acids (VFAs) in (a) the influent and (b) the effluent during acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v).

Figure 5-3: (a) Biogas production rate and (b) pH value during acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v).
Figure 5-4: Concentration of (a) soluble COD (SCOD) and (b) total (TS) and volatile solids (VS) during methanogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v). .................................................................115
Figure 5-5: (a) Biogas and methane production rates and (b) pH value during methanogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v). .................................................................116
Figure 5-6: Concentration of volatile fatty acids (VFAs) in (a) the influent and (b) the effluent during methanogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v).117
Figure 5-7: Concentration of (a) soluble COD (SCOD) and (b) total (TS) and volatile solids (VS) during acidogenesis of CW/LCM mixture (90/10, v/v). .................118
Figure 5-8: Variation of volatile fatty acids (VFAs) in (a) the influent and (b) the effluent during acidogenesis of CW/LCM mixture (90/10, v/v). ......................119
Figure 5-9: (a) Biogas production rate and (b) pH value during acidogenesis of CW/LCM mixture (90/10, v/v). ..........................................................................119
Figure 5-10: Concentration of (a) soluble COD (SCOD) and (b) total (TS) and volatile solids (VS) during methanogenesis of CW/LCM mixture (90/10, v/v).120
Figure 5-11: (a) Biogas and methane production rates and (b) pH value during methanogenesis of CW/LCM mixture (90/10, v/v). ........................................121
Figure 5-12: Variation of volatile fatty acids (VFAs) in (a) the influent and (b) the effluent during methanogenesis of CW/LCM mixture (90/10, v/v). .............122
Figure 5-13: Concentration of (a) soluble COD (SCOD) and (b) total (TS) and volatile solids (VS) during acidogenesis of OMW/CW mixture (80/20, v/v). ..124
Figure 5-14: Variation of volatile fatty acids (VFAs) in (a) the influent and (b) the effluent during acidogenesis of OMW/CW mixture (80/20, v/v). ................124
Figure 5-15: (a) Biogas production rate and (b) pH value during acidogenesis of OMW/CW mixture (80/20, v/v). .................................................................125
Figure 5-16: Concentration of (a) soluble COD (SCOD) and (b) total (TS) and volatile solids (VS) during methanogenesis of OMW/CW mixture (80/20, v/v). .......................................................................................126
Figure 5-17: (a) Biogas and methane production rates and (b) pH value during methanogenesis of OMW/CW mixture (80/20, v/v). ........................................126
Figure 5-18: Variation of volatile fatty acids (VFAs) in (a) the influent and (b) the effluent during methanogenesis of OMW/CW mixture (80/20, v/v). ............127
Figure 5-19: Concentration of (a) soluble COD (SCOD) and (b) total (TS) and volatile solids (VS) during acidogenesis of OMW/LCM mixture (20/80, v/v).128
Figure 5-20: Variation of volatile fatty acids (VFAs) in (a) the influent and (b) the effluent during acidogenesis of OMW/LCM mixture (20/80, v/v). ..........129
Figure 5-21: (a) Biogas production rate and (b) pH value during acidogenesis of OMW/LCM mixture (20/80, v/v) .................................................................130
Figure 5-22: Concentration of (a) soluble COD (SCOD) and (b) total (TS) and volatile solids (VS) during methanogenesis of OMW/LCM mixture (20/80, v/v). .......................................................................................131
Figure 5-23: (a) Biogas and methane production rates and (b) pH value during methanogenesis of OMW/LCM mixture (20/80, v/v). .................................................. 132
Figure 5-24: Variation of volatile fatty acids (VFAs) in (a) the influent and (b) the effluent during methanogenesis of OMW/LCM mixture (20/80, v/v). ............. 132
Figure 6-1: Evolution of (a) biogas and methane production rates and (b) main volatile fatty acids concentration during LCM treatment in a single-stage process. .............................................................................................................. 146
Figure 6-2: Evolution of (a) soluble COD concentration and (b) total and volatile solids during LCM treatment in a single-stage process. ............................................ 146
Figure 6-3: Evolution of (a) biogas and methane production rates and (b) main volatile fatty acids concentration during CW treatment in a single-stage process. .............................................................................................................. 147
Figure 6-4: Evolution of (a) soluble COD concentration and (b) total and volatile solids during CW treatment in a single-stage process. ............................................ 148
Figure 6-5: Evolution of (a) biogas production rate, (b) main volatile fatty acids concentration and (c) total and volatile solids concentration during acidogenesis of CW treatment in a two-stage process. ........................................ 151
Figure 6-6: Evolution of (a) biogas and methane production rates and (b) main volatile fatty acids concentration during methanogenesis of CW treatment in a two-stage process. .............................................................................................................. 151
Figure 6-7: Evolution of (a) soluble COD concentration and (b) total and volatile solids during methanogenesis of CW treatment in a two-stage process. .......... 152
Figure 7-1: (a) Biogas and hydrogen evolution at STP conditions, (b) consumption of total carbohydrates and (c) main metabolic products, as a function of different pH values tested. ........................................................................................................ 167
Figure 7-2: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of OMW / CW / LCM mixture (55 / 40 / 5, v/v/v), at pH 6.0. ........................................................................................................ 169
Figure 7-3: (a) Net hydrogen yield and (b) TVFAs produced at each pH value tested.  170
Figure 7-4: Correlation between the hydrogen production and the produced butyric acid in all pH values tested. Labels indicate the controlled pH value. .......... 175
Figure 7-5: (a) Cumulative hydrogen production (experimental data and modified Gompertz model simulation) during batch acidogenesis of OMW / CW / LCM mixture (55 / 40 / 5, v/v/v), (b) specific hydrogen production potential (SHPP) and (c) maximum specific hydrogen production rate (SHPRm) at the pH values tested in this study. ........................................................................................................ 176
Figure 7-6: (a) Biogas and hydrogen production and (b) total and soluble carbohydrates concentration during acidogenesis for each HRT tested. .............. 178
Figure 7-7: Consumption of hydrogen in batch mode at HRT of 3 d. .......................... 180
Figure 7-8: (a) Evolution of main volatile fatty acids and lactic acid and (b) total (TS) and volatile solids (VS) concentration during acidogenesis for each HRT tested. .................................................................................................................181

Figure 7-9: (a) Variation of main volatile fatty acids (VFA) and (b) biogas and methane evolution during methanogenesis for each HRT tested. .........................................................183

Figure 7-10: (a) Total COD (TCOD), soluble COD (SCOD), total organic carbon (TOC), soluble organic carbon (SOC) and TVFA (expressed in units of COD) evolution and (b) total (TS) and volatile solid (VS) concentration during methanogenesis for each HRT tested. ........................................................................185

Figure 7-11: Cumulative methane production during the BMP assay from the mixture (55% OMW, 40% CW and 5% LCM) and blank sample. Errors bars represent the standard deviation for the replicates. ..............................................................................187

Figure 7-12: Cumulative methane production (experimental data and modified Gompertz model simulation) during BMP assay of OMW / CW / LCM mixture (55 / 40 / 5, v/v/v). .................................................................................................................188

Figure 8-1: Effect of pH on (a) carbohydrates consumption and (b) main soluble end-products. ..........................................................................................................................203

Figure 8-2: Effect of pH on (a) biogas and hydrogen production and (b) hydrogen yield. ..........................................................................................................................204

Figure 8-3: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and lactic acid and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of agro-waste mixture (in ratio of 55:40:5), at pH 5.5. ......206

Figure 8-4: Cumulative hydrogen production (experimental data and modified Gompertz model simulation) at different pH values tested. ..........................................................210

Figure 8-5: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and lactic acid and (c) gaseous products (biogas and hydrogen) using heat-pretreated enriched anaerobic sludge, at pH 5.5...........................................212

Figure 8-6: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and lactic acid and (c) gaseous products (biogas and hydrogen) using half initial substrate concentration, at pH 5.5...........................................214

Figure 8-7: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and lactic acid and (c) gaseous products (biogas and hydrogen) using ensiled sorghum, at pH 5.5. .............................................................................................................215

Figure 8-8: Evolution of (a) biogas and hydrogen production rate and (b) main end-products during acidogenic reactor operation at different HRT...............................................220

Figure 8-9: Effect of HRT on (a) hydrogen production rate, (b) hydrogen content and (c) hydrogen yield, during acidogenic reactor operation..............................................221

Figure 8-10: Evolution of (a) total and soluble carbohydrates concentration, (b) total and soluble COD concentration and (c) total and volatile solids concentration during acidogenic reactor operation at different HRT...............................................222
Figure 8-11: Evolution of (a) biogas and methane production rate, (b) methane content and (c) main volatile fatty acids concentration during methanogenesis operated at different HRT. ................................................................................. 224
Figure 8-12: Effect of HRT on (a) methane production rate, (b) methane content and (c) methane yield. .............................................................................................................................. 226
Figure 8-13: Evolution of (a) pH, (b) total, soluble COD and TVFA (expressed in COD units) concentration and (b) total and volatile solids concentration during methanogenesis operated at different HRT. ......................................................................... 228
Figure 8-14: Cumulative methane production during the BMP assay from the mixture (55% ES3, 40% CW and 5% LCM) and blank sample. Errors bars represent the standard deviation for the replicates. ................................................................. 229
Figure 8-15: Cumulative methane production (experimental data and modified Gompertz model simulation) during BMP assay of ES3 / CW / LCM mixture (55 / 40 / 5, v/v/v). ............................................................................................. 230
Figure 9-1: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of OMW (SOMW:55), at pH 6.0.......................................................................................... 241
Figure 9-2: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of CW (SCW:40), at pH 6.0. .................................................................................. 242
Figure 9-3: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of LCM (SLCM:30), at pH 6.0. ................................................................. 243
Figure 9-4: Cumulative hydrogen production (experimental data and modified Gompertz model simulation) during the batch acidogenesis of each substrate (OMW, CW and LCM) at controlled pH 6.0.................................................................................. 244
Figure 9-5: Comparative hydrogen yield between OMW, CW, LCM, theoretical calculated mixture yield and experimental mixture yield (The experimental mixture yield was obtained from Section 7.4.1). ................................................................. 245
Figure 9-6: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of FS2 stalks (SFS2:55), at pH 5.5. ................................................................. 246
Figure 9-7: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of CW (SCW:40), at pH 5.5. ................................................................. 247
Figure 9-8: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of LCM (SLCM:30), at pH 5.5.......................................................................................... 248
Figure 9-9: Cumulative hydrogen production (experimental data and modified Gompertz model simulation) during the batch acidogenesis of each substrate (FS2, CW and LCM) at controlled pH 5.5. ................................................................. 249
Figure 9-10: Comparative hydrogen yield between FS2, CW, LCM, theoretical calculated mixture yield and experimental mixture yield (The experimental mixture yield was obtained from Section 8.3.3). ......................................................... 250
Figure 9-11: Biogas and CH₄ production in each mixture (□ : biogas (full-strength), ■ : biogas (half-strength), Δ : CH₄ (full-strength), ▲ : CH₄ (half-strength)). ........................................ 252
Figure 9-12: Cumulative methane production (experimental data and modified Gompertz model simulation) during the batch methanogenesis of a) mixture A and b) mixture B. ......................................................................................................................... 253
Figure 9-13: Evolution of main volatile fatty acids during methanogenesis of each mixture. .............................................................................................................................................................................. 255
Figure 10-1: Effect of pH on (a) biogas and hydrogen production and (b) carbohydrates consumption. .................................................................................................................................................................. 264
Figure 10-2: Effect of pH on (a) main soluble end-products and (b) hydrogen yield. 265
Figure 10-3: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of mixture (95% FS1 and 5% LCM), at pH 5.0. 267
Figure 10-4: Cumulative hydrogen production (experimental data and modified Gompertz model simulation) at different pH values tested. ..................................................... 268
Figure 10-5: (a) Consumption of carbohydrates, b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of mixture (95% ES3 and 5% LCM), at pH 5.0. 269
Figure 10-6: Evolution of (a) biogas and hydrogen production rates, (b) hydrogen content and (c) main soluble end-products during acidogenesis for each HRT tested. ......................................................................................................................... 272
Figure 10-7: Evolution of (a) methane production rate and pH, (b) main volatile fatty acids concentration and (c) total, soluble COD and TVFA (expressed in units of COD) concentration during methanogenesis for each HRT tested. .......... 274
Figure 10-8: Cumulative methane production during the BMP assay from the mixture (95% ES3 and 5% LCM) and blank sample. Errors bars represent the standard deviation for the replicates................................................................. 275
Figure 10-9: Cumulative methane production (experimental data and modified Gompertz model simulation) during BMP assay of ES3 / LCM mixture (95 / 5, v/v). ......................................................................................................................... 276
Figure 11-1: Schematic description of ADM1 model including biochemical processes (1) acidogenesis from sugars, (2) acidogenesis from amino acids, (3) acetogenesis from LCFA, (4) acetogenesis from propionate, (5) acetogenesis from butyrate and valerate, (6) acetoclastic methanogenesis and (7) hydrogenotrophic methanogenesis. .......................................................................................... 283
Figure 11-2: Experimental and model prediction of (a) biogas and methane production and (b) pH value during the methanogenesis of LCM. .......................... 290
Figure 11-3: Experimental and model predicted (a) main volatile fatty acids concentrations and (b) soluble and particulate COD during the methanogenesis of LCM. 291

Figure 11-4: Experimental and model prediction of (a) biogas and methane production and (b) pH value during the methanogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v). 293

Figure 11-5: Experimental and model predicted (a) main volatile fatty acids concentrations and (b) soluble and particulate COD during the methanogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v). 293

Figure 11-6: Experimental and model prediction of (a) biogas and methane production and (b) pH value during the methanogenesis of CW/LCM mixture (90/10, v/v). 295

Figure 11-7: Experimental and model predicted (a) main volatile fatty acids concentrations and (b) soluble and particulate COD during the methanogenesis of CW/LCM mixture (90/10, v/v). 295

Figure 11-8: Experimental and model prediction of (a) biogas and methane production and (b) pH value during the methanogenesis of OMW/LCM mixture (20/80, v/v). 297

Figure 11-9: Experimental and model predicted (a) main volatile fatty acids concentrations and (b) soluble and particulate COD during the methanogenesis of OMW/LCM mixture (20/80, v/v). 297

Figure 11-10: Experimental and model prediction of (a) biogas and methane production and (b) pH value during the methanogenesis of ES3/CW/LCM mixture (55/40/5, v/v/v). 300

Figure 11-11: Experimental and model predicted (a) main volatile fatty acids concentrations and (b) soluble COD during the methanogenesis of ES3/CW/LCM mixture (55/40/5, v/v/v). 300

Figure 12-1: ζ potential, COD, and TSS reduction of supernatant phase in coagulation/flocculation experiments with different concentration of poly-(diallyldimethylammonium chloride). 308

Figure 12-2: Flux results throughout the ultrafiltration (UF) membrane. 310

Figure 13-1: Construction cost of concrete storage tank as a function of waste feed. 344

Figure 13-2: Cost of (a) stainless steel and (b) concrete digester as a function of the volume. 345

Figure 13-3: Cost of (a) stirrers, (b) heat exchanger and (c) biogas membrane as a function of digester volume. 346

Figure 13-4: (a) production of electric energy and (b) cost of CHP machine as a function of waste feed. 347

Figure 13-5: Construction cost of settling tank as a function of waste feed. 347

Figure 13-6: (a) cost of solar drying unit and (b) the amount of fry matter as a function of waste feed. 348
Figure 13-7: Cost of one or two (a) concrete and (b) stainless steel digesters as a function of the volume.

Figure A-1: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v), at pH 4.5.

Figure A-2: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v), at pH 5.0.

Figure A-3: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v), at pH 5.5.

Figure A-4: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v), at pH 6.5.

Figure A-5: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v), at pH 7.0.

Figure A-6: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v), at pH 7.5.

Figure A-7: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and lactic acid and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of agro-waste mixture (in ratio of 55:40:5), at pH 5.0.

Figure A-8: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and lactic acid and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of agro-waste mixture (in ratio of 55:40:5), at pH 6.0.

Figure A-9: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and lactic acid and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of agro-waste mixture (in ratio of 55:40:5), at pH 6.5.

Figure A-10: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of mixture (95% FS1 and 5%LCM), at pH 4.5.

Figure A-11: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of mixture (95% FS1 and 5%LCM), at pH 5.5.
Figure A-12: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of mixture (95% FS1 and 5%LCM), at pH 6.0. ......374
List of Pictures

Picture 2-1: The fields where the different sorghums were cultivated.................................47
Picture 2-2: (a) 1-L continuous reactor, (b) reactor’s geared motor drive unit and (c) automatic tailor-made device used in continuous experiments..............................50
Picture 2-3: (a) Steel reactor and (b) glass flasks used for batch experiments.........................51
Picture 2-4: (a) Experimental set-up and (b) orbital shaking water bath used in BMP assay....................................................................................................................52
Picture 4-1: (a) Chemical pretreated FS1 with H₂SO₄ solution (1.5%) and (b) orbital shaking water bath for chemical pretreatment procedure. .................................81
Picture 12-1: Coagulation/flocculation with the use of poly-(diallyldimethylammonium chloride)........................................................................................................306
Picture 12-2: (a) Membrane filtration module (1: input, 2: concentrate, 3: permeate and 4: pressure indicator) and (b) lab-scale experimental set-up. ...............................307
Picture 12-3: (a) Waste sedimentation and (b) agglomeration of the formed flocs. ..................309
Picture 12-4: From left to right: the feed (UF), the concentrate (UF), the permeate (UF) or feed (NF), the concentrate (NF) and the permeate (NF) .............................311
Picture 12-5: (a) plastic pots and (b) Eisenia foetida earthworms used in vermicomposting.................................................................313
Picture 13-1: (a) typical bunker silo and (b) storage tanks for liquid feedstock. .......................322
Picture 13-2: (a) typical cutting pump and (b) typical centrifugal pump used in a biogas facility .................................................................................................................324
Picture 13-3: (a) typical positive displacement pump and (b) design of a progressive cavity pump. ........................................................................................................325
Picture 13-4: Main configurations for feeding the digester with solid feedstock. ...............325
Picture 13-5: (a) typical stainless steel and (b) typical concrete made anaerobic digesters. ..............................................................................................................................327
Picture 13-6: (a) vertical digester covered by a double gas proof membrane and (b) horizontal continuous flow digester ..........................................................327
Picture 13-7: (a) vertical shaft agitator, (b) blade agitatorand (c) vortex fans. ..........................328
Picture 13-8: The sphere-like double-membrane biogas holders were manufactured and installed by JDV Equipment Corp., Dover, N.J. ........................................330
Picture 13-9: A biogas flare. .................................................................................................330
Picture 13-10: (a) biological desulphurization and (b) typical biogas dehumidification systems .............................................................................................................331
Picture 13-11: Containerized CHP generator (Weltec Biopower GmbH). .............................332
Picture 13-12: Typical hot water pipeline circuit in an AD facility .............................................333
Picture 13-13: (a) typical inline flame arrester and (b) biogas safety relief valve. .................334
Picture 13-14: (a) diaphragm valves and (b) knife gate valves. ..................................................334
Picture 13-15: (a) butterfly valves and (b) ball valves. .........................................................335
Picture 13-16: (a) diaphragm filter press, (b) decanter centrifuge and (c) belt filter press. ..................................................................................................................336
Picture 13-17: (a) aerated static piles and (b) in-vessel composting. ....................337
Picture 13-18: (a) aerated windrow composting and (b) compost turner for windrows.........................................................................................................................337
Picture 13-19: (a) ultrasonic flow meter and (b) Pt100 thermometer. ....................339
Picture 13-20: (a) on-line device and (b) portable device for gas composition measurement. ............................................................................................................339
1.1 Energy

Energy drives human life. It is crucial for continued human development and for human life. There is a need for a secure and accessibly supply of energy for the sustainability of modern societies. Now, energy has become an integral part of human life for almost every activity, e.g. domestic, transport, industrial, medical, etc. So, there is a need for energy security for sustainability of the growing world population. Energy resources will play an important role in the world’s future. Energy is considered a prime agent in the generation of wealth and a significant factor in economic development. Conventional energy sources based on oil, coal, and natural gas have proven to be highly effective drivers of economic progress, but at the same time damaging to the environment and to human health.

Continuation of the use of fossil fuels (conventional energy sources) is set to face multiple challenges namely (i) depletion of fossil fuel reserves, (ii) global warming and other environmental concerns, (iii) geopolitical and military conflicts, and (iv) continuing fuel price rise. These problems will create an unsustainable situation (Tiwari and Mishra, 2011). The energy crisis and environmental degradation are currently two vital issues for global sustainable development. It is now accepted that the dependence on fossil fuels - over 80% of energy consumption - contributes not only to climate change and global warming, but also to a rapid exhaustion of natural energy sources (Ni et al., 2006).
1.2 Renewable energy sources

Renewable energy sources (RES) are also often called alternative sources of energy. There are many alternative new and renewable energy sources which can be used instead of fossil and conventional fuels. The energy resources have been split into three categories: fossil fuels, renewable resources, and nuclear resources (Demirbas, 2005). Renewable energy resources such as biomass, wind, solar, hydropower and geothermal energy are abundant, inexhaustible and environmentally friendly. Renewable energy resources that use domestic resources have the potential to provide energy services with zero or almost zero emissions of both air pollutants and greenhouse gases. Renewable energy technologies produce marketable energy by converting natural phenomena into useful forms of energy. These technologies use the sun’s energy and its direct and indirect effects on the earth (solar radiation, wind, falling water and various plants, i.e. biomass), gravitational forces (tides), and the heat of the earth’s core (geothermal) as the resources from which energy is produced (Kalogirou, 2004). The contribution of renewable energy to global energy needs has continued to grow in recent years, stimulated by policy initiatives in an increasing number of countries. The main advantages of RES are as follows:

- They are practically inexhaustible sources of energy and contribute to reducing dependence on conventional energy resources.
- They are an answer to the energy problem for the stabilization of carbon dioxide emissions and other greenhouse gases. The deployment of renewables in the New Policies Scenario saves some 4.1 gigatonnes (Gt) of CO$_2$ emissions in 2035 compared with the 2010 fuel mix at the same level of total generation (IEA, 2013). In addition, by replacing energy generation plants which use conventional resources, they lead to a reduction in the emission of other pollutants, such as sulfur and nitrogen oxides which cause acid rain.
- They are domestic sources of energy and contribute to increasing energy independence and security of energy supply at the national level.
- They are geographically dispersed, leading to the decentralization of the energy system, making it possible for energy needs to be met at a local and regional level, thus relieving infrastructure systems and reducing losses from energy transmission.
- They provide opportunities for the rational use of energy sources because they cover a wide range of users’ energy needs (i.e. solar energy for low temperature heat, wind energy for electricity production).
- They usually have low operating costs which are not influenced by fluctuations in the international economy and especially in prices for conventional fuels.
- RES investments create a significant number of new jobs, especially at the local level.
- In many cases, they can be a catalyst for the renewal of economically and socially depressed areas and a magnet for local development through the promotion of relevant investments (for example, greenhouses using geothermal energy).
Several strategies are considered but all scenarios investigated include the increase of renewable energy in the energy mix. The European Commission (EC) aims at reducing greenhouse gas emissions, diversifying energy supply, reducing the dependence on unreliable and volatile fossil fuel markets, and creating new technologies and improving trade balances. For example, it also targets reaching a 20% share of energy from renewable sources by 2020 and at least 55% of renewable energy in gross final energy consumption in 2050 (EC, 2013). According to renewable energy progress report, Greece should be able to produce 18% of primary energy using RES by 2020 (in 2005, energy production from RES was already at 6.9%, and in 2010, it was 9.7%). These goals are headline targets of the European 2020 strategy for growth, since they contribute to Europe's industrial innovation and technological leadership as well as reducing emissions, improving the security of our energy supply and reducing our energy import dependence (EC, 2013). In the European Union, the RES installed capacity reached 325 GW, with a main contribution from hydropower (147 GW), wind (94 GW), solar (52 GW) and biomass (31 GW).

The Greece is a country endowed with particular regard renewables because of climate and geography. It enjoys high solar radiation throughout the year and in most of the sunshine lasts more than 2700 hours a year. Several also areas of mainland and island Greece have stable and strong winds on an ongoing basis. Because of the terrain, in several points of the interior, especially in the western part, there are suitable conditions, encouraging the creation of small and large dams, which means production of electricity through hydropower. Moreover, Greece as a primarily agricultural country has enough reserves of biomass suitable for energy production.

1.3 Biomass

The term "biomass" refers to organic matter that has stored energy through the process of photosynthesis. It exists in one form as plants and may be transferred through the food chain to animals' bodies and their wastes, all of which can be converted for everyday human use through processes such as combustion, which releases the carbon dioxide stored in the plant material (http://www.altenergy.org). The biomass resource can be considered as organic matter, in which the energy of sunlight is stored in chemical bonds. Typically photosynthesis converts less than 1% of the available sunlight to stored, chemical energy. When the bonds between adjacent carbon, hydrogen and oxygen molecules are broken by digestion, combustion, or decomposition, these substances release their stored, chemical energy. Biomass has always been a major source of energy for mankind and is presently estimated to contribute of the order 10–14% of the world’s energy supply (McKendry, 2002a).

Biomass has been recognized as a major world renewable energy source to supplement declining fossil fuel resources (Demirbas, 2007). Biomass appears to be an attractive feedstock for three main reasons. First, it is a renewable resource that could be sustainably developed in the future. Second, it appears to have formidably positive
environmental properties resulting in no net releases of carbon dioxide (CO₂) and very low sulfur content. Third, it appears to have significant economic potential provided that fossil fuel prices increase in the future (Demirbas, 2007). Biomass is a viable renewable resource and includes organic and animal wastes, wastewater, agricultural residues, energy crops and industrial wastes that can be used for the production of power, heat and biofuels (Claassen et al., 1999; Ni et al., 2006; Antonopoulou et al., 2008).

Biomass can be converted into useful forms of energy using a number of different processes. Factors that influence the choice of conversion process are: the type and quantity of biomass feedstock; the desired form of the energy, i.e. end-use requirements; environmental standards; economic conditions; and project specific factors. In many situations it is the form in which the energy is required that determines the process route, followed by the available types and quantities of biomass (McKendry, 2002b).

Conversion of biomass to energy is undertaken using two main process technologies: thermo-chemical and bio-chemical/biological (McKendry, 2002b). Mechanical extraction (with esterification) is the third technology for producing energy from biomass, e.g. rapeseed methyl ester (RME) bio-diesel. Currently the cost of bio-diesel compared with fossil fuel makes the technology uncompetitive but increasing environmental pressures to improve air quality, especially in cities, may change this situation in the near future. Within thermo-chemical conversion four process options are available: combustion, pyrolysis, gasification and liquefaction. Bio-chemical conversion encompasses two process options: digestion (production of biogas, a mixture of mainly methane and carbon dioxide) and fermentation (production of ethanol). A brief review of the main conversion processes is presented (McKendry, 2002b):

**Combustion:**
The burning of biomass in air, i.e. combustion, is used over a wide range of outputs to convert the chemical energy stored in biomass into heat, mechanical power, or electricity using various items of process equipment, e.g. stoves, furnaces, boilers, steam turbines, turbo-generators, etc. Combustion of biomass produces hot gases at temperatures around 800–1000°C. It is possible to burn any type of biomass but in practice combustion is feasible only for biomass with a moisture content <50%, unless the biomass is pre-dried. High moisture content biomass is better suited to biological conversion processes.

**Gasification:**
Gasification is the conversion of biomass into a combustible gas mixture by the partial oxidation of biomass at high temperatures, typically in the range 800–900°C. The low calorific value (CV) gas produced (about 4–6 MJ/N·m³) can be burnt directly or used as a fuel for gas engines and gas turbines. The product gas can be used as a feedstock (syngas) in the production of chemicals (e.g. methanol).
**Pyrolysis:**
Pyrolysis is the conversion of biomass to liquid (termed bio-oil or bio-crude), solid and gaseous fractions, by heating the biomass in the absence of air to around 500°C. Pyrolysis can be used to produce predominantly bio-oil if flash pyrolysis is used, enabling the conversion of biomass to bio-crude with an efficiency of up to 80%. The bio-oil can be used in engines and turbines and its use as a feedstock for refineries is also being considered.

**Fermentation:**
Fermentation is used commercially on a large scale in various countries to produce ethanol from sugar crops (e.g. sugar cane, sugar beet) and starch crops (e.g. maize, wheat). The biomass is ground down and the starch converted by enzymes to sugars, with yeast then converting the sugars to ethanol. The solid residue from the fermentation process can be used as cattle-feed and in the case of sugar cane, the bagasse can be used as a fuel for boilers or for subsequent gasification. The conversion of lignocellulosic biomass (such as wood and grasses) is more complex, due to the presence of longer-chain polysaccharide molecules and requires acid or enzymatic hydrolysis before the resulting sugars can be fermented to ethanol.

**Anaerobic Digestion (AD):**
AD is the conversion of organic material directly to a gas, termed biogas, a mixture of mainly methane and carbon dioxide with small quantities of other gases such as hydrogen sulphide. The biomass is converted by bacteria in an anaerobic environment, producing a gas with an energy content of about 20–40% of the lower heating value of the feedstock. AD is a commercially proven technology and is widely used for treating high moisture content organic wastes, i.e. 80–90% moisture. Biogas can be used directly in spark ignition gas engines and gas turbines and can be upgraded to higher quality i.e. natural gas quality, by the removal of CO₂.

**Mechanical extraction:**
Extraction is a mechanical conversion process used to produce oil from the seeds of various biomass crops, such as oilseed rape, cotton and groundnuts. The process produces not only oil but also a residual solid or ‘cake’, which is suitable for animal fodder. Three tons of rapeseed are required per ton of rapeseed oil produced. Rapeseed oil can be processed further by reacting it with alcohol using a process termed esterification to obtain RME or bio-diesel. RME is used in some European countries as a supplementary transport fuel.
1.4 Agro-industrial wastes

Growing interest in processes that involve the conversion of biomass to renewable energy, such as anaerobic digestion, has stimulated research in this field and a considerable number of research projects have been developed to assess ideal digestion conditions for different substrates such as agro-industrial wastes and energy crops. Certain agro-industries such as olive mills, cheese factories and dairy farms represent a considerable share of environmental problem in the Mediterranean countries producing large amounts of wastewaters i.e. cheese whey and cow manure.

1.4.1 Olive Mill Wastewater

Mediterranean people have been growing olive trees and extracting olive oil for thousands of years. Olive oil is produced from olive trees, each olive tree yielding between 15 and 40 kg of olives per year. Worldwide olive oil production for the year 2002 was $2.5 \times 10^6$ tons (Paraskeva and Diamadopoulos, 2006). According to recent statistics of the International Olive Oil Council (November 2010), the major world producers of olive oil are in the EU (74.3%), accounting for 46.2% in Spain, 15.2% in Italy, 10.6% in Greece, and 1.9% in Portugal. Outside the EU, yet in the Mediterranean area, the relevant olive oil producers are: Morocco (5.9%), Syria and Tunisia (each 5.0%), and Turkey (4.9%). The majority of the world consumption of olive oil is also in the Mediterranean area: Italy (23.5%), Spain (19.1%) and Greece (7.8%). The USA, with 9.0% of the total world olive oil consumption, plays also an important role in olive oil business (Justino et al., 2012).

Despite the health and economical benefits of the production and consumption of OMW, producing countries have to deal with two serious environmental issues: the high water consumption, and the extremely high toxicity of resulting effluents. There are about 25000 olive mills worldwide (Paraskeva and Diamadopoulos, 2006). The traditional process of olive oil extraction by batches has been discontinued for some time, and nowadays the extraction of olive oil can be obtained by one of two different continuous processes: the two-phase or the three-phase systems (Justino et al., 2012). The two-phase and the three-phase extraction processes generate two main wastes: a brownish-black liquid effluent called olive oil mill wastewater (OMW), and a solid waste, generally called pomace or husk (Obied et al., 2005).

OMWs have been spread, without any valorization treatment, into soil or nearby streams and rivers, for many years, being very harmful to soil microflora, plants and freshwater species (Aggelis et al., 2003; Roig et al., 2006). The management and treatment of OMW have been considered as the main goal of the majority of research studies in this field. In order to minimize these environmental impacts, olive mills have been obliged to treat or even reduce substantially their wastes.

Wastewater arising from olive processing is one of the strongest industrial effluents, with chemical oxygen demand (COD) values of up to 220 g/L and corresponding
biochemical oxygen demand (BOD) values of up to 100 g/L (Paraskeva and Diamadopoulos, 2006). The wastewater arising from the milling process amounts to 0.5–1.5 m³ per 1000 kg of olives depending on the process. The discontinuous process produces less but more concentrated wastewater (0.5–1 m³ per 1000 kg) than the centrifugation process (1–1.5 m³ per 1000 kg) (Hamdi, 1996). However, the complex physico-chemical composition of OMW represents a technical difficulty to achieve efficient treatments, since its recalcitrant compounds-rich composition is highly variable, depending on many factors such as the type of olive oil extraction process, the local and seasonal nature of oil production, the climatic conditions and cultivation methods (Roig et al., 2006; McNamara et al., 2008).

Besides its strong organic content (BOD₅ 35–110 g/L, COD 45–170 g/L, suspended solids (SS) 1–9 g/L), olive mill wastewater (OMW) contains high concentrations of recalcitrant compounds such as lignin and tannins which give it a characteristic dark color (52–180 g/L Pt-Co units), but, most importantly, it contains phenolic compounds and long-chain fatty acids which are toxic to microorganisms and plants (Paixo and Anselmo, 2002; Paraskeva and Diamadopoulos, 2006). The phenolic compounds can be either simple phenols and flavonoids, or polyphenols which result from polymerisation of the simple phenols. The concentration of phenolic compounds in OMW varies greatly from 0.5 to 24 g/L (Borja et al., 2006). The high recalcitrant organic load and the associated toxicity make the treatment of OMW imperative. Problems arise also from the fact that olive oil production is seasonal and so the treatment process should be flexible enough to operate in a non-continuous mode, otherwise storage of the wastewater will be required.

### 1.4.2 Cheese Whey

The dairy industry is one of the main sources of industrial effluent generation in Europe (Demirel et al., 2005). This industry is based on the processing and manufacturing of raw milk into products such as yogurt, ice cream, butter, cheese and various types of desserts by means of different processes, such as pasteurization, coagulation, filtration, centrifugation, chilling, etc. (Rivas et al., 2010). Moreover, the wastewater management, climate, operating conditions and types of cleaning-in-place also influence the dairy effluents characterization (Pattnaik et al., 2008). The volume of effluents produced in the cheese manufacturing industry has increased with the increase in cheese production. For the production of 1 kg of cheese, 10 kg of milk are needed, originating 9 kg of cheese whey. Worldwide, 40.7 × 10⁶ tons per year of cheese whey are produced (Prazeres et al., 2012).

According to FAO (Food and Agricultural Organization) cheese is one of the main agricultural products worldwide. The European Union dominates its production and consumption, followed by the United States. Whatever type of cheese (Parmesan, Mozzarella, Gouda, Danish blue, Brie, Camembert, Feta, Serpa, etc.), the making factories generate effluents that represent a significant environmental impact (Ghaly and...
Singh, 1989). From an environmental point of view, among the dairy effluent key parameters, a considerable high organic load should be highlighted. Cheese effluents exhibit COD values in the interval 0.8–102 g/L and BOD values in the range 0.6–60 g/L leading to a high consumption of dissolved oxygen in water bodies (Carvalho et al., 2013). The lactose and fat contents can be considered as the main responsible for COD and BOD. With their very high concentration of organic matter, these effluents may create serious problems of organic burden on the local municipal sewage treatment systems (Janczukowicz et al., 2008). Comparing the organic load of domestic wastewater with organic load of cheese whey, we can conclude that the load is equivalent to the pollution load of one hundred times the volume of common domestic wastewaters (Mockaitis et al., 2006). The BOD/COD ratio is normally above 0.5 constituting a substrate easily biodegradable by anaerobic or aerobic digestions (Prazeres et al., 2012).

Total suspended solids have values within 1.3–22.0 g/L. The high CW salinity (conductivity in the proximity of 8 mS/cm) is the consequence of the type of whey produced in the process and NaCl addition during cheese production. Acidic pH (3.8-6.5) favors the filamentous biomass growth (Ghaly, 1996). The low buffering capacity of CW is responsible for the rapid acidification in biological treatments (Castelló et al., 2009). The main mineral components (>50%) are NaCl, KCl and calcium salts (Dragone et al., 2009; Venetsaneas et al., 2009). Lactose is the main responsible of the organic load, (Carvalho et al., 2013) and an extensive number of microorganisms cannot directly use it as a carbon source (González Siso, 1996). In addition, microorganisms may require tight conditions and addition of some supplementary chemicals (Carvalho et al., 2013).

Other inhibiting parameters of the biological processes can be mentioned, such as free ammonia, potassium, volatile fatty acids, etc. (Appels et al., 2008). CW poses a considerable risk of eutrophication attributable to the nitrogen (0.2-1.76 kg/m³) and phosphorus (0.124-0.54 kg/m³) contents (Prazeres et al., 2012). Eutrophication leads to many water quality problems including increased purification costs, interference with the recreational and conservation value of impoundments, loss of live-stock and the possible sublethal effects of algal toxins on animals and humans. Furthermore, the ammonium nitrogen (NH₄⁺-N) value ranging from 60 to 270 mg/L can also cause toxic effects to aquatic life (Farizoglu et al., 2007). In addition, cheese effluent composition can be approached to the following ratio for carbon, nitrogen and phosphorus C/N/P ≈ 200/3.5/1 which may be considered as deficient in terms of nitrogen components for aerobic or anaerobic processes (Prazeres et al., 2012).

From the previous statements, it is obvious that cheese whey cannot be directly discharged to the environment without an adequate treatment and/or valorization. From the valorization point of view, cheese whey is a nutrient-rich effluent. Cheese whey contains about 93-94% of water and the following nutrients from the original milk: lactose, soluble proteins, minerals, lactic acid and fats. Additionally, significant amounts of other components, such as citric acid, non-protein nitrogen compounds (urea
and uric acid), vitamins (B group), etc. are also present in the composition of CW (Carvalho et al., 2013). Without an appropriate treatment, these effluents pose serious environmental hazards (Rivas et al., 2011). Biological and physicochemical processes are usually suggested to deal with dairy effluents (Kushwaha et al., 2010).

1.4.3 Liquid Cow Manure

Recently, considerable interest has developed concerning the use of livestock manure as an alternative renewable source of energy. This is due to the continuous economic and environmental concerns faced by farmers and governments (Demirer and Chen, 2005). Dairy manure is one of the most polluting agro-industrial wastewaters (Rico et al., 2011). Intensive dairy farming produces large amounts of manure which, when not properly managed due to its high organic matter, nitrogen and phosphorous concentrations, can cause severe environmental problems such as eutrophication of water bodies (Carpenter et al., 1998), groundwater contamination (Hao and Chang, 2002), air pollution by volatilization of ammonia and other compounds (Ryden et al., 1987) and soil degradation when manure is applied in excess (Nasir et al., 2012). With environmental regulations becoming more stringent, regulatory compliance has become a matter of increasing concern to livestock industries, and there is a need to install more effective waste treatment facilities (Sakar et al., 2009). There is also a concern regarding methane emissions, which contribute to the greenhouse effect (Karim et al., 2005).

The methane potential of manure comes from the digestion of the organic components present in the faeces and the straw used as bedding material, which mainly consists of carbohydrates, proteins and lipids (Møller et al., 2004). Manures often contain quantities of organic fibers, including straw bedding material, that are more difficult to degrade than the manure itself (Appels et al., 2011). It remains the foremost primary substrate for co-digestion due to its abundance and its unique properties such as high water content, good buffering capacity and the presence of almost all the essential nutrients and trace elements (Atandi and Rahman, 2012).
1.5 **Energy crops**

The current events such as the global energy crisis, increased greenhouse gas emissions, social awareness on environmental threats and international environmental agreements, have brought back the attention and interest on alternative cheap and renewable energy sources, such as the production of energy crops which can be designed as biofuel, electricity or heating feedstocks (Zegada-Lizarazu and Monti, 2012). Lately, many energy crops such as perennial grasses, woody and annual crops are being evaluated around the world for such purposes. Numerous plant species and plant residues have been tested for their methane potential. In principal, many varieties of grass, clover, cereals and maize, including whole plants, as well as rape and sunflower proved feasible for methane production. Hemp, flax, nettle, potatoes, beets, kale, turnip, rhubarb and artichoke have all been tested successfully (Murphy et al., 2011).

Crops may be used for digestion directly after harvest. For year round availability of substrates, crops are frequently stored in silage clamps. Grass, for example, may be ensiled in a clamp or pit or it may be baled. The time of harvest varies for differing crops. Grass may be cut between two and five times in a season; sugar beet is harvested later than most crops, typically between November and January. Staggered harvest improves the possibility for co-digestion of fresh crops and reduces the amount of storage capacity required. The time of harvest can influence bio-degradability, and hence the methane yield. Late harvest (with longer growing period) usually leads to higher lignin content in grasses, causing slower bio-degradation and lower methane yield.

Among annual crops, sweet sorghum (*Sorghum bicolor* L.) has emerged as a potential feedstock candidate for bioenergy purposes because of its versatility, yield potential and growth characteristics. Moreover, given the state of the art of the technological advancement of alternative renewable energy sources, the production of biofuels from energy crops such as sweet sorghum is one of the most immediate and feasible ones that could meet the food, fuel, feed, and fiber demands in the near future. However, to date the scientific information available on its cultivation and sustainability seems disperse, insufficient, and sometimes inconsistent (Zegada-Lizarazu and Monti, 2012).

In contrast to food crops that pull nutrients from the soil, energy crops actually improve soil quality. Prairie grasses, with their deep roots, build up topsoil, putting nitrogen and other nutrients into the ground. Since they are replanted only every 10 years, there is minimal plowing that causes soil to erode. Energy crops can create better wildlife habitat than food crops. Since they are native plants, they attract a greater variety of birds and small mammals than modern industrial food-producing farms. They improve the habitat for fish by increasing water quality in nearby streams and ponds (www.altenergy.org).
1.5.1 Sorghum

Sorghum is a fast growing C4 plant native to tropical zones but with a wide adaptability to different environmental conditions. It can be grown in tropical, subtropical and temperate zones, especially thanks to its relative lower agronomic requirements compared to other sugar crops such as sugarcane or sugar beet. However, even though several biomass sorghum hybrids have been recently developed and improved for the production of lignocellulosic, sugar, and starch feedstocks (Rooney et al., 2007), its development as an energy crop is far behind the development of maize, sugar beet, or sugarcane (Smith et al., 1987) because of the little knowledge on sweet sorghum management and recent breeding history. Moreover, the heritability of sugar content in the plant stems is a complex process, thus developing new breeds and agronomic management practices would enhance its productivity.

Among the cultivated sorghums, bicolor sorghums represent types that have been selected not only for grain production but also for fiber, forage and sugar production (Dogget, 1998). The later type is characterized by genotypes that produce tall juicy stalks rich in sugars, predominately sucrose and with variable levels of glucose and fructose. In general the content of nonstructural carbohydrates is higher in sweet sorghum types than in forage or fiber ones. The fiber and forage types are predominantly composed by structural carbohydrates (Table 1.1). However all types of sorghums produce lignocellulose that could serve as feedstock for second generation biofuels. In the case of sweet types, the high content in stem soluble sugars and structural carbons (obtained from cellulose and hemicellulose components of the bagasse) could be used both for first and second generation ethanol. Currently, genotypes with a high cellulosic production potential are being developed. Sweet and forage/fiber sorghum types, however, are not completely distinct between them as they share many common characteristics (Monk et al., 1984) and it is not a typical to find hybrids classified as forage/fiber but rich in soluble sugars and vice versa. Moreover, among the sweet types, sugar and syrup sorghum subtypes have been developed by breeders, with the former ones containing mainly sucrose, while the syrup types produce a mixture of glucose and fructose that not crystallize (Monk et al., 1984). However, sorghum shows flexibility in producing similar amounts of starch, sugars or cellulose in the grains and stems (Rooney et al., 2007). Then, the sweet sorghum ideotype grown for biofuel production will depend on the environmental conditions and the type of conversion process that will be used. Once the best ideotype has been identified for the production of first or second generation biofuels, there are numerous sorghum characteristics that can be manipulated by traditional or improved agronomic approaches and incorporated as needed in order to optimize its yields.

Moreover, compared to other crops potentially used for energy such as sugarcane, sugar beet or maize, sweet sorghum shows a much wider adaptability to different environments and soil conditions (Ratnavathi et al., 2011). Unlike maize, sweet sorghum is resistant to drought, and has a higher water and nutrient use efficiency.
While, compared to sugarcane, it has a shorter growing cycle (120-150 days), it is propagated by seeds, and produces high amounts of starch in grains along with soluble sugar in the stalks. Furthermore, June and February plantings are ideal to obtain maximum biomass and high sugar content in sweet sorghum (Ratnavathi et al., 2011).

Table 1-1: Sorghum types, conventional uses and possible applications (Zegada-Lizarazu and Monti, 2012).

<table>
<thead>
<tr>
<th>Sorghum type</th>
<th>Main characteristics</th>
<th>Typical/potential end use</th>
<th>Application for bioenergy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain sorghum</td>
<td>Dwarf varieties (50-80 cm) rich in starch in the grains.</td>
<td>Grain harvested for food and fodder.</td>
<td>Grain can be used as a starch and cellulose feedstock for 1st and 2nd generation biofuels. Crop residues can be used as cellulose feedstock for 2nd generation biofuels.</td>
</tr>
<tr>
<td>Sweet sorghum</td>
<td>Varieties with thick and long stalks and high content of sugars in the stem (about 53 and 37% are structural and non-structural carbohydrates, respectively).a</td>
<td>Juice harvested for molasses, syrup and sugar.</td>
<td>Sweet sorghum juice can be used to produce ethanol.</td>
</tr>
<tr>
<td>Forage sorghum</td>
<td>Varieties with high protein and fiber content (between 59 and 63% are structural carbohydrates, and 22-28% are non-structural carbohydrates).</td>
<td>Biomass harvested for fodder.</td>
<td>Biomass can be used as a cellulose feedstock for 2nd generation biofuels.</td>
</tr>
<tr>
<td>Fiber sorghum</td>
<td>Tall varieties with fine stems and rich in cellulose and hemicelluloses (about 77 and 20% are structural and non-structural carbohydrates, respectively).</td>
<td>Cellulose for paper production.</td>
<td>Biomass used for combustion and 2nd generation biofuels.</td>
</tr>
</tbody>
</table>

---

*aStructural carbohydrates include hemicellulose, cellulose, and lignin, while the non-structural carbohydrates include sucrose, glucose, fructose and starch.
1.6 Lignocellulosic biomass

Lignocellulose is the most abundant renewable biomass; its annual production has been estimated in $1 \times 10^{10}$ MT worldwide (Sánchez and Cardona, 2008), since the expansion of agro-industrial activity has led to the accumulation of a large quantity of lignocellulosic residues from wood (e.g. poplar trees), herbaceous (e.g. switchgrass), agricultural (e.g. corn stover, and wheat and rice straw), forestry (e.g. sawdust, thinning, and mill waste), municipal solid wastes (e.g. waste paper) and various industrial wastes all over the world (Mudhoo, 2012). Lignocellulosic material consists of mainly three different types of polymers, namely cellulose, hemicellulose and lignin, which are associated which each other (Fengel and Wegener, 1984).

Cellulose $(C_{6}H_{10}O_{5})_n$ is a linear polymer of cellobiose (glucose – glucose dimer) strongly linked via β-1,4 glycosidic bonds (Fengel and Wegener, 1984), that can only be broken with specific enzymes, cellulases. Cellulose chains are able to elongate up to more than 104 glucose units (mean number of glucose units: 7000 – 15000) depending on the source of biomass (Mudhoo, 2012). The cellulose in a plant consists of parts with a crystalline (organized) structure, and parts with a, not well-organized, amorphous structure. The cellulose strains are ‘bundled’ together and form so called cellulose fibrils or cellulose bundles. These cellulose fibrils are mostly independent and weakly bound through hydrogen bonding (Laureano-Perez et al., 2005).

Hemicellulose, though in some cases represent a high percentage of the lignocellulosic biomass (Mudhoo, 2012), are less studied than cellulose. Hemicellulose is a complex carbohydrate structure that consists of different polymers like pentoses (like xylose and arabinose), hexoses (like mannose, glucose and galactose), and sugar acids. The dominant component of hemicellulose from hardwood and agricultural plants, like grasses and straw, is xylan, while this is glucomannan for softwood (Fengel and Wegener, 1984; Saha, 2003). As opposed to cellulose, hemicellulose (20–40% of the dry biomass) has a branched, amorphous structure made of short lateral chains of different sugars and is therefore easily hydrolyzed (Mudhoo, 2012). Hemicellulose serves as a connection between the lignin and the cellulose fibers and gives the whole cellulose–hemicellulose–lignin network more rigidity (Laureano-Perez et al., 2005). The solubility of the different hemicellulose compounds is in descending order: mannose, xylose, glucose, arabinose, and galactose. It increases with temperature and also depends on other parameters, such as moisture content and pH (Mudhoo, 2012).

Lignin is, after cellulose and hemicellulose, one of the most abundant polymers in nature and is present in the cellular wall (Hendriks and Zeeman, 2009). It is responsible for the cross-linking between the polysaccharides (cellulose and hemicellulose) into a rigid woody structure. It is an amorphous heteropolymer consisting of three different phenylpropane units (p-coumaryl, coniferyl and sinapyl alcohol) that are held together by different kind of linkages. The main purpose of lignin is to give the plant structural support, impermeability, and resistance against microbial attack and oxidative stress. The amorphous heteropolymer is also non-water soluble and optically inactive; all this
makes the degradation of lignin very tough (Fengel and Wegener, 1984; Mudhoo, 2012).

The fermentable organic matter of lignocellulosic biomass is bonded with lignin, thus being inaccessible for microbial attack. The carbohydrate polymers usually reach 60-70% of the total dry biomass, with cellulose (glucose polymer) and hemicellulose (pentose polymers) representing 40-50% and 25-35% respectively, whereas lignin accounts for about 15-20% of the dry matter (Sambusiti et al., 2014). Lignin cannot be anaerobically digested, but this is not the main obstacle in the process. In lignocellulosic biomass, lignin is tightly bound to the carbohydrate polymers i.e. cellulose and hemicellulose, thus rendering some fraction of them non-accessible to further hydrolysis and fermentation (Tong et al., 1990). These bonds are mainly hydrogen bonds and also covalent bonds and can be broken down only by certain organisms. A schematic presentation of the lignocellulosic cell wall structure is presented in Fig. 1.1.

Figure 1-1: Schematic presentation of a lignocellulose structure. Cellulose microfibrils with crystalline and paracrystalline (amorphous) regions are embedded into a matrix consisting of lignin and hemicellulose in the cell wall (Kallioinen, 2014).
Therefore, the sugars that compose the polysaccharides are not directly available for bioconversion (Thomsen, 2005). Since lignocelluloses are in focus as the raw materials for bio-products such as biogas or bioethanol, there is a conflict in our interest and nature’s interest in their decomposition. It is therefore a challenge to digest lignocelluloses; and pretreatment is necessary to enhance their digestibility (Fig 1.2). There are several pretreatment processes, which have quite different efficiency in improving the digestion process. It is therefore important to understanding the nature of plants resistance against microbial decomposition.

Figure 1-2: Pretreatment of lignocellulosic material prior to bioethanol and biogas production.

1.6.1 Goals of pretreatment

An effective and economical pretreatment should meet the following requirements: (a) production of reactive cellulosic fiber for enzymatic attack, (b) avoiding destruction of hemicellulose and cellulose, (c) avoiding formation of possible inhibitors for hydrolytic enzymes and fermenting microorganisms, (d) minimizing the energy demand, (e) reducing the cost of size reduction for feedstocks, (f) reducing the cost of material for construction of pre-treatment reactors, (g) producing less residues, (h) consumption of little or no chemical and using a cheap chemical.

These properties, along with others including low pretreatment catalyst cost or inexpensive catalyst recycle, and generation of higher-value lignin co-product form a basis of comparison for various pre-treatment options. Pretreatment results must be balanced against their impact on the cost of the downstream processing steps and the trade-off between operating costs, capital costs, and biomass costs (Ladisch et al., 1983; Wyman, 1995; Delgenes et al., 1996; Lynd et al., 1996; Wyman, 1996, 1999; Palmqvist and Hahn-Hagerdal, 2000). The process itself utilizes pretreatment additives and/or energy to form solids that are more reactive than native material and/or generate soluble oligo- and monosaccharides.
Chapter 1  Introduction

The objective of pretreatments stage is to modify the structure of complex materials (usually cellulosic) with decreasing degree of polymerization, the weakening of the bonds of lignin with carbohydrates and increased surface area of particles constitute the remainder. The nature of the apparent association of lignin and carbohydrates is still under discussion. Some pretreatments result in almost no change in the composition of the feedstock, whereas some others dissolve hemicellulose or lignin or both; and consequently the composition is changed. In addition to the composition of biomass, its physicochemical structure may also altered by changing the molecular weight and crystallinity of cellulose, biomass porosity and particle size (Fig 1.3). However, the effect of pretreatment, in general, is evaluated on the basis of improved digestibility; while less attention has been paid to the pretreatment effect on the anatomical and structural level of biomass organization. For this purpose, greater attention must be given to the understanding of how the pretreatments can overcome the natural recalcitrance of biomass and what are the factors that are the most critical in biomass digestibility enhancement.

**Figure 1-3:** Effect of pretreatment on accessibility of degrading enzymes (Taherzadeh and Karimi, 2008).
1.6.2 Pretreatment technologies for lignocellulosic biomass

Several methods have been introduced for pretreatment of lignocellulosic materials prior to enzymatic hydrolysis or digestion (Alvira et al., 2010). These pretreatments at very different conditions (widely varying chemicals, time, temperature, irradiation, etc.) have been investigated across a variety of biomass feedstock. The outcome of the pretreatment depends on the biomass composition and on the pretreatment itself, because each pretreatment has its own effect on the main components of lignocellulosic material (Hendricks and Zeeman, 2009). Pretreatment strategies can be physical, chemical, enzymatic or biological, or a combination of these (Kumar et al., 2009). The methods of pretreatment of lignocellulosic materials are summarized in Tables 1.2 and 1.3. In this section, we review these methods, although not all of them have yet developed enough to be feasible for applications in large-scale processes.

Table 1-2: Physical pretreatment processes of lignocellulosic materials (Taherzadeh and Karimi, 2008).

<table>
<thead>
<tr>
<th>Pretreatment method</th>
<th>Processes</th>
<th>Studied application</th>
<th>Possible changes in biomass</th>
<th>Notable remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical pretreatments</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milling:</td>
<td>- Ball milling</td>
<td></td>
<td></td>
<td>- Most of the methods are highly energy-demanding</td>
</tr>
<tr>
<td></td>
<td>- Two-roll milling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Hammer milling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Colloid milling</td>
<td></td>
<td></td>
<td>- Most of them cannot remove the lignin</td>
</tr>
<tr>
<td></td>
<td>- Vibro energy milling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irradiation:</td>
<td>- Gamma-ray irradiation</td>
<td>Ethanol</td>
<td>- Increase in accessible surface area and pore size</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Electron-beam irradiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Microwave irradiation</td>
<td>Ethanol and biogas</td>
<td>- Decrease in cellulose crystallinity</td>
<td>- It is preferable not to use these methods for industrial applications</td>
</tr>
<tr>
<td>Others:</td>
<td>- Hydrothermal</td>
<td>Ethanol and biogas</td>
<td>- Decrease in degrees of polymerization</td>
<td>- No chemicals are generally required for these methods</td>
</tr>
<tr>
<td></td>
<td>- High pressure steaming</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Expansion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Extrusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Pyrolysis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1-3: Chemical, physicochemical and biological pretreatment processes of lignocellulosic materials (Taherzadeh and Karimi, 2008).

<table>
<thead>
<tr>
<th>Pretreatment method</th>
<th>Processes</th>
<th>Studied application</th>
<th>Possible changes in biomass</th>
<th>Notable remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Explosion:</td>
<td>-Steam explosion&lt;br&gt;-Ammonia fiber explosion (AFEX)&lt;br&gt;-CO₂ explosion&lt;br&gt;-SO₂ explosion</td>
<td>Ethanol and biogas</td>
<td>-Increase in accessible surface area&lt;br&gt;-Partial or nearly complete delignification&lt;br&gt;-Decrease in cellulose crystallinity&lt;br&gt;-Decrease in degrees of polymerization&lt;br&gt;-Partial or complete hydrolysis of hemicelluloses</td>
<td>-These methods are among the most effective and include the most promising processes for industrial applications&lt;br&gt;-Usually rapid treatment rate&lt;br&gt;-Typically need harsh conditions&lt;br&gt;-There are chemical requirements</td>
</tr>
<tr>
<td>Alkali:</td>
<td>-Sodium hydroxide&lt;br&gt;-Ammonia&lt;br&gt;-Ammonium Sulfite</td>
<td>Ethanol and biogas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid:</td>
<td>-Sulfuric acid&lt;br&gt;-Hydrochloric acid&lt;br&gt;-Phosphoric acid</td>
<td>Ethanol and biogas</td>
<td>-Partial or nearly complete delignification&lt;br&gt;-Decrease in degrees of polymerization</td>
<td></td>
</tr>
<tr>
<td>Gas:</td>
<td>-Chlorine dioxide&lt;br&gt;-Nitrogen dioxide&lt;br&gt;-Sulfur dioxide</td>
<td>Ethanol and biogas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidizing agents:</td>
<td>-Hydrogen peroxide&lt;br&gt;-Wet oxidation&lt;br&gt;-Ozone</td>
<td>Ethanol and biogas</td>
<td>-Partial or complete hydrolysis of hemicelluloses</td>
<td></td>
</tr>
<tr>
<td>Solvent extraction of lignin:</td>
<td>-Ethanol-water&lt;br&gt;-Benzene-water&lt;br&gt;-Ethylene glycol&lt;br&gt;-Butanol-water&lt;br&gt;-Swelling agents</td>
<td>Ethanol</td>
<td>-Delignification&lt;br&gt;-Reduction in degree of polymerization of cellulose&lt;br&gt;-Partial hydrolysis of hemicellulose</td>
<td>-Low energy requirement&lt;br&gt;-No chemical requirement&lt;br&gt;-Mild environmental conditions&lt;br&gt;-Very low treatment rate</td>
</tr>
</tbody>
</table>
1.6.2.1 Biological pretreatments

Fungal pretreatment has been previously explored to upgrade lignocellulosic materials for feed and paper applications. Recently, this environmentally friendly approach has received renewed attention as a pretreatment method for enhancing enzymatic saccharification of lignocellulosic biomass in ethanol production processes. Biological pretreatments employ microorganisms mainly brown, white and soft-rot fungi which degrade lignin and hemicellulose and very little of cellulose, more resistant than the other components (Sánchez, 2009). Lignin degradation by white-rot fungi, the most effective for biological pretreatment of lignocellulosic materials, occurs through the action of lignin-degrading enzymes such as peroxidases and laccases (Kumar et al., 2009a).

In general, such processes offer advantages such as low-capital cost, low energy, no chemicals requirement, and mild environmental conditions. However, the main drawback to develop biological methods is the low hydrolysis rate obtained in most biological materials compared to other technologies (Sun and Cheng, 2002).

1.6.2.2 Physical pretreatments

Physical pretreatment can increase the accessible surface area and size of pores, and decrease the crystallinity and degrees of polymerization of cellulose. Different types of physical processes such as milling (e.g. ball milling, two-roll milling, hammer milling, colloid milling, and vibro energy milling) and irradiation (e.g. by gamma rays, electron beam or microwaves) can be used to improve the enzymatic hydrolysis or biodegradability of lignocellulosic waste materials.

1.6.2.2.1 Milling

The objective of the mechanical pretreatment is a reduction of particle size and crystallinity of lignocellulosic in order to increase the specific surface and reduce the degree of polymerization. This can be produced by a combination of chipping, grinding or milling depending on the final particle size of the material (10–30 mm after chipping and 0.2–2 mm after milling or grinding) (Sun and Cheng, 2002). Different milling processes (ball milling, two-roll milling, hammer milling, colloid milling and vibro energy milling) can be used to improve the enzymatic hydrolysis of lignocellulosic materials (Taherzadeh and Karimi, 2008). The power requirement of this pretreatment is relatively high depending on the final particle size and the biomass characteristics. Taking into account the high energy requirements of milling and the continuous rise of energy prices, it is likely that this process is not economically feasible (Hendriks and Zeeman, 2009).
1.6.2.2.2 Irradiation

Irradiation by e.g. gamma rays, electron beam and microwaves can improve enzymatic hydrolysis of lignocelluloses. The combination of the radiation and other methods such as acid treatment can further accelerate enzymatic hydrolysis (Mamar and Hadjadj, 1990). Irradiation has enhanced enzymatic degradation of cellulose into glucose. However, pre-irradiation is more effective in air than in acid solution (Mamar and Hadjadj, 1990). Kumakura and Kaetsu (1983) studied the effect of irradiation for pretreatment of bagasse prior to its enzymatic hydrolysis. The pretreated bagasse resulted in double yield of glucose by the hydrolysis compared to the untreated one. The cellulose component of the lignocellulose materials can be degraded by irradiation to fragile fibers and low molecular weight oligosaccharides and even cellobiose (Kumakura and Kaetsu, 1983). It could be due to preferential dissociation of the glucoside bonds of the cellulose molecular chains by irradiation in the presence of lignin. However, the irradiation methods are expensive and have difficulties in industrial application.

Ultrasound is a means used for pretreatment in biogas production. It can be used for disintegration of waste-activated sludge and aquaculture effluents (Chu et al., 2002). In this method, the sludge flocs are disintegrated and the bacterial cells’ walls are disrupted (Chu et al., 2002). Several factors such as ultrasonic density and intensity, sludge pH and sludge concentration have impact on the disintegration (Wang et al., 2005). In addition to sonication, other methods such as cavitation, repeated freezing and defreezing, and heating at low temperatures of e.g. 60-170°C for 5-30 min or high temperatures of 180-200°C for 10 s, can improve cell disruption and lysing (Dohányos et al., 1997). The other physical methods such as γ-irradiation, microwaves and electrical pulses have also been used to improve formation of biogas from waste materials.

1.6.2.2.3 Extrusion

Extrusion process is a novel and promising physical pretreatment method for biomass conversion to ethanol production. In extrusion, the materials are subjected to heating, mixing and shearing, resulting in physical and chemical modifications during the passage through the extruder. Screw speed and barrel temperature are believed to disrupt the lignocellulose structure causing defibrillation, fibrillation and shortening of the fibers, and, in the end, increasing accessibility of carbohydrates to enzymatic attack (Karunanithy et al., 2008). The different bioreactor parameters must be taken into account to achieve the highest efficiency in the process. In recent studies application of enzymes during extrusion process is being considered as a promising technology for ethanol production.
1.6.2.3 Physico-chemical pretreatments

Pretreatments that combine both chemical and physical processes are referred to as physicochemical processes (Taherzadeh and Karimi, 2008). We review the most important processes of this group in this section.

1.6.2.3.1 Steam explosion

Among the physico-chemical processes, steaming with or without explosion (autohydrolysis) has received substantial attention in pretreatment for both ethanol and biogas production. The pretreatment removes most of the hemicellulose, thus improving the enzymatic digestion. In steam explosion, the pressure is suddenly reduced and makes the materials undergo an explosive decompression. High pressure and consequently high temperature, typically between 160 and 260°C, for a few seconds (e.g. 30 s) to several minutes (e.g. 20 min), were used in steam explosion (Varga et al., 2004). The steam explosion process is well documented and was tested in lab- and pilot processes by several research groups and companies. Its energy cost is relatively moderate, and it satisfies all the requirements of the pretreatment process.

1.6.2.3.2 Steam explosion with addition of SO\textsubscript{2}

Steam pretreatment can be performed with addition of sulfur dioxide (SO\textsubscript{2}), while the aim of adding this chemical is to improve recovering both cellulose and hemicellulose fractions. The treatment can be carried out by 1-4% SO\textsubscript{2} (w/w substrate) at elevated temperatures, e.g. 160-230°C, for a period of e.g. 10 min (Eklund et al., 1995). Eklund et al. (1995) studied steam pretreatment of willow with the addition of SO\textsubscript{2} or H\textsubscript{2}SO\textsubscript{4} in order to recover both cellulose and hemicellulose. The maximum glucose yield, 95%, was obtained when the willow was treated with 1% SO\textsubscript{2} at 200°C. However, the yield of xylose recovery by SO\textsubscript{2} was not as high as pretreatment with dilute sulfuric acid.

1.6.2.3.3 CO\textsubscript{2} explosion

Supercritical carbon dioxide has been considered as an extraction solvent for non-extractive purposes, due to several advantages such as availability at relatively low cost, non-toxicity, no flammability, easy recovery after extraction, and environmental acceptability (Zheng, 1996). Supercritical carbon dioxide displays gas-like mass transfer properties, besides a liquid-like solvating power. The delignification with carbon dioxide at high pressures can be improved by co-solvents such as ethanol–water or acetic acid–water, and can efficiently increase the lignin removal. Carbon dioxide molecules should be comparable in size to those of water and ammonia, and should be able to penetrate small pores accessible to water and ammonia molecules.

1.6.2.3.4 Ammonia fiber explosion (AFEX)

AFEX is one of the alkaline physico-chemical pretreatment processes. Here the biomass is exposed to liquid ammonia at relatively high temperature (e.g. 90-100°C) for
a period of e.g. 30 min, followed by immediate reduction of pressure. The effective parameters in the AFEX process are ammonia loading, temperature, water loading, blowdown pressure, time, and number of treatments. The AFEX process produces only a pretreated solid material, while some other pretreatments such as steam explosion produce a slurry that can be separated in a solid and a liquid fractions (Mosier et al., 2005b).

The AFEX process can either modify or effectively reduce the lignin fraction of the lignocellulosic materials, while the hemicellulose and cellulose fractions may remain intact. One of the major advantages of AFEX pretreatment is no formation of some types of inhibitory by-products, which are produced during the other pretreatment methods, such as furans in dilute-acid and steam explosion pretreatment. However, part of phenolic fragments of lignin and other cell wall extractives may remain on the cellulosic surface. However, there are some disadvantages in using the AFEX process compared to some other processes. AFEX is more effective on the biomass that contains less lignin, and the AFEX pretreatment does not significantly solubilize hemicellulose compared to other pretreatment processes such as dilute-acid pretreatment.

1.6.2.3.5 Liquid hot water (LHW)

Liquid hot water is another hydrothermal treatment which does not require rapid decompression and does not employ any catalyst or chemicals. Pressure is applied to maintain water in the liquid state at elevated temperatures (160–240°C) and provoke alterations in the structure of the lignocellulose. The objective of the liquid hot water is to solubilize mainly the hemicellulose, to make the cellulose more accessible and to avoid the formation of inhibitors. The slurry generated after pretreatment can be filtered to obtain two fractions: one solid cellulose-enriched fraction and a liquid fraction rich in hemicellulose derived sugars. To avoid the formation of inhibitors, the pH should be kept between 4 and 7 during the pretreatment because at this pH hemicellulosic sugars are retained in oligomeric form and monomers formation is minimized. Therefore the formation of degradation products is also lower (Mosier et al., 2005a).

1.6.2.3.6 Microwave-chemical pretreatment

The microwave-chemical pretreatment resulted in a more effective pretreatment than the conventional heating chemical pretreatment by accelerating reactions during the pretreatment process (Taherzadeh and Karimi, 2008). Zhu et al. (2006) examined three microwave-chemical processes for pretreatment of rice straw: (a) microwave/alkali, (b) microwave/acid/alkali and (c) microwave/acid/alkali/H2O2. They found that xylose could not be recovered during the first pretreatment process, but could be recovered as crystalline xylose during the second and third pretreatment. The enzymatic hydrolysis of pretreated rice straw showed that the pretreatment by microwave/acid/alkali/H2O2 had the highest hydrolysis rate and glucose content in the hydrolyzate.
1.6.2.4 Chemical pretreatments

1.6.2.4.1 Alkaline hydrolysis

The effect that some bases have on lignocellulosic biomass is the basis of alkaline pretreatments, which are effective depending on the lignin content of the biomass. Alkali pretreatments increase cellulose digestibility and they are more effective for lignin solubilization, exhibiting minor cellulose and hemicellulose solubilization than acid or hydrothermal processes (Carvalheiro et al., 2008).

Alkali pretreatment can be performed at room temperature and times ranging from seconds to days. It is described to cause less sugar degradation than acid pretreatment and it was shown to be more effective on agricultural residues than on wood materials (Kumar et al., 2009a). Nevertheless, possible loss of fermentable sugars and production of inhibitory compounds must be taken into consideration to optimize the pretreatment conditions. Sodium, potassium, calcium and ammonium hydroxides are suitable alkaline pretreatments. NaOH causes swelling, increasing the internal surface of cellulose and decreasing the degree of polymerization and crystallinity, which provokes lignin structure disruption (Taherzadeh and Karimi, 2008). NaOH has been reported to increase hardwood digestibility from 14% to 55% by reducing lignin content from 24–55% to 20% (Kumar et al., 2009a).

Ca(OH)$_2$, also known as lime, has been widely studied. Lime pretreatment removes amorphous substances such as lignin, which increases the crystallinity index. Lignin removal increases enzyme effectiveness by reducing non-productive adsorption sites for enzymes and by increasing cellulose accessibility (Kim and Holtzapple, 2006). Lime also removes acetyl groups from hemicellulose reducing steric hindrance of enzymes and enhancing cellulose digestibility (Mosier et al., 2005b). Lime has been proven successfully at temperatures from 85–150°C and for 3–13 h with corn stover (Kim and Holtzapple, 2006) or poplar wood (Chang et al., 2001). Pretreatment with lime has lower cost and less safety requirements compared to NaOH or KOH pretreatments and can be easily recovered from hydrolysate by reaction with CO$_2$ (Mosier et al., 2005b).

1.6.2.4.2 Acid hydrolysis

The main objective of the acid pretreatments is to solubilize the hemicellulosic fraction of the biomass and to make the cellulose more accessible to enzymes. This type of pretreatments can be performed with concentrated or diluted acid but utilization of concentrated acid is less attractive for ethanol production due to the formation of inhibiting compounds. Furthermore, equipment corrosion problems and acid recovery are important drawbacks when using concentrated acid pretreatments. The high operational and maintenance costs reduce the interest of applying the concentrated acid pretreatment at commercial scale (Wyman, 1996).

Diluted acid pretreatment appears as more favourable method for industrial applications and have been studied for pretreating wide range of lignocellulosic biomass. Different types of reactors such as percolation, plug flow, shrinking-bed, batch
and countercurrent reactors have been applied for pretreatment of lignocellulosic materials (Taherzadeh and Karimi, 2008). It can be performed at high temperature (e.g. 180°C) during a short period of time; or at lower temperature (e.g. 120°C) for longer retention time (30–90 min). It presents the advantage of solubilizing hemicellulose, mainly xylan, but also converting solubilized hemicellulose to fermentable sugars. Nevertheless, depending on the process temperature, some sugar degradation compounds such as furfural and HMF and aromatic lignin degradation compounds are detected, and affect the microorganism metabolism in the fermentation step (Saha et al., 2005). Anyhow, this pretreatment generates lower degradation products than concentrated acid pretreatments.

High hydrolysis yields have been reported when pretreating lignocellulosic materials with diluted H$_2$SO$_4$ which is the most studied acid. Hydrochloric acid, phosphoric acid and nitric acid have also been tested (Mosier et al., 2005a). Organic acids such as fumaric or maleic acids are appearing as alternatives to enhance cellulose hydrolysis for ethanol production. In this context, both acids were compared with sulfuric acid in terms of hydrolysis yields from wheat straw and formation of sugar degradation compounds during pretreatment.

1.6.2.4.3 Organosolv

Organosolvation is a promising pretreatment strategy, since it has demonstrated its potential for lignocellulosic materials (Papatheofanous et al., 1995). Numerous organic or aqueous solvent mixtures can be utilized, including methanol, ethanol, acetone, ethylene glycol and tetrahydrofurfuryl alcohol, in order to solubilize lignin and provide treated cellulose suitable for enzymatic hydrolysis (Zhao et al., 2009). Comparing to other chemical pretreatments the main advantage of organosolv process is the recovery of relatively pure lignin as a by-product (Zhao et al., 2009). Removal of solvents from the system is necessary using appropriate extraction and separation techniques, e.g., evaporation and condensation, and they should be recycled to reduce operational costs. Solvents need to be separated because they might be inhibitory to enzymatic hydrolysis and fermentative microorganisms (Sun and Cheng, 2002). The high commercial price of solvents is another important factor to consider for industrial applications. For economic reasons, among all possible solvents, the low-molecular weight alcohols with lower boiling points such as ethanol and methanol are favored.

1.6.2.4.4 Ozonolysis

Ozone is a powerful oxidant that shows high delignification efficiency (Sun and Cheng, 2002). This lignin removal increases the yield in following enzymatic hydrolysis. The pretreatment is usually performed at room temperature and normal pressure and does not lead to the formation of inhibitory compounds that can affect the subsequent hydrolysis and fermentation. Ozonolysis has been applied on several agricultural residues such as wheat straw and rye straw increasing in both cases the enzymatic hydrolysis yield after ozonolysis pretreatment (Garcia-Cubero et al., 2009).
Despite of some interesting results further research has to be performed regarding ethanol production from lignocellulosic materials pretreated with ozone. An important drawback to consider is the large amounts of ozone needed, which can make the process economically unviable (Sun and Cheng, 2002).

1.6.2.4.5 Wet oxidation

Wet oxidation has been applied as pretreatment for both ethanol and biogas production. In this process, the materials are treated with water and air or oxygen at temperatures above 120°C (e.g. 148- 200°C) for a period of e.g. 30 min (Garrote et al., 1999). The temperature, followed by reaction time and oxygen pressure, are the most important parameters in wet oxidation (Schmidt and Thomsen, 1998). The process is exothermic, and therefore it becomes self-supporting with respect to heat while the reaction is initiated (Schmidt and Thomsen, 1998). Wet oxidation of the hemicellulose fraction is a balance between solubilization and degradation. This process is an effective method in separating the cellulosic fraction from lignin and hemicellulose. Oxygen participates in the degradation reactions and allows operation at comparatively reduced temperatures by enhancing generation of organic acids. However, the control of reactor temperature is critical because of the fast rates of reaction and heat generation (Garrote et al., 1999). The main reactions in wet oxidation pretreatment are the formation of acids from hydrolytic processes, as well as oxidative reactions.

Table 1.4 summarizes the most important effects of the different pretreatment methods, discussed previously. The table suggests that increasing the surface area is one of the major approaches of a pretreatment by solubilization of the hemicellulose and/ or lignin and/ or altering the lignin.

<table>
<thead>
<tr>
<th>Method of pretreatment</th>
<th>Increase accessible surface area</th>
<th>Decrystallization cellulose</th>
<th>Solubilization hemicellulose</th>
<th>Solubilization lignin</th>
<th>Formation furfural/ HMF</th>
<th>Alteration lignin structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ST/SE</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LHW (batch)</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LHW (flow through)</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acid</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaline</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Oxidative</td>
<td>+</td>
<td>ND</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Thermal+acid</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Thermal+alkali (lime)</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Thermal+oxidative</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Thermal+alkali+oxid</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ammonia (AFEX)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CO₂ explosion</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

+: major effect; -: minor effect; ND: No Determined.
1.7 Anaerobic Digestion

1.7.1 Background

Increasing awareness of global warming and rising energy prices have led to a growing interest for renewable energy during recent years. Biogas, consisting mainly of methane, is an energy carrier with many advantages. It can be produced from a variety of substrates, preferably from different types of wastes, in the process of anaerobic digestion (AD). The technique is widely used at waste water treatment plants (WWTPs) for reducing the waste volume and stabilizing the sludge produced from waste water treatment. The process can also be applied to treat e.g. household and agricultural waste, turning them into valuable resources and solving a waste handling problem. Greenhouse gas reduction is not only attained by the replacement of fossil fuels, but digesting household waste, sludge and manure prevents direct emission of the strong greenhouse gases methane and nitrous oxide to the atmosphere. The residues from the digestion process can be used as fertilizer on farmland, and the carbon dioxide produced from the methane combustion is thus taken up by plants. Plants which once again can be used for biogas production, thus the carbon cycle is closed.

The key to effective practical applications of AD technology lies in regulating and optimizing the internal environment of an enclosed bioreactor vessel such that the ideal conditions for the process are produced and maintained. Under these circumstances, in the absence of free oxygen, anaerobic bacteria convert the large organic molecules into biogas (Mata-Alvarez, 2003). The produced biogas is mainly composed of methane (CH₄) and carbon dioxide (CO₂). It can be used in combined heat and power plants (CHP) to produce both electricity injected in the grid, and heat for local needs (Doušková et al., 2010). It is a process found in many naturally occurring anoxic environments including watercourses, sediments, waterlogged soils and the mammalian gut. It can also be applied to a wide range of feedstocks including industrial and municipal waste waters, agricultural, municipal, food industry wastes, and plant residues (Ward et al., 2008). The interest in the process is mainly due to the following two reasons (Angelidaki et al., 2003):

- A high degree of reduction of organic matter is achieved with a small increase -in comparison to the aerobic process - in the bacterial biomass.

- The production of biogas, which can be utilized to generate different forms of energy (heat and electricity) or be processed for automotive fuel.

The evaluation of biogas and methane production through anaerobic digestion from energy crops or agricultural wastes is not new and is being prompted in recent years, when the number of anaerobic digesters in the EU has increased dramatically. In early 2010, about 5900 biogas plants with an installed electrical capacity of 2300 MWₑ were
operational. Within the next five years, more than 3000 biogas plants with an electrical capacity of more than 1700 MW_{el} will be constructed (Zuber, 2010).

1.7.2 The benefits of anaerobic digestion

The production of biogas through anaerobic digestion offers significant advantages over other forms of waste treatment. Anaerobic digestion is a microbial conversion method that occurs in an aqueous environment, meaning that biomass sources containing high water levels (even containing less than 40% dry matter) can be processed without any pretreatment (Ward et al., 2008). This is not the case for most other conversion technologies. Combustion, for example, only offers a net positive energy balance if the water content of the biomass or waste is below 60% and even then, most of the energy stored in the biomass is used for evaporation of the contained water. Also, the energetic efficiency of pyrolysis and gasification decreases considerably with high water content, and the presence of water in the produced bio-oil is undesirable (Van de Velden et al., 2010). The use of these technologies thus necessitates an energy consuming pre-drying step for wet types of biomass and waste.

Less biomass sludge is produced in comparison to aerobic treatment technologies. Furthermore, more effective pathogen removal (Sahlström, 2003) was achieved. This is especially true for multi-stage digesters (Sahlström, 2003; Kunte et al., 2004) or if a pasteurization step is included in the process.

The valorization of the produced biogas (consisting of ca. 65% CH_{4}, 35% CO_{2} and trace gases such as H_{2}S, H_{2} and N_{2}) is energy efficient and environmentally friendly because of the low emission of hazardous pollutants. In most cases, biogas is valorized energetically in a CHP (combined heat and power) installation for the simultaneous generation of heat and electricity. These installations typically offer an electrical efficiency of 33% and a thermal efficiency of 45%. As pointed out by various studies (Smet et al., 1999), the emissions of volatile organic compounds (VOCs) are very limited since 99% of the volatile compounds are completely oxidized during combustion. This is in contrast to incinerators that suffer from the emission of hazardous compounds like dioxins, and hence require extensive flue gas purification. Alternatively, the biogas can be upgraded to natural gas purity and injected in the natural gas grid (Appels et al., 2008).

The produced slurry (digestate) is nitrogen rich and can in most cases (depending on the nature of the biomass) be utilized in agriculture as a nutrient fertilizer and/or organic amendment (Tambone et al., 2009). A more novel application is to transform the digestate into biochar, which can be further employed as soil enhancer or an adsorbent for purification of wastewater or flue gas (Inyang et al., 2010).

Additionally, one advantage of anaerobic digestion is that a wide variety of organic substrates can be used to produce energy (Weiland, 2010). The feedstock of an anaerobic digester can be liquid or solid materials and residues, originating mainly from food and feed industries, agriculture or households.
1.7.3 Biochemical process

Anaerobic degradation of organic material to methane and carbon dioxide is a complex system of biochemical reactions. The reactions are commonly divided into four groups of processes; hydrolysis, acidogenesis, acetogenesis and methanogenesis (Fig 1.4). The individual degradation steps are carried out by different consortia of microorganisms, which partly stand in syntrophic interrelation and place different requirements on the environment (Angelidaki et al. 1993). To obtain a stable and efficient process all four reaction steps need to function, as the processes are connected.

Anaerobic bioconversion of complex organic material to methane requires four major steps and five physiologically distinct groups of microorganisms. Elements of the food web of methanogenic anaerobic digestion are expected to occur also for biological hydrogen production, microbial fuel cells and biochemical production.

![Diagram of anaerobic digestion process for biogas production.](image)

**Figure 1-4:** Outline of anaerobic digestion process for biogas production.
As shown in Fig 1.4, complex organic polymers (e.g. proteins, polysaccharides) are hydrolyzed to monomers by fermentative bacteria (a), which ferment the monomers to a mixture of low-molecular-weight organic acids and alcohols. These fermentation products are further oxidized to acetic acid and hydrogen by obligatory hydrogen-producing acetogenic bacteria (b) through a process called acetogenesis. Acetogenesis also includes acetate production from hydrogen and carbon dioxide by acetogens and homoacetogens (c). Hydrogen-producing acetogenic bacteria (b) grow in syntrophic associations with hydrogenotrophic methanogens (d), which keep the hydrogen partial pressure low enough to allow acetogenesis to become thermodynamically favorable (this process is referred to as interspecies hydrogen transfer) (Trauer et al., 1977). Finally, acetoclastic methanogens (e) convert the acetate to methane and carbon dioxide (methanogenesis). Although ~70% of methane produced in many natural and engineered systems is due to acetoclastic methanogens, it is increasingly clear that many stressed and thermophilic systems use an alternative pathway: syntrophic oxidation of acetate to carbon dioxide and hydrogen by acetogenic or homoacetogenic bacteria (c) coupled to hydrogen consumption by hydrogenotrophic methanogens (Schnürer et al., 1999; Agnenent et al., 2002).

1.7.4 Types of anaerobic digesters

There are many ways in which anaerobic digestion systems may be categorized as will be briefly discussed below. However, it is important to realize that, irrespective of their individual construction, they all fundamentally consist of isolated vessels of some kind, designed to exclude air and maintain internal conditions at the optimum for bacterial action. It is possible to describe systems treating slurries of 15% total dry solids (TDS) or less as ‘wet’, or ‘dry’ if their TDS exceeds this figure. Alternatively, the temperature range at which they are operated can be used, thus leading to defining AD systems as either mesophilic or thermophilic.

The loading regime adopted can also be a useful means of distinguishing digester types for some purposes, allowing a distinction to be drawn between ‘batch’ and ‘continuous’ systems. The former are filled in a single go, then permitted to digest the contents before being emptied and recharged, while the latter have a continuous cycle of new biowaste being added and processed material being drawn off.

However these are, in effect, operational criteria and as such, though useful in themselves, they can tend to unite dissimilar technologies within essentially artificial groupings, giving little clue as to which best suits what type of biowaste. For this, an examination of aspects of the digester design and engineering principles can often provide a better insight, as in the following descriptions of some of the major examples (Evans and Furlong, 2003).
**Anaerobic baffled reactor (ABR)**
These generally feature a horizontal flow of biowaste through the digester vessel, and are suitable for a wide range of materials. The Valorga process, with its patented gas recirculation and mixing system, is based on this approach.

**Anaerobic fixed film reactor (AFFR)**
These digesters have a fixed growth plate on which a bacterial biofilm is established, digestion taking place on this surface. They are ideal for relatively weak biowastes with low solids content, but are of less use in other applications.

**Completely mixed contact reactor (CMCR)**
In this design, the biomass derived during processing is recycled after dewatering to increase the retention time for the solids. This approach is typically used to treat high strength, industrial biowastes.

**Continuously stirred tank reactor (CSTR)**
Intended to treat slurries and liquid biowastes, this system is essentially the same as the preceding CMCR design, but without the need for the solids’ recycle.

**Fluidised bed reactor (FBR)**
Sometimes termed the expanded bed reactor, this system relies on an internal microbial growth medium, which is fluidised by the waste liquid circulating within it. Accordingly, they are only suitable for liquid biowastes or very dilute slurries.

**Multi-phasic processes (MPP)**
Physically separating the stages of AD into different reactors, these systems are principally of use as experimental tools for increasing understanding of the pathways and mechanisms of the anaerobic digestion process. They are featured here for completeness, but they do not generally have any commercial application.

**Upflow anaerobic sludge blanket (UASB)**
These systems hold relatively high numbers of active bacteria, which makes them suitable for treating biowastes of low solids content and they are commonly used to process high-strength industrial waste liquids or light suspensions.

From all of this discussion, it should be obvious that, however proprietary AD technologies are classified, no one type is universally ideal or superior, each having certain characteristics which make it appropriate for particular wastes and less suitable for others. This means, of course, that although comparisons of the various approaches
are of great interest to potential users, in practice they are difficult to make in any meaningful way. Even certified data from an operating plant can only be taken as broadly indicative of how a similar one might perform elsewhere, especially in respect of breakdown efficiency and biogas generation or quality.

1.7.5 Process parameters

The biogas process as a complex biological process is influenced by several environmental factors. The interdependence of the bacteria is a key factor of the biogas process. Under conditions of unstable operation, intermediates such as volatile fatty acids and alcohols accumulate at different rates depending on the substrate and the type of perturbation causing instability. Thus, changes in the concentration of intermediates indicate disturbance of the biogas process (Angelidaki et al., 2003). Moreover, efficient AD requires the development and maintenance of an optimized internal environment to facilitate biological activity. This is of particular importance in the commercial setting and a number of both physical and chemical factors must be taken into account to achieve it (Evans and Furlong, 2003). The most important factors that can influence the balance of the system are:

Temperature

Temperature is one of the main environmental factors affecting bacterial growth. Anaerobic bacteria are affected in the same way as the aerobic ones. Growth rates often increase with increasing temperature up to a certain limit, while there is a rapid decrease in growth as the temperature approaches the upper limit for survival of the bacterium (Angelidaki et al., 2003). As mentioned previously, in commercial systems, digesters are operated at around 35°C (mesophilic) or 55°C (thermophilic). Irrespective of which approach is adopted for any particular application, a relatively constant temperature is essential for the process to run at its greatest efficiency. Poor stability was previously believed to be associated with thermophilic temperatures. However, many years of experience with full-scale biogas plants operating at thermophilic temperature, have demonstrated that this is not the case. Methanogenesis is also possible under psychrophilic conditions (below 25°C) but at lower process rates. Anaerobic bacteria can adapt quite easily to low temperatures, and high rate anaerobic treatment has been achieved at psychrophilic conditions, when bacteria have been immobilized or otherwise retained in the process (Angelidaki et al., 2003).

pH

The anaerobic digestion process is limited to a relatively narrow pH interval from approx. 6.0 to 8.5; a pH value outside this range can lead to imbalance. Each of the microbial groups involved in anaerobic degradation has a specific pH optimum and can grow in a specific pH range. The methanogens and acetogens have pH
optimum at approx. 7.0, while acidogens have lower pH optimum around 6.0. Methanogens at pH lower than 6.6 grow very slowly.

In an anaerobic reactor, instability will as a rule lead to accumulation of VFA, which can lead to a drop in pH (acidification). However, accumulation of VFA will not always be expressed as a pH drop due to the buffer capacity of some waste types. In manure there is a surplus of alkalinity, which means that the VFA accumulation shall exceed a certain point before this can be detected as a significant change in pH. This means that when a drop in pH in the reactor is eventually observed, the concentration of volatile fatty acids is most probably very high and the process may already have been affected. There are many factors which influence pH. It is especially organic acids and carbon dioxide which lower pH, while ammonia will contribute to an increase of pH. Other compounds contributing to the buffering capacity are hydrogen sulfide and phosphate.

**Feedstock**

The particle size and nature of the material to be treated play an important role. The ease of breakdown is largely defined by the characteristics of the biowaste material to be treated, but generally finer particles allow for better processing and a homogeneous slurry or suspension is the ideal feedstock for AD. It must be stressed, however, that some biowaste types, particularly the likes of lignin-rich, woody material, are relatively resistant to this process.

**Retention period**

Although the amount of biowaste degraded depends on its character, the availability of bacteria and the time allowed for processing, temperature governs both the rate of breakdown itself and the particular bacterial species present in the digester. Hence, there is a direct relationship between temperature and the retention period. Some AD technologies have attempted to shorten the retention period by separating the stages of the process within the digester. The separation of the acidogenic and methanogenic stages permits each to be optimized and this has been well demonstrated at laboratory scale using a completely mixed digester, with phase-isolation being achieved by pH manipulation. Despite the greater efficiency, higher biogas yield and enhanced process stability claimed, it has seen little large-scale use, probably as a result of the higher cost implications of such a system.

**Loading rate**

Loading depends on the characteristics of the waste, its degree of wetness, digester volume, the expected retention period and similar system design parameters. It is typically expressed as the chemical oxygen demand per cubic meter of digester void-space (COD/m³) or, for continuous or semi-continuous process, per unit time (COD/day, COD/hr).
Agitation

The agitation of the digester contents has a number of benefits, one of the most obvious being that it helps to mix up material, evening out any localized concentrations, thus also helping to stop the formation of ‘dead zones’ or scum. In addition, it increases the waste’s availability to the bacteria, helps remove and disperse metabolic products and also acts to ensure a more uniform temperature within the digester. There have been some suggestions that efficient mixing enhances methane production, but the evidence is inconclusive, so it seems likely that this may only be of noticeable benefit for some systems or operational regimes.

Wetness

Anaerobic digestion is a wet process and any biowaste which is too dry in its natural state will require the addition of a suitable liquid, typically water, recycled AD process liquor or slurries, either sewage or agricultural, before processing can begin. In order to minimize digester size, so-called ‘dry’ systems have tended to dominate the commercial world, but the relatively thicker contents inevitably demand more energy to mix effectively, off-setting much of the advantage. Comparisons of ‘wet’ or ‘dry’ approaches, like those of mesophilic or thermophilic processes, generally yield no clear winner. Each system has particular advantages and applications for certain kinds of biowaste, and selecting the right one for any given use is almost always best done on this basis.

Toxins

A number of compounds are toxic to the anaerobic microorganisms (Chen et al., 2008). Methanogens are commonly considered to be the most sensitive to toxicity of the microorganisms in anaerobic digestion. However, the process can acclimatize, and higher concentrations of the toxicant can be tolerated after a period of adaptation. The most common inhibitor for the anaerobic process is ammonia. In anaerobic digestion ammonia originates from soluble ammonia in the influent, from protein degradation and other compounds such as urea. Many substrates used for anaerobic treatment often contain ammonia in toxic concentrations. Such substrates include pig and poultry manure, slaughterhouse waste, potato, juice, highly proteinaceous sludge, wastewater from shale oil and coal liquefaction processes. The results concerning ammonia-N inhibitory level are conflicting, as they depend on parameters such as pH, temperature and adaptation of the inocula. According to Koster and Lettinga (1988), as ammonia concentrations were increased in the range of 4051–5734 mg NH₃–N/L, acidogenic populations in the granular sludge were hardly affected while the methanogenic population lost 56.5% of its activity.

Anaerobic treatment of wastewater containing high sulfate concentrations can cause inhibition as a result of the formation of hydrogen sulfide. It has been reported that total hydrogen sulfide concentrations of 100 to 300 mg/L or free hydrogen sulfide
concentrations of 50 to 150 mg/L caused severe inhibition resulting in complete cessation of biogas production.

The light metal ions including sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) are present in the influent of anaerobic digesters. They may be released by the breakdown of organic matter (such as biomass), or added as pH adjustment chemicals (Grady et al., 1999). They are required for microbial growth and, consequently, affect specific growth rate like any other nutrient. While moderate concentrations stimulate microbial growth, excessive amounts slow down the growth, and even higher concentrations can cause severe inhibition or toxicity (Soto et al., 1993).

The carbon/nitrogen (C/N) ratio is also important for process stability. A C/N ratio of 25 to 32 has been reported to have a positive effect on the methane yield. At lower C/N ratios the risk of excess nitrogen not needed for biomass synthesis and therefore becoming inhibitory increases. Opposite, a very high C/N ratio would lead to N deficiency for biomass synthesis. Waste with very high COD concentration and low content of nitrogen such as olive mill effluents has been shown not to be able to be degraded alone. Addition of either nitrogen or co-digestion with wastes with a lower C/N ratio was needed in order to digest olive mill effluents successfully.

Long chain fatty acids (LCFA), such as oleate and stearate, have been found to be toxic to the anaerobic process (Kim et al., 2004). No adaptation to the fatty-acid toxicity was observed. However, the presence of particulate material can increase the resistance of the process to long-chain fatty acids as LCFA are absorbed on the particulate material and thus not active as inhibitor. For other organic compounds such as phenols, chloroform and formaldehyde a reversible toxicity has been observed.

Heavy metals are toxic for anaerobic microorganisms in concentrations in the range $10^{-3}$ to $10^{-4}$ M. However, experiments have shown that acclimatization to high heavy metal concentrations can occur and often the level of heavy metals would become an environmental problem before affecting the process. In a reactor, the actual concentration of soluble metal ions is normally low due to precipitation of insoluble metal salts, e.g., as sulfides. It has been shown that less than 2% of the metals may be in the soluble form.

**1.7.6 Co-digestion**

Co-digestion is the simultaneous anaerobic digestion of a mixture of two or more substrates. This technology is an attractive option to improve the yields of the anaerobic digestion of wastes due to the positive synergisms established in the digestion medium; a fact that increases the economic viability of the biogas plants (Mata-Alvarez et al., 2000). The main advantage of this technology-based system is an improved methane yield created by the supply of additional nutrients to the mixture. Moreover, co-digestion technology could lead to the following benefits (Mata-Alvarez et al., 2000; Alatriste-Mondragón et al., 2006): (1) dilution of inhibitory and/or toxic compounds, (2)
increase of the organic content inside the digester, better utilization of the digester volume, (3) enhancement of the digestate stabilization, (4) accomplishment of the required moisture contents in the digester feed, with an easier handling of blended wastes, (5) high reduction of the emission of greenhouse gases to the atmosphere and (6) economic advantages from the fact of sharing equipment and cost. However, some drawbacks exist as well: (1) the high cost of waste transfer from the co-substrate generation point to the anaerobic plant, (2) the risk of spreading poisonous substances originated from the industrial or municipal waste and (3) the harmonization of different policies of the waste generators. What is more, co-digestion will change the digestion behavior and the quality of the digestate; furthermore, the addition of unknown co-substrate should be prevented. In order to better the results of the co-digestion and to detect the amounts of inhibitory or toxic compounds, which can lead to a process breakdown or decrease the methane production, it is necessary to carry out several laboratory experiments such as the biodegradability test and/or the lab-scale digester (Braun et al., 2002). A literature review of the most recent attempts in the last years is presented on the selection of co-substrates, its contribution in greenhouse gas (GHG) reduction and the optimization of the process.
1.8 References


Chapter 1

Introduction


Sites

http://www.altenergy.org
Chapter 2.

Materials and Methods

2.1 Materials

2.1.1 Agro-industrial wastes

The raw wastewaters used in all experiments included olive-mill wastewater (OMW), cheese whey (CW) and liquid cow manure (LCM) were collected from small local plants in the area of Patras (Western Greece). In particular, OMW was obtained from a local olive oil-mill (Panitsas N. & Co.) using a three-phase decanter centrifugation process for extraction of olive oil. CW was provided from a cheese factory (AVIGAL S.A.) located in the same region producing mainly “feta” cheese with daily production of 30 m$^3$ of wastewaters. LCM was collected from a dairy farm (Kaimakas D.) breeding 230 cows. Because of the fact that OMW and CW are characterized by seasonal availability and high tendency for fermentation, all wastewater samples were collected fresh and stored immediately in the freezer at −18ºC until subsequent use throughout the experimentation period.

2.1.2 Sweet Sorghum

Sweet sorghum used in this dissertation corresponded to six different types (three fresh and three ensiled sorghums). They were cultivated through biological farming techniques according to European Regulation EC 2091/91 and were collected from different fields in Achaia or Aitoloakarnania (Western Greece) and were harvested in different seasons. Table 2.1 presents the six sorghum biomasses with their characteristics, whereas Picture 2.1 shows the position of the fields. The two ensiled sorghums (ES2 and ES3) were originated from the fresh ones (FS2 and FS3) respectively, after the ensiling procedure. The fresh chopped sorghum was ensiled by enclosetment for 60 days in 30 L plastic bins at ambient temperature.
Table 2-1: Different sorghum used with their main characteristics.

<table>
<thead>
<tr>
<th>Type of sorghum</th>
<th>Cultivation field</th>
<th>Geo-coordinates</th>
<th>Harvest period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Sorghum (FS1) <em>bicolor L. Moench var. Keller</em></td>
<td>University of Patras</td>
<td>38°17’51.14”N 21°48’2.28”E</td>
<td>November 2009</td>
</tr>
<tr>
<td>Ensiled Sorghum (ES1) <em>Sugargraze, forage sorghum</em></td>
<td>Neochori Mesologiou</td>
<td>38°24’18.72”N 21°16’45.73”E</td>
<td>November 2009</td>
</tr>
<tr>
<td>Fresh Sorghum (FS2) <em>bicolor L. Moench var. Keller</em></td>
<td>University of Patras</td>
<td>38°17’51.14”N 21°48’2.28”E</td>
<td>October 2010</td>
</tr>
<tr>
<td>Ensiled Sorghum (ES2) <em>bicolor L. Moench var. Keller</em></td>
<td>University of Patras</td>
<td>38°17’51.14”N 21°48’2.28”E</td>
<td>October 2010</td>
</tr>
<tr>
<td>Fresh Sorghum (FS3) <em>Sudangrass hybrid-HoneyGraze BMR</em></td>
<td>Farm near Patras (Western Greece)</td>
<td>38°06’42.27”N 21°38’26.37”E</td>
<td>November 2011</td>
</tr>
<tr>
<td>Ensiled Sorghum (ES3) <em>Sudangrass hybrid-HoneyGraze BMR</em></td>
<td>Farm near Patras (Western Greece)</td>
<td>38°06’42.27”N 21°38’26.37”E</td>
<td>November 2011</td>
</tr>
</tbody>
</table>

After harvesting, the sweet sorghums were chopped into pieces (particle size ranged between 1 and 3 cm), were dried at 55°C, then ground into 1 mm particle size with a kitchen blender and sieved to powder of < 315 μm diameter. Biomass particle size reduction can alter the inherent ultrastructure of lignocellulosic biomass, increase the accessible surface area, reduce the degree of cellulose crystallinity, and decrease the degree of cellulose polymerization for improved digestibility (Kratky and Jirout, 2011). Using energy crops and other high-strength organic wastes for co-digestion, size reduction might be very important because microbial hydrolysis of lignocellulose is a slow and difficult process. The effect of mechanical pretreatment on methane production and hydrolysis kinetics was investigated by many authors. Sambusiti et al. (2013), for example, found no significant differences in terms of methane yields and kinetic constants of ensiled sorghum forage, milled into 2, 1, 0.5 and 0.25 mm particle sizes. On the other hand, Sharma et al. (1998) found biogas production of agricultural and forest residues (e.g. wheat straw, rice straw, mirabilis leaves, and dump grass) increased with a decrease of particle size (from 30 to 0.088 mm), but negligible differences in biogas yields occurred at particle sizes of between 0.088 and 0.40 mm.
Materials and Methods

Chapter 2

Picture 2-1: The fields where the different sorghums were cultivated.
2.2 Experimental set-up

The experimental configurations used in all experiments was continuous (CSTR) experiments (two or single-stage system), or batch experiments or biochemical methane potential assays. Each experimental set-up was briefly described above.

2.2.1 Continuous anaerobic digestion experiments

Anaerobic digestion experiments were carried out in two CSTR reactors, one used for acidogenesis and the other one for methanogenesis. A schematic description of bioreactors setup for hydrogen and methane production, in a two-stage system, is shown in Fig. 2.1. The two anaerobic reactors were made with a double wall, cylindrical in shape (Picture 2.2(a)), entirely of stainless steel (INOX 316), having a total volume of 1 L and 5 L, respectively and were operated at constant temperature of 37 ± 0.2°C via a thermocouple controller. Agitation was performed at 70 rpm ensuring homogeneous mixing by a geared motor drive unit which was installed on the top of each reactor (Picture 2.2(b)). Each reactor’s feedstock was stored in a tank placed in a refrigerator to maintain constant temperature at 4°C and was fed to the reactor via a precise peristaltic pump after being sparged with nitrogen gas to remove dissolved oxygen. Gas production was measured separately in each one of the reactors by two automatic tailor-made devices comprising of a combination of an engine oil filled U-tube, an electron – valve and a counter. The measurement was based on counting the number of displacements of constant oil volume by the produced biogas in each biogas line (Picture 2.2(c)). Anaerobic conditions in both anaerobic reactors were ensured by sparging with nitrogen gas their liquid content at the beginning of each experiment. In some experiments, a pH-controller was used in the acidogenic reactor, via automatic control (using a Hach PID-controller), in order to keep constant the pH throughout the experimentation phase. The addition of a proper solution (e.g. mixture of NaOH/KOH) was carried out via a peristaltic pump. There were also experiments in a single-stage system using only the methanogenic reactor which operated in the same experimental set-up.
Figure 2-1: Schematic diagram of the two-stage system used in this study for combined hydrogen and methane production.
2.2.2 Batch experiments

Batch experiments were carried out either in steel reactors (Picture 2.3(a)) or in glass flasks (Picture 2.3(b)). In particular, steel reactors were made as mentioned previously (Section 2.2.1), with total volume of 1-L and were operated in batch mode under controlled temperature, pH and stirring rate conditions (Section 2.2.1). On the other hand, the glass flasks (with total volume capacity of 500 mL) were shaken in an orbital shaking water bath (Grant OLS200) at 80 rpm at constant mesophilic temperature (37°C). They were closed by rubber caps where two output ports were installed, one for sampling and the other one for biogas outlet which was collected in a glass syringe.
The working volume of the reactor was different between the experiments. The amount of anaerobic sludge used as inoculum was every time 15 or 20% (v/v) of working volume, while the remaining consisted of the tested substrate. Prior to experiment startup, the contained liquor in each reactor was flushed with nitrogen gas for 5 min in order to remove oxygen from the headspace and maintain anaerobic conditions.

**Picture 2-3:** (a) Steel reactor and (b) glass flasks used for batch experiments.

### 2.2.3 Biochemical Methane Potential assay

Biochemical methane potential (BMP) is an experimental procedure developed to determine the methane production of a given organic substrate during its anaerobic decomposition. The BMP assay has proved to be a relatively simple and reliable method to obtain the extent and rate of organic matter conversion to methane (Owen et al., 1979; Chynoweth et al., 1993). The information provided by BMP is valuable when evaluating potential substrates and optimizing the design and operation of anaerobic digesters. A vast amount of literature has been related to BMP assays proving the extent to which this test has been used to evaluate a wide variety of substrates (Gunaseelan, 2004; Labatut et al., 2011). The anaerobic digestion of substrates was performed in batch mode using the biochemical methane potential (BMP) assay. Methane potential of wastes is defined as the ultimate specific methane production, for indefinite degradation time. In practice the degradation time is definite and the methane potential is estimated...
by extrapolation of a methane time degradation curve. Methane potential can be expressed specifically per amount of waste (mL CH₄/g waste), volume of waste (mL CH₄/mL waste), per mass volatile solids added (mL CH₄/g VS) or COD added (mL CH₄/g COD). The volume is usually expressed in standard pressure (1 atm) and temperature (0°C) conditions (STP conditions). The BMP protocol followed in this study was based on the principles described by Owen et al. (1979) and revised by others (Chynoweth et al., 1993).

2.2.3.1 BMP procedure

In this study, different agro-industrial organic wastes or sweet sorghum were subjected to BMP assay. Each substrate was used in duplicates, whereas two additional bottles containing only inoculum were included to account for background (i.e. endogenous) methane production (blank). Briefly, known amounts of substrate and active anaerobic inoculum (20% v/v) were added to 160-mL serum bottles (Picture 2.4(a)). Additional defined media containing nutrients and vitamins for mixed anaerobic cultures were also added (Owen et al., 1979) and the pH was measured. The serum bottles were flushed for 5 min with nitrogen gas and then sealed immediately using butyl rubber septum and aluminum crimp caps. Once sealed, the bottles were placed in an orbital shaking water bath (Grant OLS200) at 80 rpm (Picture 2.4(b)) and maintained at a constant mesophilic temperature (37°C).

Picture 2-4: (a) Experimental set-up and (b) orbital shaking water bath used in BMP assay.
2.3 Analytical Methods

Physicochemical characterization was performed in all raw agro-wastes and experimental samples. For the measurement of TSS and soluble compounds (soluble organic carbon, lactic acid, VFAs etc), the insoluble residue was separated from the supernatant via Whatman® glass microfiber filters, Grade GF/F. The determination procedure for each characteristic is described below:

2.3.1 pH

The off-line pH measurements were carried out using an electrode (Thermo Scientific Orion 3-star benchtop pH meter).

2.3.2 Electrical Conductivity

The measurement of the electrical conductivity was performed using a portable electrode, (Type Vernier).

2.3.3 Solids

Total solids (TS), volatile solids (VS) and suspended solids (TSS, VSS) were determined according to Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Total solids were measured after sample drying at 105 °C (Section 2540 B, D), while volatile solids after sample ignition at 550 °C (Section 2540 E).

2.3.4 COD

Total and soluble COD (TCOD and SCOD) were determined according to Standard Methods for the Examination of Water and Wastewater (APHA, 1998). In particular, total COD was measured using ‘Open Reflux, Titrimetric Method’ (Section 5220 B), whereas soluble COD in accordance with ‘Closed Reflux, Colorimetric Method’ (Section 5220 D).

2.3.5 BOD

Biochemical oxygen demand (BOD₅) was determined according to Standard Methods for the Examination of Water and Wastewater (APHA, 1998). In particular, BOD₅ was measured using ‘5-Day BOD Test’ (Section 5210 B). The method consists of filling with sample, to overflowing, an airtight bottle of the specified size and incubating it at the specified temperature for 5 d. Dissolved oxygen is measured initially and after incubation, and the BOD₅ is computed from the difference between initial and final dissolved oxygen.
2.3.6 **Organic carbon**

    Total and soluble organic carbon (TOC and SOC) were analyzed with a Carbon TOC-V module (Shimadzu). In the TOC-V, carrier gas is controlled using a pressure regulator and mass flow controller. Carrier gas flows at 150 mL/min to the combustion tube, which has been filled with an oxidation catalyst and heated to 680ºC. The TC of a sample is burned in the combustion tube to form carbon dioxide. The carrier gas, containing the carbon dioxide and other combustion products, flows from the combustion tube to a dehumidifier, where it is cooled and dehydrated. Then it passes through a halogen scrubber before it reaches the cell of a non-dispersive infrared NDIR gas analyzer, where the carbon dioxide is detected. The analog detection signal of the NDIR gas analyzer forms a peak, and the area of this peak is measured by a data processor.

2.3.7 **Carbohydrates**

    For the determination of carbohydrates, a colored sugar derivative was produced through the addition of L-tryptophan, sulfuric and boric acid, which was subsequently measured colorimetrically in a Cary 50 UV/VIS spectrophotometer (Varian) at the wavelength of 520 nm (Joseffson, 1983).

2.3.8 **Phenols**

    Total phenolic compounds were determined spectrophotometrically in centrifuged and filtered samples according to the Folin–Ciocalteu method (Waterman and Mole, 1994) using syringic acid as standard solution.

2.3.9 **Nitrogen**

    Ammonia and total Kjeldahl nitrogen (TKN) were analyzed by Kjeldahl methods (Section 4500-N_\text{org} B, C), according to *Standard Methods for the Examination of Water and Wastewater* (APHA, 1998).

2.3.10 **Proteins**

    The protein content was determined from the total nitrogen content (Kjeldahl method) with a conversion factor of 6.25 (Panagiotopoulos et al., 2010; Sambusiti et al., 2012).

2.3.11 **Alkalinity**

    For alkalinity measurement was utilized a “Titration Method” (Section 2320 B), according to *Standard Methods for the Examination of Water and Wastewater* (APHA, 1998).
2.3.12 Total Potassium

The determination of potassium in test samples was performed by the technique of atomic emission, using the atomic absorption apparatus (Perkin Elmer AAnalyst800).

2.3.13 Phosphorus

Phosphorus measurement was achieved according to Standard Methods for the Examination of Water and Wastewater (APHA, 1998) after using two general procedural steps: conversion of the phosphorus form of interest to dissolved orthophosphate, and colorimetric determination of dissolved orthophosphate with the ‘‘Ascorbic Acid Method’’ (Section 4500-P, E).

2.3.14 Oil and Grease

Oil and Grease were determined according to Standard Methods for the Examination of Water and Wastewater (APHA, 1998) using a ‘‘Soxhlet Extraction Method’’ (Section 5520 D) with n-Hexane as an extraction solvent.

2.3.15 Volatile fatty acids (VFAs)

Composition analysis of volatile fatty acids (VFA) was performed in a gas chromatograph (Agilent Technologies 7890A). For quantification of volatile fatty acids (VFAs) liquid samples were removed from the fermentor, transferred immediately to 2-mL vials where 1 mL of sample was acidified with 30 μL of 20% H₂SO₄ and then centrifuged (>3000 rpm) for 15 min to remove biomass. The supernatant was filtered and transferred to 2-mL septum-capped vials. The gas chromatograph was equipped with a flame ionization detector (FID) using helium as carrier gas. A capillary column (DB–FFAP, 30 m in length, 0.25 mm I.D. and 0.25 μm film) was used for determining the concentration of the individual volatile fatty acids, i.e. acetic, propionic, isobutyric, butyric, isovaleric, valeric and caproic acid. The oven was programmed at 110ºC (held for 5 min) to 250ºC (held for 6 min) at a rate of 15ºC/min. The operating temperature of the injector and detector was set at 250 and 300ºC, respectively.

2.3.16 Alcohols

Composition analysis of alcohols (i.e. ethanol), was performed in a gas chromatograph (Agilent Technologies 7890A) equipped with a flame ionization detector (FID) using helium as carrier gas with a capillary column (DB–FFAP, 30 m in length, 0.25 mm I.D. and 0.25 μm film). The procedure of sample preparing was the same with VFAs procedure. For ethanol determination the operating temperature of the injector and detector was kept at 175 and 250ºC, respectively. The oven temperature was gradually increased from 35ºC (held for 4 min) to 120ºC at a rate of 10ºC/min and then to 240ºC (held for 7 min) at a rate of 27ºC/min.
2.3.17 Lactic acid

Lactic acid in the culture medium was measured with a DIONEX ICS3000 ion chromatography system using a thermostated (30°C) Dionex IonPac analytical column (AS19 length 4 x 250 mm and 7.5 mm I.D) and a guard column (4x50 mm length and 12 mm I.D) and an electron conductivity detector (Dionex). Analysis was performed by applying an elution gradient with KOH solution, as mobile phase, at a flow rate of 0.8 mL/min. The eluent gradient was programmed to result in a 3 mM KOH solution during equilibration and analysis and a 70 mM KOH solution during column regeneration. The total running time of analysis was 28 min and the gradient profile as follows: 3 mM KOH for 14 min, 70 mM KOH in 3 min and maintained for 4 min and 3 mM KOH in 0.5 min until the end of run (28 min). The injection volume was 10 μL.

2.3.18 Monosugars determination

Monosugars were analyzed by HPLC (Agilent Technologies 1200 series) using an Evaporative Light Scattering Detector (ELSD). A separation column (Phenomenex, Rezex RPM-Monosaccharide Pb+2 (8%), 300 x 7.8 mm) and a guard pre-column (SecurityGuard™ Carbo-H+, 50 x 7.8 mm I.D., 8 μm cartridges) were used at adjusted constant column temperature (85°C). An aqueous mobile phase of H₂O (18 MΩ cm) was used at a flow rate of 0.6 mL/min. The temperature of the heated drift tube was 60°C at nitrogen pressure 3.5 bar with detector gain = 7. Solution samples were filtered (0.2 μm Nylon Whatman) prior to HPLC analysis and were injected using a glass syringe (SGE Analytical Science) and a fixed-volume 20 μL loop injector.

2.3.19 Lignin

Lignin content of dried extracted material was determined according to the strong acid hydrolysis method of NREL (Sluiter et al., 2011). Sample (0.3 g) was first hydrolyzed with 3 mL 72% sulfuric acid at 30°C for 1 h. The mixture was diluted with 84 mL ultrapure water and treated for 1 h at 121°C in an autoclave. The insoluble residue was separated from the supernatant via Whatman® glass microfiber filters, Grade GF/F. This insoluble residue was washed with 50 mL of deionized water and then placed in a crucible. The crucible and microfiber filters were dried at 105°C during 24 h to determine by weighting the amount of acid insoluble lignin (AIL).

2.3.20 Cellulose and Hemicellulose

Cellulose and hemicellulose content of dried extracted material were measured according to the strong acid hydrolysis method of NREL (Sluiter et al., 2011). The liquid fraction, which remained after the determination of acid insoluble lignin (Section 2.3.19) was neutralized by calcium carbonate and after centrifugation in 2 mL Eppendorf® tubes, was filtrated at 0.2 μm (Nylon Whatman®). Then, the supernatant was transferred to a vial prior to the analysis by high performance liquid
chromatography (HPLC). Structural carbohydrates (i.e. glucose, xylose, arabinose) were measured by HPLC (Agilent Technologies 1200 series) as described at Section 2.3.18.

The system was calibrated with glucose, xylose, arabinose and fructose standards (Sigma–Aldrich). Thereafter, cellulose and hemicellulose contents were estimated as follows (Eq. (2.1) and (2.2)):

\[
\text{Cellulose} \, (\% \text{ VS}) = \frac{\text{Glucose} \, (\% \text{ VS})}{1.11}
\]  
\[
\text{Hemicellulose} \, (\% \text{ VS}) = \frac{[\text{Xylose} \, (\% \text{ VS}) + \text{Arabinose} \, (\% \text{ VS})]}{1.13}
\]

where: 1.11 is the ratio of the molecular weights of glucose to glucan (180/162) and 1.13 is the ratio of the molecular weights of xylose and arabinose to xylan (150/132).

### 2.3.21 Elemental composition analysis

Another approach for characterization involves the quantification of the content of certain elements (C, O, H, N and S). Elemental composition analysis was performed on freeze-dried samples. The elemental analyzer contains a combustion furnace, which was maintained during the analysis at 1020°C. In the combustion furnace there was a quartz column for oxidation and reduction of the solid sample. The carrier gas was helium (constant flow of 100 mL/min), which was enriched with pure oxygen in order to achieve a strong oxidizing environment for solid material combustion. The solid samples were oxidized to gases CO₂, H₂O, NOₓ and SO₃ and then after reduction a gas mixture consisted of N₂, CO₂, H₂O and SO₂ was obtained. Finally, the gas mixture was analyzed in gas chromatography equipped with a packed column (Porapack Q) and a thermal conductivity detector (TCD).

### 2.3.22 Gaseous products

The produced biogas was measured by automatic tailor-made devices comprising of a combination of an engine oil filled U-tube, an electron–valve and a counter. The measurement was based on counting the number of displacements of constant oil volume by the produced biogas in each biogas line. The biogas was sampled with a 1-mL gas-tight syringe. Biogas composition (hydrogen, methane and carbon dioxide) was analyzed by gas chromatography (Agilent Technologies 7890A) equipped with a capillary column (HP-PLOT/Q, 30 m in length, 0.53 mm I.D. and 40 μm packing film) and a thermal conductivity detector (TCD) using nitrogen as carrier gas. The temperature of injector and detector was kept at 250°C and the oven temperature was gradually increased from 80°C (held for 6 min) to 200°C (held for 2 min) at a rate of 50°C/min.

Total biogas, hydrogen and methane volumes, produced during each experiment, were converted at standard temperature and pressure conditions (i.e. STP = 0°C and 1 atm).
2.4 References


Chapter 3.

Quantitative and qualitative characteristics of the main agro-wastes in Western Greece

3.1 Quantitative characteristics

Certain agro-industries such as olive mills, cheese factories and dairy farms represent a considerable share of the environmental problem in the Mediterranean countries producing large amounts of wastewaters i.e. olive mill wastewaters (OMW), cheese whey (CW), liquid cow manure (LCM) and pig manure (PM). Although technical solutions for their treatment do exist, their application is still lacking in these agro-industries due to their regional spatial distribution, their small to medium-size family character and also their periodic operation. In collaboration with local Prefectures, namely Achaia, Ionian Islands, Preveza and Lefkada, the following quantitative characteristics were collected (Table 3.1-3.4) in order to identify the recommendation of the wastes through daily production for two different periods in the year. Furthermore, the daily production of the same kind of wastes was collected from the Italian province of Bari (Table 3.5).

<table>
<thead>
<tr>
<th>Waste</th>
<th>Average Daily Production (m³/d)</th>
<th>Percentage Recommendation¹ (%)</th>
<th>Percentage Recommendation² (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig-Cow manure</td>
<td>5</td>
<td>0.8</td>
<td>4.3</td>
</tr>
<tr>
<td>Olive mill wastewater</td>
<td>511</td>
<td>81.4</td>
<td>-</td>
</tr>
<tr>
<td>Cheese Whey</td>
<td>112</td>
<td>17.8</td>
<td>95.7</td>
</tr>
<tr>
<td>Total</td>
<td>628</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

¹: Recommendation during period of November - January
²: Recommendation during period of February - June
### Chapter 3: Quantitative and qualitative characteristics of the main agro-wastes in Western Greece

Table 3-2: Prefecture of Ionian Islands.

<table>
<thead>
<tr>
<th>Waste</th>
<th>Average Daily Production (m³/d)</th>
<th>Percentage Recommendation¹ (%)</th>
<th>Percentage Recommendation² (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig-Cow manure</td>
<td>3</td>
<td>4.5</td>
<td>10.3</td>
</tr>
<tr>
<td>Olive mill wastewater</td>
<td>37</td>
<td>56.1</td>
<td>-</td>
</tr>
<tr>
<td>Cheese Whey</td>
<td>26</td>
<td>39.4</td>
<td>89.7</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

¹: Recommendation during period of October - March  
²: Recommendation during period of April - May

Table 3-3: Prefecture of Preveza.

<table>
<thead>
<tr>
<th>Waste</th>
<th>Average Daily Production (m³/d)</th>
<th>Percentage Recommendation¹ (%)</th>
<th>Percentage Recommendation² (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig-Cow manure</td>
<td>964</td>
<td>77.5</td>
<td>99.3</td>
</tr>
<tr>
<td>Olive mill wastewater</td>
<td>274</td>
<td>22.0</td>
<td>-</td>
</tr>
<tr>
<td>Cheese Whey</td>
<td>6</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Total</td>
<td>1244</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

¹: Recommendation during period of November - February  
²: Recommendation during period of March - June

Table 3-4: Prefecture of Lefkada.

<table>
<thead>
<tr>
<th>Waste</th>
<th>Average Daily Production (m³/d)</th>
<th>Percentage Recommendation¹ (%)</th>
<th>Percentage Recommendation² (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow manure</td>
<td>2.5</td>
<td>3.1</td>
<td>100</td>
</tr>
<tr>
<td>Olive mill wastewater</td>
<td>78</td>
<td>96.9</td>
<td>-</td>
</tr>
<tr>
<td>Cheese Whey</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>80.5</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

¹: Recommendation during period of November - March  
²: Recommendation during period of March - October
Table 3-5: Prefecture of Centro Ricerche Bonomo (Italian province of Bari).

<table>
<thead>
<tr>
<th>Waste</th>
<th>Average Daily Production (m³/d)</th>
<th>Percentage Recommendation (%)</th>
<th>Percentage Recommendation² (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow manure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olive mill wastewater</td>
<td>693</td>
<td>61.5</td>
<td>-</td>
</tr>
<tr>
<td>Cheese Whey</td>
<td>434</td>
<td>38.5</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>1127</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

1: Recommendation during period of November - February
2: Recommendation during period of March - October

3.2 Qualitative characteristics

Moreover the main objective of this part was the characterization of agro-wastes such as olive mill wastewater (OMW), cheese whey (CW) and animal manures (LCM, PM), which were taken during whole wastes production and were analyzed by easily applicable physicochemical methods (Table 3.6). From the characterization of various types of waste conducted, may be mentioned in conclusion the following characteristics per type of waste:

The olive mill wastewater (OMW) is characterized by:
- High organic load and solids concentration
- High concentration of phenolics (toxicity)
- Low concentration of nitrogen
- Low pH (~5.0)

The cheese whey (CW) is characterized by:
- High organic load concentration
- High concentration of carbohydrates
- Low concentration of nitrogen
- Low pH (<6.0) (quick acidification)
- Low content of solids
- Higher phosphorus content than other wastes

The animal manures (LCM or PM) are characterized by:
- High organic load and solids concentration
- High nitrogen content
- Low content of soluble phosphorus
- Neutral pH

Table 3.7 presents the main characteristics from Italian wastes (3 types of OMW and 4 types of CW). OMWs were taken from 3 different regions (Monopoli, Turi and Andria), whereas CWs were either whey or buttermilk from Biancolat industry or Corato region.
Table 3-6: Chemical composition of different types of agro-wastes from different regions in Western Greece.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>OMW Achaia</th>
<th>OMW Ionian Islands</th>
<th>OMW Preveza</th>
<th>OMW Lefkada</th>
<th>CW Achaia</th>
<th>CW Ionian Islands</th>
<th>CW Preveza</th>
<th>LCM Achaia</th>
<th>LCM Ionian Islands</th>
<th>LCM Preveza</th>
<th>PM Preveza</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-</td>
<td>5.09 ± 0.08</td>
<td>5.08 ± 0.05</td>
<td>4.85 ± 0.03</td>
<td>5.29 ± 0.21</td>
<td>6.17 ± 0.14</td>
<td>6.07 ± 0.63</td>
<td>5.02 ± 0.29</td>
<td>7.03 ± 0.04</td>
<td>6.42 ± 0.40</td>
<td>6.87 ± 0.07</td>
<td>7.27 ± 0.24</td>
</tr>
<tr>
<td>TSS</td>
<td>gL</td>
<td>40.29 ± 3.30</td>
<td>44.02 ± 5.32</td>
<td>32.90 ± 2.36</td>
<td>38.54 ± 11.75</td>
<td>10.09 ± 1.00</td>
<td>10.92 ± 3.54</td>
<td>1.01 ± 0.17</td>
<td>50.91 ± 15.75</td>
<td>33.90 ± 27.58</td>
<td>71.22 ± 3.67</td>
<td>1.79 ± 0.21</td>
</tr>
<tr>
<td>VSS</td>
<td>gL</td>
<td>33.29 ± 3.59</td>
<td>42.48 ± 5.11</td>
<td>31.22 ± 2.70</td>
<td>31.33 ± 11.07</td>
<td>9.05 ± 1.04</td>
<td>8.10 ± 3.87</td>
<td>0.88 ± 0.23</td>
<td>34.65 ± 10.55</td>
<td>27.85 ± 23.52</td>
<td>56.65 ± 7.92</td>
<td>0.11 ± 0.22</td>
</tr>
<tr>
<td>SCOD</td>
<td>gL</td>
<td>61.36 ± 5.45</td>
<td>79.26 ± 6.11</td>
<td>28.44 ± 3.11</td>
<td>53.83 ± 12.28</td>
<td>55.19 ± 2.94</td>
<td>59.91 ± 7.17</td>
<td>4.00 ± 0.59</td>
<td>20.95 ± 0.77</td>
<td>9.67 ± 2.24</td>
<td>14.52 ± 1.97</td>
<td>1.74 ± 0.90</td>
</tr>
<tr>
<td>TCOD</td>
<td>gL</td>
<td>143.24 ± 14.24</td>
<td>147.89 ± 16.33</td>
<td>108.40 ± 12.90</td>
<td>100.52 ± 22.49</td>
<td>73.44 ± 2.62</td>
<td>72.77 ± 18.40</td>
<td>6.65 ± 1.14</td>
<td>54.51 ± 5.90</td>
<td>49.93 ± 18.80</td>
<td>66.18 ± 8.32</td>
<td>5.07 ± 1.71</td>
</tr>
<tr>
<td>BOD₅</td>
<td>gL</td>
<td>39.65 ± 1.69</td>
<td>24.00 ± 1.94</td>
<td>10.50 ± 1.99</td>
<td>25.36 ± 18.89</td>
<td>34.70 ± 1.39</td>
<td>38.33 ± 4.04</td>
<td>3.06 ± 0.86</td>
<td>16.19 ± 5.91</td>
<td>7.53 ± 1.63</td>
<td>9.44 ± 2.51</td>
<td>1.26 ± 0.74</td>
</tr>
<tr>
<td>Total Carbohydrates*</td>
<td>gL</td>
<td>20.69 ± 4.81</td>
<td>37.48 ± 2.67</td>
<td>27.83 ± 2.99</td>
<td>15.23 ± 4.68</td>
<td>30.95 ± 8.74</td>
<td>46.05 ± 8.03</td>
<td>0.16 ± 0.12</td>
<td>7.82 ± 2.11</td>
<td>1.98 ± 0.37</td>
<td>5.43 ± 0.68</td>
<td>0.29 ± 0.04</td>
</tr>
<tr>
<td>Soluble Carbohydrates*</td>
<td>gL</td>
<td>18.19 ± 3.13</td>
<td>31.90 ± 2.58</td>
<td>2.53 ± 0.98</td>
<td>12.23 ± 6.03</td>
<td>31.01 ± 5.51</td>
<td>40.15 ± 3.27</td>
<td>0.03 ± 0.03</td>
<td>0.76 ± 0.18</td>
<td>0.15 ± 0.05</td>
<td>0.37 ± 0.09</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>Total nitrogen, TKN</td>
<td>gL</td>
<td>0.78 ± 0.08</td>
<td>0.42 ± 0.07</td>
<td>0.49 ± 0.03</td>
<td>0.22 ± 0.24</td>
<td>0.78 ± 0.12</td>
<td>1.02 ± 0.17</td>
<td>0.34 ± 0.38</td>
<td>2.94 ± 0.73</td>
<td>1.99 ± 0.70</td>
<td>1.96 ± 0.51</td>
<td>1.07 ± 0.21</td>
</tr>
<tr>
<td>Ammonium nitrogen</td>
<td>gL</td>
<td>0.11 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.10 ± 0.02</td>
<td>0.25 ± 0.01</td>
<td>0.10 ± 0.03</td>
<td>1.56 ± 0.11</td>
<td>1.55 ± 0.67</td>
<td>1.29 ± 0.43</td>
<td>0.74 ± 0.62</td>
</tr>
<tr>
<td>Total Phosphorus</td>
<td>gL</td>
<td>0.38 ± 0.04</td>
<td>0.51 ± 0.05</td>
<td>0.31 ± 0.03</td>
<td>0.29 ± 0.02</td>
<td>0.27 ± 0.06</td>
<td>0.30 ± 0.06</td>
<td>0.09 ± 0.02</td>
<td>0.46 ± 0.20</td>
<td>0.42 ± 0.21</td>
<td>0.42 ± 0.18</td>
<td>0.13 ± 0.17</td>
</tr>
<tr>
<td>Soluble Phosphorus</td>
<td>gL</td>
<td>0.22 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>0.12 ± 0.01</td>
<td>0.27 ± 0.03</td>
<td>0.21 ± 0.02</td>
<td>0.20 ± 0.05</td>
<td>0.07 ± 0.02</td>
<td>N.D.</td>
<td>0.06 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.08 ± 0.09</td>
</tr>
<tr>
<td>Phenols*</td>
<td>gL</td>
<td>5.49 ± 1.19</td>
<td>8.71 ± 0.46</td>
<td>3.16 ± 0.22</td>
<td>4.43 ± 0.81</td>
<td>0.11 ± 0.05</td>
<td>0.13 ± 0.08</td>
<td>N.D.</td>
<td>0.95 ± 0.59</td>
<td>0.25 ± 0.13</td>
<td>0.48 ± 0.05</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td>Oil and grease</td>
<td>gL</td>
<td>12.51 ± 2.96</td>
<td>8.60 ± 1.25</td>
<td>11.26 ± 3.95</td>
<td>3.78 ± 1.93</td>
<td>1.24 ± 1.02</td>
<td>0.40 ± 0.22</td>
<td>0.14 ± 0.11</td>
<td>2.04 ± 1.05</td>
<td>5.54 ± 3.57</td>
<td>1.79 ± 0.89</td>
<td>0.55 ± 0.27</td>
</tr>
</tbody>
</table>

* In equivalent glucose;  In equivalent syringic acid; OMW: Olive Mill Wastewater; CW: Cheese Whey; LCM: Liquid Cow Manure; PM: Pig Manure
Table 3-7: Chemical composition of agro-wastes in Italian province of Bari.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>OMW Monopoli</th>
<th>OMW Frantoio Turi</th>
<th>OMW Frantoio Andria</th>
<th>CW Latticello Biancolat</th>
<th>CW Siero Burro Biancolat</th>
<th>CW Scotta Corato</th>
<th>CW Siero Corato</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-</td>
<td>5.54</td>
<td>5.84</td>
<td>5.55</td>
<td>6.73</td>
<td>4.12</td>
<td>5.88</td>
<td>4.70</td>
</tr>
<tr>
<td>TSS</td>
<td>g/L</td>
<td>23.85</td>
<td>10.62</td>
<td>9.52</td>
<td>8.13</td>
<td>13.49</td>
<td>4.51</td>
<td>15.00</td>
</tr>
<tr>
<td>VSS</td>
<td>g/L</td>
<td>21.30</td>
<td>9.20</td>
<td>8.75</td>
<td>6.11</td>
<td>13.21</td>
<td>4.14</td>
<td>14.28</td>
</tr>
<tr>
<td>SCOD</td>
<td>g/L</td>
<td>144.92</td>
<td>125.85</td>
<td>87.70</td>
<td>12.16</td>
<td>19.07</td>
<td>28.80</td>
<td>57.64</td>
</tr>
<tr>
<td>TCOD</td>
<td>g/L</td>
<td>170.76</td>
<td>168.22</td>
<td>144.86</td>
<td>14.29</td>
<td>35.51</td>
<td>32.45</td>
<td>82.24</td>
</tr>
<tr>
<td>Total Carbohydrates(^a)</td>
<td>g/L</td>
<td>85.40</td>
<td>64.60</td>
<td>41.80</td>
<td>8.85</td>
<td>11.04</td>
<td>29.40</td>
<td>53.40</td>
</tr>
<tr>
<td>Soluble Carbohydrates(^a)</td>
<td>g/L</td>
<td>77.20</td>
<td>55.60</td>
<td>35.3</td>
<td>8.80</td>
<td>9.94</td>
<td>27.40</td>
<td>50.10</td>
</tr>
<tr>
<td>Total nitrogen, TKN</td>
<td>g/L</td>
<td>0.06</td>
<td>0.09</td>
<td>0.06</td>
<td>0.17</td>
<td>0.35</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Ammonium nitrogen</td>
<td>g/L</td>
<td>0.05</td>
<td>0.08</td>
<td>0.05</td>
<td>0.04</td>
<td>0.07</td>
<td>0.02</td>
<td>0.09</td>
</tr>
<tr>
<td>Total Phosphorus</td>
<td>g/L</td>
<td>0.56</td>
<td>0.70</td>
<td>0.38</td>
<td>0.15</td>
<td>0.21</td>
<td>0.24</td>
<td>0.47</td>
</tr>
<tr>
<td>Soluble Phosphorus</td>
<td>g/L</td>
<td>0.28</td>
<td>0.43</td>
<td>0.11</td>
<td>0.11</td>
<td>0.16</td>
<td>0.17</td>
<td>0.19</td>
</tr>
<tr>
<td>Phenols(^b)</td>
<td>g/L</td>
<td>9.50</td>
<td>11.70</td>
<td>7.00</td>
<td>0.05</td>
<td>0.06</td>
<td>0.02</td>
<td>0.20</td>
</tr>
<tr>
<td>Oil and grease</td>
<td>g/L</td>
<td>4.76</td>
<td>4.05</td>
<td>3.94</td>
<td>6.52</td>
<td>5.00</td>
<td>4.25</td>
<td>5.98</td>
</tr>
</tbody>
</table>

\(^a\) In equivalent glucose, \(^b\) In equivalent syringic acid, OMW: Olive Mill Wastewater, CW: Cheese Whey
3.3 **Biochemical Methane Potential**

Bio-methane potential (BMP) tests are widely used in many studies concerning the anaerobic digestion of organic solids. The BMP assay can be used as an index of the anaerobic biodegradation potential as it is the experimental value of the maximum quantity of methane produced per gram of VS. Initially, three agro-industrial wastes (OMW, CW and LCM) were selected in order to determine their biochemical methane potential through the BMP assay. The main characteristics of the studied substrates are shown in Table 3.8.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>OMW</th>
<th>CW</th>
<th>LCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-</td>
<td>5.36</td>
<td>6.32</td>
<td>8.82</td>
</tr>
<tr>
<td>TS</td>
<td>g/L</td>
<td>73.50</td>
<td>64.40</td>
<td>20.30</td>
</tr>
<tr>
<td>VS</td>
<td>g/L</td>
<td>55.97</td>
<td>55.72</td>
<td>12.47</td>
</tr>
<tr>
<td>Total COD</td>
<td>g/L</td>
<td>85.23</td>
<td>77.26</td>
<td>21.37</td>
</tr>
<tr>
<td>Soluble COD</td>
<td>g/L</td>
<td>69.35</td>
<td>43.18</td>
<td>12.16</td>
</tr>
<tr>
<td>Total carbohydrates&lt;sup&gt;a&lt;/sup&gt;</td>
<td>g/L</td>
<td>34.70</td>
<td>41.00</td>
<td>2.13</td>
</tr>
<tr>
<td>Soluble carbohydrates&lt;sup&gt;a&lt;/sup&gt;</td>
<td>g/L</td>
<td>24.97</td>
<td>33.75</td>
<td>0.59</td>
</tr>
<tr>
<td>Total phenols&lt;sup&gt;b&lt;/sup&gt;</td>
<td>g/L</td>
<td>7.71</td>
<td>N.D</td>
<td>N.D</td>
</tr>
<tr>
<td>Total nitrogen, TKN</td>
<td>g/L</td>
<td>0.42</td>
<td>0.95</td>
<td>2.52</td>
</tr>
<tr>
<td>Ammonium nitrogen</td>
<td>g/L</td>
<td>0.11</td>
<td>0.12</td>
<td>0.59</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>mg/L</td>
<td>234.55</td>
<td>229.30</td>
<td>126.50</td>
</tr>
<tr>
<td>Soluble phosphorus</td>
<td>mg/L</td>
<td>118.60</td>
<td>115.99</td>
<td>116.38</td>
</tr>
<tr>
<td>Oil and grease</td>
<td>g/L</td>
<td>9.06</td>
<td>0.66</td>
<td>0.71</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>g CaCO&lt;sub&gt;3&lt;/sub&gt;/L</td>
<td>0.85</td>
<td>0.58</td>
<td>6.40</td>
</tr>
<tr>
<td>Carbon</td>
<td>%</td>
<td>53.45</td>
<td>41.94</td>
<td>41.55</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>%</td>
<td>6.65</td>
<td>5.15</td>
<td>5.37</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>%</td>
<td>0.90</td>
<td>1.36</td>
<td>1.90</td>
</tr>
<tr>
<td>Sulfur</td>
<td>%</td>
<td>N.D</td>
<td>N.D</td>
<td>N.D</td>
</tr>
<tr>
<td>Ash</td>
<td>%</td>
<td>23.85</td>
<td>13.48</td>
<td>38.57</td>
</tr>
<tr>
<td>Oxygen</td>
<td>%</td>
<td>15.15</td>
<td>38.07</td>
<td>12.61</td>
</tr>
</tbody>
</table>

<sup>a</sup> In equivalent glucose; <sup>b</sup> In equivalent syringic acid

Fig. 3.1 depicts three distinctive biogas and methane production patterns from three different substrates during the course of BMP assay. Also the cumulative production, using only the inoculum (blank), was illustrated (Fig. 3.1(d)). Fig. 3.1(a) presents the cumulative production of olive mill wastewater (OMW). Fig. 3.1(b) shows the steep biogas and methane production pattern of cheese whey (CW) - a substrate mostly
composed of easily-degradable sugars (Table 3.8), which achieve its maximum potential in 50 days. Fig. 3.1(c) depicts the cumulative production of liquid cow manure (LCM), a slowly-degradable substrate due to its composition. The lignocellulosic matrix of LCM is primarily responsible for its low degradability. The calculated methane production of OMW, CW and LCM, after subtraction of the methane produced from the blank experiment, was 64.52, 107.01 and 51.29 mL of CH₄, respectively.

Figure 3-1: Cumulative biogas and methane production during the BMP assay from a) Olive Mill Wastewater (OMW), b) Cheese Whey (CW), c) Liquid cow Manure (LCM) and d) blank sample. Errors bars represent the standard deviation for the replicates.

Moreover, BMP tests were conducted to determine the suitability of the previous three substrates (OMW, CW and LCM) for co-digestion of them with different mix ratios. In particular, the two or three substrates were co-digested at different SOMW:S_LCM, S_CW:S_LCM, S_OMW:S_CW and S_OMW:S_LCM:S_LCM ratios ranging from 0% to 100% v/v at progressive variations of 25%. 

65
Fig. 3.2 illustrates the BMP production curves from co-digestion of OMW with LCM. A slow biogas and methane production rate was observed when the highest percent of OMW (S_{OMW}:75%) used. It appears to be highly inhibited, likely due to higher initial phenolics concentration in the mixture. During the BMP assay, the effluent phenolics concentration decreased, and removal efficiencies of 80.49%, 82.46% and 58.19% were obtained increasing the OMW fraction (from 25% to 75%), respectively. According to Gelegenis et al. (2007), co-digestion of OMW with other waste alleviates the effect of inhibitory factors. Angelidaki and Ahring (1997) and Marques et al. (1998) demonstrated that the lack of ammonia needed, as nitrogen source for synthesis of bacterial biomass and as an important pH buffer, could be responsible for the problems encountered when anaerobic degradation of OMW alone is attempted. They showed that the amount of nitrogen needed to obtain a stable degradation of OMW could be provided by cattle manure, swine manure or piggery effluent during co-degradation of OMW and manure.

**Figure 3-2:** Cumulative biogas and methane production during the BMP assay from mixtures with ratio a) S_{OMW}:S_{LCM}:75:25, b) S_{OMW}:S_{LCM}:50:50 and c) S_{OMW}:S_{LCM}:25:75. Errors bars represent the standard deviation for the replicates.
Co-digestion of CW with LCM was also carried out at three different mixture ratios (SCW:SLCM of 75:25, 50:50 and 25:75). The combination of SCW and SLCM resulted in high methanogenic performances (Fig. 3.3). A higher production rate was observed using the mixture with ratio SCW:SLCM=25:75. This higher rate could be explained by the high contribution of LCM in the mixture which is a substrate rich in nitrogen. In the co-digestion process with high biodegradable substrates, such as cheese whey, the ammonia contained in livestock manure could turn from a cause of inhibition (Chen et al., 2008) into a positive element for the biological process since the ammonia can supply the requested buffer capacity (Angelidaki and Ahring, 1993). Furthermore, several studies have demonstrated that the co-digestion of CW with LCM can maintain favourable pH and improve biogas production (Ghaly, 1996; Gelegenis et al., 2007). Manure is frequently applied in co-digestion with other wastes that are characterized by low nitrogen concentrations (Ward et al., 2008). Co-digestion of animal manure with various biomass substrates increases the biogas yield and offers a number of advantages for the management of manure and organic wastes (Holm-Nielsen et al., 2009).

Figure 3-3: Cumulative biogas and methane production during the BMP assay from mixtures with ratio a) SCW:SLCM-75:25, b) SCW:SLCM-50:50 and c) SCW:SLCM-25:75. Errors bars represent the standard deviation for the replicates.
Mixing and digesting OMW with other wastes offers several advantages such as: (a) reduction of feed COD and total phenols concentration, (b) no need to add nutrients (i.e. nitrogen and phosphorous) if OMW are mixed with wastes rich in nutrients and (c) the possibility of running a year-round treatment plant based on the co-digestion of seasonally generated effluents (Mantzavinos and Kalogerakis, 2005). The co-digestion of OMW with CW was also studied (S\textsubscript{OMW}:S\textsubscript{CW} of 75:25, 50:50 and 25:75) in order to assess the performance in terms of productivity and the role of co-digestion (Fig. 3.4). The highest production rate was observed using the mixture with ratio S\textsubscript{OMW}:S\textsubscript{CW}-75:25 (Fig. 3.4(a)) with the highest COD reduction (85.03%), whereas the phenols degradation (49.64%) was similar at three different ratios. A high COD reduction (93%) has been reported by Martinez-Garcia et al. (2007) co-digesting a mixture of OMW (75%) and CW (25%) in a continuous two-stage system.

**Figure 3-4:** Cumulative biogas and methane production during the BMP assay from mixtures with ratio a) S\textsubscript{OMW}:S\textsubscript{CW}-75:25, b) S\textsubscript{OMW}:S\textsubscript{CW}-50:50 and c) S\textsubscript{OMW}:S\textsubscript{CW}-25:75. Errors bars represent the standard deviation for the replicates.
Moreover, co-digestion of OMW with CW and LCM was also carried out at three different mixture ratios (S_{OMW}:S_{CW}:S_{LCM} of 50:25:25, 25:50:25 and 25:25:50). The combination of three wastes resulted in high methanogenic performances (Fig. 3.5) with high COD degradation. The maximum production was achieved in 30 days. The highest productivity was 93.76 mL using the mixture of 50% OMW with 25% CW and 25% LCM. Similar productivity was achieved (93.46 mL) using the mixture of 25% OMW with 25% CW and 50% LCM, whereas the COD degradation was slight higher (92.88%) using the mixture with the highest percent of CW (S_{OMW}:S_{CW}:S_{LCM} of 25:50:25) compared to the other mixtures. A significant increase of degradation was observed using mixtures consist of three wastewaters in comparison with mixtures of two wastes.

Figure 3-5: Cumulative biogas and methane production during the BMP assay from mixtures with ratio a) S_{OMW}:S_{CW}:S_{LCM}-50:25:25, b) S_{OMW}:S_{CW}:S_{LCM}-25:50:25 and c) S_{OMW}:S_{CW}:S_{LCM}-25:25:50. Errors bars represent the standard deviation for the replicates.
The final methane yields from the BMP experiments are shown in Table 3.9. Even without buffer addition, the pH remained neutral during the assays, with all treatments having no significant decreases in pH during digestion. Cumulative methane production was normalized by two ways: (1) by methane yield of each substrate using the initial VS (mL CH₄/g VS added), and (2) by volumetric methane production potential, using the volumetric loading of each substrate (mL CH₄/mL added), as the majority of the farmers and producers manage their systems on a volumetric basis but researchers normalize results using VS (Moody et al., 2011) or COD (Owen et al., 1979). The results were not normalized by COD, as Moody et al. (2011) recommended normalizing with VS for agricultural wastes due to the variability associated with COD concentrations of high-solids materials.

Table 3-9: Methane yield, as obtained from the BMP assay of each agro-industrial waste and co-digested mixtures.

<table>
<thead>
<tr>
<th>Substrate-Mixture</th>
<th>Methane yield (mL CH₄/g VS added)</th>
<th>Methane yield (mL CH₄/mL added)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S_OMW-100</td>
<td>345.03 ± 28.61</td>
<td>35.84 ± 2.97</td>
</tr>
<tr>
<td>S_CW-100</td>
<td>399.29 ± 35.63</td>
<td>36.38 ± 3.25</td>
</tr>
<tr>
<td>S_LCM-100</td>
<td>216.41 ± 30.12</td>
<td>19.66 ± 2.73</td>
</tr>
<tr>
<td>S_OMW:S_LCM-75:25</td>
<td>526.92 ± 53.73</td>
<td>31.47 ± 3.20</td>
</tr>
<tr>
<td>S_OMW:S_LCM-50:50</td>
<td>496.19 ± 63.00</td>
<td>39.70 ± 5.04</td>
</tr>
<tr>
<td>S_OMW:S_LCM-25:75</td>
<td>564.25 ± 51.34</td>
<td>31.85 ± 2.89</td>
</tr>
<tr>
<td>S_CW:S_LCM-75:25</td>
<td>498.88 ± 36.92</td>
<td>25.18 ± 1.86</td>
</tr>
<tr>
<td>S_CW:S_LCM-50:50</td>
<td>592.73 ± 110.27</td>
<td>23.71 ± 4.41</td>
</tr>
<tr>
<td>S_CW:S_LCM-25:75</td>
<td>424.11 ± 106.11</td>
<td>25.92 ± 6.48</td>
</tr>
<tr>
<td>S_OMW:S_CW-75:25</td>
<td>595.06 ± 66.20</td>
<td>51.29 ± 5.72</td>
</tr>
<tr>
<td>S_OMW:S_CW-50:50</td>
<td>504.69 ± 72.25</td>
<td>38.76 ± 5.54</td>
</tr>
<tr>
<td>S_OMW:S_CW-25:75</td>
<td>524.96 ± 38.27</td>
<td>31.18 ± 2.27</td>
</tr>
<tr>
<td>S_OMW:S_CW:S_LCM-50:25:25</td>
<td>578.75 ± 65.55</td>
<td>45.74 ± 5.18</td>
</tr>
<tr>
<td>S_OMW:S_CW:S_LCM-25:50:25</td>
<td>541.20 ± 27.80</td>
<td>34.02 ± 1.74</td>
</tr>
<tr>
<td>S_OMW:S_CW:S_LCM-25:25:50</td>
<td>467.30 ± 21.20</td>
<td>40.05 ± 1.82</td>
</tr>
</tbody>
</table>

A summary of the average methane yields of all substrates analyzed in this study is presented in Fig. 3.6. Considering mono-digestion, cheese whey (CW) produced the largest amount of methane (36.38 mL CH₄/mL substrate; 399.29 mL CH₄/g VS added), followed by the olive mill wastewater (OMW) (35.84 mL CH₄/mL substrate; 345.03 mL CH₄/g VS added) and liquid cow manure (LCM) (18.57 mL CH₄/mL substrate;
216.41 mL CH$_4$/g VS added) (Table 3.9-Fig. 3.6). Peak daily methane production values for all substrates were reached within the first 50 days. Then, the methane percent was steady, with no significant differences in the biogas. According to Labatut et al. (2011), substrates rich in lipids and easily-degradable carbohydrates yield the highest methane potential, while more recalcitrant substrates with a high lignocellulosic fraction have the lowest. Cow manure had a methane production potential ranging from 213 to 242 mL CH$_4$/g VS added, according to Moody et al. (2011), similar to the value reported in this study (216.41 mL CH$_4$/g VS added).

As you can see in Fig. 3.6, the co-digestion of these substrates improves the methane yield. The methane yields of the co-digested substrates was higher than expected based on the calculated combined value from the individual treatments, likely due to synergistic effects. Synergistic effects may arise from the contribution of additional alkalinity, trace elements, nutrients, enzymes, or any other amendment which a substrate by itself may lack, and could result in an increase in substrate biodegradability and therefore, biomethane potential (Labatut et al., 2011). Many studies have demonstrated that the biogas production rate can be enhanced 0.8–5.5 times when co-digesting domestic food waste with dairy manure compared to digesting dairy manure alone (Callaghan et al., 1999; Li et al., 2010). Labatut et al. (2011) compared 30 possible co-digestion substrates and concluded that substrates highly rich in lipids, carbohydrates, or proteins with high volatile solids content are good candidates for co-digestion.

![Figure 3-6: Methane yields at STP, as obtained from the BMP assay of three mono- and twelve co-digested substrates.](image-url)
From the co-digestion of CW with LCM, the mixture ratio of S_{CW}:S_{LCM}-50:50 presented the highest methane yield (592.73 mL CH\textsubscript{4}/g VS added). Bertin et al (2013) reported improvement of methane yield to 320 ± 9 mL CH\textsubscript{4}/g VS, of co-digestion of the same mixture ratio (S_{CW}:S_{LCM}-50:50), that is 2.5 the value obtained by cow manure and 27 times the value obtained by cheese whey when used alone. However, the methane production fell when the S_{CW} fraction was higher than 60% with simultaneous acidification. Moreover, Kavacik and Topaloglu (2010) obtained the highest productivity when treated 50% cheese whey with 50% dairy manure (diluted 1:1) and then suggested that co-digestion of them is more advantageous than processing each one separately. On the other hand, Kougiass et al. (2014) studied the influence of the waste ratio of OMW and swine manure in batch experiments and reported that the maximum methane production and VS removal occurred at 40% OMW in the mixture. Further increase in OMW fraction caused instability of the anaerobic process.

Moreover, a typical method for calculating theoretical methane yield (TMY) was applied based on elemental compositions of organic substrates (Table 3.8) using Buswell formula (Buswell and Mueller, 1952; Li et al., 2013) as shown in Eqs. (3.1) and (3.2). Anaerobic biodegradability (BD) of the substrate could be calculated based on the experimental methane yield (EMY) and theoretical methane yield (TMY) as follows (Elbeshbishy et al., 2012): BD = EMY/TMY. The methane production potential (EMY and TMY) and biodegradability of different substrates and mixtures calculated using elemental contents are shown in Table 3.10.

\[
C_nH_aO_bN_c + \left( n - \frac{a}{2} - \frac{b}{4} + \frac{3c}{4} \right) H_2O \\
\rightarrow \left( \frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8} \right) CH_4 + \left( \frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8} \right) CO_2 + cNH_3
\] (3.1)

\[
TMY \left( \frac{mL \, CH_4}{g \, VS} \right) = \frac{22.4 \times 1000 \times \left( \frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8} \right)}{12n + a + 16b + 14c}
\] (3.2)
Table 3-10: Methane production potential and biodegradability for each agro-industrial waste and co-digested mixtures.

<table>
<thead>
<tr>
<th>Substrate-Mixture</th>
<th>EMY (mL/g VS)</th>
<th>TMY (mL/g VS)</th>
<th>BD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S&lt;sub&gt;OMW&lt;/sub&gt;-100</td>
<td>345.03 ± 28.61</td>
<td>821.09</td>
<td>42.02</td>
</tr>
<tr>
<td>S&lt;sub&gt;CW&lt;/sub&gt;-100</td>
<td>399.29 ± 35.63</td>
<td>454.08</td>
<td>87.93</td>
</tr>
<tr>
<td>S&lt;sub&gt;LCM&lt;/sub&gt;-100</td>
<td>216.41 ± 30.12</td>
<td>783.42</td>
<td>27.62</td>
</tr>
<tr>
<td>S&lt;sub&gt;OMW&lt;/sub&gt;:S&lt;sub&gt;LCM&lt;/sub&gt;-75:25</td>
<td>526.92 ± 53.73</td>
<td>811.67</td>
<td>64.92</td>
</tr>
<tr>
<td>S&lt;sub&gt;OMW&lt;/sub&gt;:S&lt;sub&gt;LCM&lt;/sub&gt;-50:50</td>
<td>496.19 ± 63.00</td>
<td>802.25</td>
<td>61.85</td>
</tr>
<tr>
<td>S&lt;sub&gt;OMW&lt;/sub&gt;:S&lt;sub&gt;LCM&lt;/sub&gt;-25:75</td>
<td>564.25 ± 51.34</td>
<td>792.84</td>
<td>71.17</td>
</tr>
<tr>
<td>S&lt;sub&gt;CW&lt;/sub&gt;:S&lt;sub&gt;LCM&lt;/sub&gt;-75:25</td>
<td>498.88 ± 36.92</td>
<td>536.42</td>
<td>93.00</td>
</tr>
<tr>
<td>S&lt;sub&gt;CW&lt;/sub&gt;:S&lt;sub&gt;LCM&lt;/sub&gt;-50:50</td>
<td>592.73 ± 110.27</td>
<td>618.75</td>
<td>95.79</td>
</tr>
<tr>
<td>S&lt;sub&gt;CW&lt;/sub&gt;:S&lt;sub&gt;LCM&lt;/sub&gt;-25:75</td>
<td>424.11 ± 106.11</td>
<td>701.09</td>
<td>60.49</td>
</tr>
<tr>
<td>S&lt;sub&gt;OMW&lt;/sub&gt;:S&lt;sub&gt;CW&lt;/sub&gt;-75:25</td>
<td>595.06 ± 66.20</td>
<td>729.34</td>
<td>81.59</td>
</tr>
<tr>
<td>S&lt;sub&gt;OMW&lt;/sub&gt;:S&lt;sub&gt;CW&lt;/sub&gt;-50:50</td>
<td>504.69 ± 72.25</td>
<td>637.59</td>
<td>79.16</td>
</tr>
<tr>
<td>S&lt;sub&gt;OMW&lt;/sub&gt;:S&lt;sub&gt;CW&lt;/sub&gt;-25:75</td>
<td>524.96 ± 38.27</td>
<td>545.83</td>
<td>96.18</td>
</tr>
<tr>
<td>S&lt;sub&gt;OMW&lt;/sub&gt;:S&lt;sub&gt;CW&lt;/sub&gt;:S&lt;sub&gt;LCM&lt;/sub&gt;-50:25:25</td>
<td>578.75 ± 65.55</td>
<td>719.92</td>
<td>80.39</td>
</tr>
<tr>
<td>S&lt;sub&gt;OMW&lt;/sub&gt;:S&lt;sub&gt;CW&lt;/sub&gt;:S&lt;sub&gt;LCM&lt;/sub&gt;-25:50:25</td>
<td>541.20 ± 27.80</td>
<td>628.17</td>
<td>86.16</td>
</tr>
<tr>
<td>S&lt;sub&gt;OMW&lt;/sub&gt;:S&lt;sub&gt;CW&lt;/sub&gt;:S&lt;sub&gt;LCM&lt;/sub&gt;-25:25:50</td>
<td>467.30 ± 21.20</td>
<td>710.50</td>
<td>65.77</td>
</tr>
</tbody>
</table>

EMY: experimental methane yield; TMY: theoretical methane yield; BD: biodegradability estimated by using EMY divided TMY.

Using mono-substrates, the highest (87.93%) and lowest (27.62%) biodegradability could be obtained for CW and LCM, respectively. Feedstocks that contained high energy density components (such as lipids) and easily-degradable substrates (such as cheese whey) had higher methane production potential and biodegradability than lignocellulosic biomass with high content of fibers (such as manure). The highest (96.18%) biodegradability could be obtained for mixture of OMW and CW (S<sub>OMW</sub>:S<sub>CW</sub>-25:75). Furthermore, it is noticeable that the lower the substrate biodegradability is, the poorer the estimation is.

3.4 Bacterial growth model

The cumulative biomethane production profile from each batch experiment was fitted to a modified Gompertz bacterial growth model (Eq. 3.3) using OriginPro version 8. This equation has been widely used to model gas production data (Mohd Yasin et al., 2011; Kafle et al., 2013).
where \( M(t) \) is the cumulative methane production (mL); \( P \) is the maximum methane production potential (mL); \( R_m \) is the maximum methane production rate (mL/d); \( \lambda \) is the lag-phase duration (d); \( t \) is the time (d) and \( e \) is \( \exp(1) = 2.71828 \). At the same time, the standard error and the coefficient of determination or correlation coefficient (\( R^2 \)) were also obtained.

Comparing each set of experimental data with the relevant model simulation, the parameters of methane production potential (\( P \)), the maximum methane production rate (\( R_m \)) and lag-phase time (\( \lambda \)) were determined (Table 3.11). The correlation coefficient (\( R^2 \)) ranged between 0.915 and 0.995. The maximum rate (8.00 ± 2.22 mL/d) was obtained using the mixture of three wastes at a ratio \( \text{SOMW}:\text{SCW}:\text{SLCM}-25:50:25 \). Moreover the minimum lag-phase (2.62 ± 1.57 d) was observed using the mixture of \( \text{SOMW}:\text{SCW}-75:25 \), whereas the maximum (34.78 ± 1.70 d) was obtained using the OMW as a substrate. The high lag-phase may be occurred due to phenols concentration as inhibitory compounds.

Table 3-11: Kinetic parameters of methane production estimated using the modified Gompertz equation.

<table>
<thead>
<tr>
<th>Substrate-Mixture</th>
<th>( P ) (mL)</th>
<th>( R_m ) (mL/d)</th>
<th>( \lambda ) (d)</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>S\text{OMW}-100</td>
<td>65.30 ± 2.76</td>
<td>3.14 ± 0.48</td>
<td>34.78 ± 1.70</td>
<td>0.975</td>
</tr>
<tr>
<td>S\text{CW}-100</td>
<td>109.11 ± 2.11</td>
<td>2.09 ± 0.09</td>
<td>10.56 ± 1.08</td>
<td>0.995</td>
</tr>
<tr>
<td>S\text{LCM}-100</td>
<td>52.26 ± 1.46</td>
<td>6.09 ± 1.63</td>
<td>7.99 ± 1.25</td>
<td>0.959</td>
</tr>
<tr>
<td>S\text{OMW}:S\text{LCM}-75:25</td>
<td>62.31 ± 6.48</td>
<td>1.84 ± 0.39</td>
<td>11.11 ± 3.44</td>
<td>0.932</td>
</tr>
<tr>
<td>S\text{OMW}:S\text{LCM}-50:50</td>
<td>68.83 ± 1.59</td>
<td>7.51 ± 1.89</td>
<td>13.36 ± 1.16</td>
<td>0.963</td>
</tr>
<tr>
<td>S\text{OMW}:S\text{LCM}-25:75</td>
<td>65.61 ± 1.66</td>
<td>6.35 ± 1.51</td>
<td>8.28 ± 1.29</td>
<td>0.957</td>
</tr>
<tr>
<td>S\text{CW}:S\text{LCM}-75:25</td>
<td>75.66 ± 5.38</td>
<td>3.98 ± 1.02</td>
<td>20.98 ± 2.49</td>
<td>0.936</td>
</tr>
<tr>
<td>S\text{CW}:S\text{LCM}-50:50</td>
<td>68.35 ± 2.66</td>
<td>4.92 ± 1.38</td>
<td>9.37 ± 1.93</td>
<td>0.938</td>
</tr>
<tr>
<td>S\text{CW}:S\text{LCM}-25:75</td>
<td>53.59 ± 1.69</td>
<td>4.41 ± 1.02</td>
<td>10.62 ± 1.56</td>
<td>0.950</td>
</tr>
<tr>
<td>S\text{OMW}:S\text{CW}-75:25</td>
<td>87.38 ± 2.46</td>
<td>2.09 ± 0.16</td>
<td>2.62 ± 1.57</td>
<td>0.983</td>
</tr>
<tr>
<td>S\text{OMW}:S\text{CW}-50:50</td>
<td>81.02 ± 1.98</td>
<td>1.76 ± 0.10</td>
<td>9.81 ± 1.21</td>
<td>0.993</td>
</tr>
<tr>
<td>S\text{OMW}:S\text{CW}-25:75</td>
<td>80.90 ± 4.11</td>
<td>2.46 ± 0.28</td>
<td>23.05 ± 1.71</td>
<td>0.979</td>
</tr>
<tr>
<td>S\text{OMW}:S\text{CW}:S\text{LCM}-50:25:25</td>
<td>79.46 ± 1.74</td>
<td>5.27 ± 0.82</td>
<td>9.65 ± 1.34</td>
<td>0.967</td>
</tr>
<tr>
<td>S\text{OMW}:S\text{CW}:S\text{LCM}-25:50:25</td>
<td>72.86 ± 1.92</td>
<td>8.00 ± 2.22</td>
<td>17.81 ± 1.29</td>
<td>0.963</td>
</tr>
<tr>
<td>S\text{OMW}:S\text{CW}:S\text{LCM}-25:25:50</td>
<td>69.47 ± 2.28</td>
<td>6.19 ± 1.70</td>
<td>11.69 ± 1.80</td>
<td>0.915</td>
</tr>
</tbody>
</table>
3.5 References


Chapter 4.

Pretreatment of sweet sorghum stalks for enhancing biofuels production

4.1 Abstract

In this work, the effect of pretreatment on the carbohydrate solubilization (saccharification) and on the biochemical methane potential (BMP) of sweet sorghum was determined. Various pretreatment methods, such as (thermal)-chemical (through alkali (NaOH) or acid (HCl, H₂SO₄) addition at concentrations of 0.5 and 1.5% w/v) and enzymatic (through the addition of the enzymes CTec2 and HTec2) were tested. The experimental results showed that the acid pretreatment of fresh sorghum led to improvement of saccharification, whereas under thermal-alkaline pretreatment a significant decrease in soluble carbohydrates concentration was observed meaning that a high portion of sugars contained in sorghum biomass was degraded or was transformed into other components. On the other hand, alkali pretreatment of ensiled sorghum led to a significant increase of saccharification yield (540%). Moreover, enzymatic hydrolysis also showed improved results in terms of glucose yield (increase up to 200%) but lower than chemical pretreatment. Furthermore, ensiled sorghum was selected for pretreatment at two alkaline solutions (0.5%NaOH-0.5%KOH and 1%NaOH-1%KOH) at different temperatures and retention times in order to evaluate the effect of pretreatment on saccharification, chemical composition, physical structure and methane production. An increase of sugars yield by 352.7 and 561.1% was observed for the two different solutions at 80°C and contact time 2 h. A delignification of 36.9 and 42.3% was achieved for the two solutions, respectively. BMP experiments showed that the alkaline pretreatment enhance methane generation compared to the raw sorghum. Finally, laboratory ensiling tests were performed in order to study the conversion profile of polysaccharides to fermentable end-products (i.e. ethanol, lactic acid) during the ensiling process. Different varieties of sorghum led to different fermentable end-products through different performances.
4.2 Introduction

Biofuels generated globally from lignocelluloses are estimated at about 30 EJ/year, compared to the total energy used worldwide of over 400 EJ/year (McKendry, 2002). Biorefineries for production of several products and by-products such as biofuels, heat and/or electricity have been in focus in the recent years (Chen et al., 2005; Zhang, 2008). In a biorefinery, biomass can be converted to useable biomaterials and/or energy carriers in an integrated manner and thereby it can maximize the economic value of the biomass used while reducing the waste streams produced (Thomsen, 2005). Development of multiple biofuels based biorefinery from lignocellulose is seen as an important possibility to increase the efficiency for materials and energy, and reduce the costs of biomass options to mitigate greenhouse gas emissions (Sheehan et al., 2003).

Although the use of energy crops converted into biogas to produce renewable energy has become a controversial practice, as it may compete for land with food crops, energy crops may be sustainably cultivated on soils unsuitable for food production (Braun et al., 2010). Among energy crops, sorghum, with a world cultivated land of 40 million ha in 2009 (FAO, 2012) and with a hectare yield as high as 25 t (dry weight) per year, represents an interesting substrate for biofuels production (Sambusiti et al., 2012). Sorghum is a warm-season, short-day annual grass and it grows best under relatively high temperatures and under sunny conditions. It requires less water than corn, so it is likely to be grown as a replacement to corn and it produces better yields than corn in hotter and drier areas. Sweet sorghum can be fractionated via sugar extraction of the stalks to a liquid fraction, rich in fermentable sugars (sorghum extract or juice) and a solid fraction, rich in cellulose and hemicellulose (sorghum bagasse) (Billa et al., 1997; Antonopoulou et al., 2008). Moreover, ensiling method is suitable storage method to preserve fresh crops, such as sweet sorghum, throughout the year for, for example, biogas production.

The main challenge in using lignocellulosic crops, as sorghum, for biogas production, is their structure and composition. Crop biomasses mainly consist of cellulose, hemicellulose and lignin. It is well known that cellulose and hemicellulose (holocellulose) are degradable by anaerobic microorganisms; nevertheless, their association with lignin, which acts as a physical barrier, prevents their degradation (Tong et al., 1990). The physical structure and chemical composition of lignocellulosic materials can be altered through various methods of pretreatment, breaking down the linkage between polysaccharides and lignin thus making holocellulose more accessible to hydrolytic enzymes (Hendriks and Zeeman, 2009).

Various methods of pretreatment have been quite intensively investigated for facilitating the enzymatic hydrolysis and consequent ethanol production from lignocellulosic substrates (Sun and Cheng, 2002), but there is less information available on the effects of pretreating crop biomass for methane production (Hendriks and Zeeman, 2009). The pretreatment methods mainly include physical methods such as...
mechanical or thermal, chemical methods, biological methods and combination of these methods (Taherzadeh and Karimi, 2008; Hendriks and Zeeman, 2009).

Chemical pretreatment such as dilute acid treatment has been tested in sweet sorghum bagasse, causing an increase in hemicellulose hydrolysis rate and lignin solubilization. Acids such as sulphuric (Antonopoulou and Lyberatos, 2013), hydrochloric (Herrera et al., 2004) and phosphoric acid, respectively (Vázquez et al., 2007) have been used so far for the treatment of sorghum, achieving high hemicellulose degradation, depending on the treatment conditions. Furthermore, alkaline pretreatment (e.g. NaOH) which belongs to chemical methods is widely used for lignocellulose pretreatment (Antonopoulou and Lyberatos, 2013).

In the enzymatic hydrolysis of pretreated biomass, cellulase enzymes including cellulases and hemicellulases are used to catalyze the depolymerization of cellulose and hemicellulose. Cellulases are a mixture of three different cellulytic enzymes: 1,4-β-D-glucan glucanohydrolase which randomly attacks and cleaves the 1,4-β-D-glucan cellobiohydrolase which releases cellobiose from the nonreducing ends of a cellulosic substrate, and β-glucosidase which hydrolyzes cellobiose to glucose (Wang, 2009). In the hydrolysis of hemicellulose, three major enzymes including endo-β-1-4-xylanase which targets β-1-4 bonds between D-xylose residues of heteroxylans and xylo-oligosaccharides, exoxylanase which releases xylobiose, and β-xylosidase which hydrolyzes xylo-oligosaccharides to xylose are involved (Saha and Bothast, 1999). With the rapid development of enzyme technology, new generations of cellulase enzymes with higher activities and specificities have emerged, which would greatly improve the economic viability of lignocellulose-to-ethanol or biogas conversion.

It should be emphasized that a particular pretreatment type, while appropriate for a particular bioconversion (such as alcoholic fermentation), may be completely inappropriate for another (such as anaerobic digestion), due to the varying sensitivity of the key microbial species involved to the inhibitory intermediates that are generated. In this work, the whole plant of sweet sorghum biomass was used and pretreatment methods, such as chemical (alkali or acid addition) and enzymatic were applied. The effectiveness of each method for carbohydrates’ solubilization was evaluated. Moreover, laboratory ensiling tests were performed in order to evaluate the performance in terms of carbohydrates conversion to fermentable end-products. Finally, the effect of ensiling procedure and alkaline pretreatment on biochemical methane potential was tested.
4.3 **Chemical pretreatment of sweet sorghum stalks**

4.3.1 **Materials**

For the chemical pretreatment investigation two varieties of sorghum were used, fresh sorghum (FS1) and ensiled sorghum (ES1). Their characteristics in terms of cultivation and harvested and also the procedure of chopping and storage are described in Section 2.1.2-Table 2.1. The chemical composition of FS1 and ES1 sorghum, after drying and milling, is given in Table 4.1. Fresh sweet sorghum (FS1) mainly consisted of polysaccharides (22% cellulose, 12% hemicellulose). The total lignin content was 9% consisting mainly of acid-insoluble Klason lignin. The ash content of the total dry matter of sweet sorghum stalks was rather low (3.8%). During the ensiling procedure soluble carbohydrates were utilized by fermentative bacteria for the production of volatile fatty acids, lactic acid and ethanol. Thus, the amount of soluble carbohydrates of ensiled sweet sorghum is lower compared to the fresh one. On the contrary, its cellulose and hemicellulose content was measured to be 33% and 18%, respectively. The ash of ensiled sweet sorghum was higher compared to the fresh one.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>FS1</th>
<th>ES1</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.4 ± 0.01</td>
<td>4.5 ± 0.02</td>
</tr>
<tr>
<td>Moisture (% wet weight)</td>
<td>74.0 ± 0.12</td>
<td>77.0 ± 0.04</td>
</tr>
<tr>
<td>TS (% wet weight)</td>
<td>26.0 ± 0.03</td>
<td>23.0 ± 0.06</td>
</tr>
<tr>
<td>VS (%TS)</td>
<td>96.2 ± 0.51</td>
<td>94.7 ± 0.11</td>
</tr>
<tr>
<td>Ash (%TS)</td>
<td>3.8 ± 0.09</td>
<td>5.3 ± 0.05</td>
</tr>
<tr>
<td>TOC (%TS)</td>
<td>57.0 ± 0.05</td>
<td>52.0 ± 0.27</td>
</tr>
<tr>
<td>Total carbohydratesb (%TS)</td>
<td>58.0 ± 0.91</td>
<td>56.0 ± 0.57</td>
</tr>
<tr>
<td>Soluble carbohydratesb (%TS)</td>
<td>28.0 ± 0.30</td>
<td>2.5 ± 0.16</td>
</tr>
<tr>
<td>Cellulose (%TS)</td>
<td>22.0 ± 1.31</td>
<td>33.0 ± 0.33</td>
</tr>
<tr>
<td>Hemicellulose (%TS)</td>
<td>12.0 ± 1.55</td>
<td>18.0 ± 0.72</td>
</tr>
<tr>
<td>Lignin (%TS)</td>
<td>9.0 ± 2.01</td>
<td>15.0 ± 1.64</td>
</tr>
<tr>
<td>Total nitrogen, TKN (%TS)</td>
<td>0.2 ± 0.01</td>
<td>0.5 ± 0.02</td>
</tr>
<tr>
<td>Proteins (%TS)</td>
<td>1.2 ± 0.06</td>
<td>3.1 ± 0.13</td>
</tr>
</tbody>
</table>

*Mean values (± standard deviation); b In equivalent glucose*
4.3.2 Chemical pretreatment procedure

Two commonly used chemical pretreatment processes based on dilute acid and alkaline, were evaluated to provide comparative performance data. Three different solutions were used, namely hydrochloric acid, sulfuric acid and sodium hydroxide. Pretreatment tests were carried out in 100 mL flasks, closed with rubber septa (Picture 4.1(a)). In each flask, fresh and ensiled sweet sorghum samples were added in HCl, H2SO4 and NaOH solutions at different dosages (0.5 and 1.5%) with a total solid concentration of 10 g TS/L and varied temperatures. Pretreatment experiments were carried out at different temperatures i.e. 25, 37, 50, 80 and 100°C and contact periods of sweet sorghum in chemical solutions varied within hours to days (1 to 2 days at 25°C and 30 to 120 min at T≥37°C) depending on the temperature selected. All pretreatment experiments were conducted in triplicates with an average standard deviation less than 5%. During the experiments, samples were continuously agitated for complete mixing, by using an orbital shaking water bath (Grant OLS200) at constant 80 rpm (Picture 4.1(b)).

Picture 4-1: (a) Chemical pretreated FS1 with H2SO4 solution (1.5%) and (b) orbital shaking water bath for chemical pretreatment procedure.

4.3.3 Results and Discussion

The effect of acid pretreatment, as well as of alkaline pretreatment, on soluble carbohydrates’ concentration expressed in glucose equivalents (accompanied by their standard deviations) using the FS1 as a substrate, is shown in Fig. 4.1. Initially room temperature (25°C) was tested for 24 and 48 h, while higher solution temperature values were selected (37, 50, 80 and 100°C) for 30 and 120 min. As shown in Fig. 4.1, using FS1 as raw material, an increase in temperature (from 25°C to 80°C) led to a significant carbohydrates increase by soaking FS1 samples in HCL and H2SO4 solutions. On the contrary, a negative effect of the NaOH solution was observed on the carbohydrates solubilization at high temperature (80°C). Moreover, Fig. 4.3 shows the amount of soluble sugars after pretreatment at varied temperature conditions, using ES1 as raw material.
Figure 4-1: The effect of acid and alkaline pretreatment of fresh sweet sorghum biomass on soluble carbohydrates concentration expressed in glucose equivalents at (a) 25°C, (b) 37°C, (c) 50°C and (d) 80°C. Dotted lines depict the initial amount of soluble sugars in fresh sweet sorghum (FS1).

In particular, at 37°C for 120 min for acid and alkaline pretreatment the augmentative amount of soluble sugars in fresh was 25% and 18% (Fig. 4.1(b)), respectively, and in ensiled sweet sorghum 200% and 500% (Fig. 4.2(b)), respectively. At 50°C, the same trend of increase was observed for both lignocellulosic materials. For acid pretreatment of FS1 and ES1 50% and 210% increase in soluble sugars was determined, respectively. For the alkaline pretreatment of FS1 and ES1, the increase in the amount of soluble sugars was 15% and 500%. Acid pretreatment of FS1 with 0.5% H₂SO₄ and HCl (at 50°C) resulted in 28% and 22% increase of soluble sugars respectively for 30 min, whether for higher contact period (120 min) the increase was 46 and 50%, respectively (Fig. 4.1(c)). For ES1, a profound increase was measured at 140 and 180% for H₂SO₄ and HCl, respectively (Fig. 4.2(c)). The alkaline pretreatment of FS1 and ES1 with
0.5% NaOH solution resulted to 18% and 540% increase, respectively (Fig. 4.1(c)). However, no significant effect on the amount of soluble sugars was observed by increasing the contact duration from 30 to 120 min. At higher pretreatment temperatures (T≥ 50°C) soluble sugars of ES1 increased by 540% at 80°C (Fig. 4.2(d)) and 580% at 100°C (data not shown). However, the alkaline pretreatment of FS1 at high temperature (80°C) showed lower amount of soluble sugars (30% reduction). Possibly at high temperature values Maillard reaction between amino acids and reducing sugars from fresh sweet sorghum reacted resulting into decrease of soluble sugars in the solution (Pirt and Whelan, 1951; Jin et al., 2009; Vavouraki et al., 2013). Antonopoulou and Lyberatos (2013) observed a significant decrease in soluble carbohydrates concentration when fresh sorghum pretreated with alkaline solution of NaOH (0.5-2%) at high temperature (121°C).

**Figure 4-2:** The effect of acid and alkaline pretreatment of ensiled sweet sorghum biomass on soluble carbohydrates concentration expressed in glucose equivalents. Dotted lines depict the initial amount of soluble sugars in ensiled sweet sorghum (ES1).
Pretreatment of sweet sorghum stalks for enhancing biofuels production

The main soluble monomeric sugars of untreated FS1 determined using HPLC were 1160 mg glucose/L, 1270 mg fructose/L and 580 mg sucrose/L (Fig. 4.3(a)). Chemical pretreatment of sweet sorghum resulted in the degradation of lignocellulosic structure material into fermentable sugars (Mosier et al., 2005) while delignification of the material was succeeded by alkaline (NaOH) pretreatment (Panagiotopoulos et al., 2010). Acid pretreatment of FS1 at both 50 and 80°C produced same amounts of soluble monomeric sugars compared to the initial amounts of soluble sugars during chemical compositional analysis of the material. Since HPLC analysis was focused only on monosaccharides and low-degree polymerized glucose determination, possible degradation of lignocellulosic material to sugars with a high degree of polymerization (DP≥4) could not be identified. However, such a high degree polymerized glucose was measured after soluble sugar analysis (Joseffson, 1983), as shown in Fig. 4.1. Alkaline pretreatment showed lower amounts of soluble monomeric sugars compared to the initial analysis (with water). For the untreated ES1 (Fig. 4.3(b)) glucose and fructose was determined (50 and 40 mg/L, respectively). Acid pretreatment of ES1 at 50°C resulted to the same amount of glucose and fructose, compared to the initial one, whereas arabinose of 45 mg/L was also determined. At 80°C, the fructose content was increased up to 77 and 84 mg/L, for H2SO4 and HCl, respectively. For the same acid pretreatment, the arabinose content was increased up to 127 and 140 mg/L. Xylose was also determined (~95 mg/L). After the alkaline pretreatment of ES1, the glucose content was increased up to 143 and 180 mg/L at 50 and 80°C, respectively. Augmentation of fructose concentration was also measured (53 and 75 mg/L at 50 and 80°C, respectively). Sugars of higher degree of polymerisation (DP2) were also resolved (360 and 380 mg/L at 50 and 80°C, respectively).

Figure 4-3: Soluble sugar determination from untreated and pretreated (a) fresh sweet sorghum (FS1) and (b) ensiled sweet sorghum (ES1) with 0.5% H2SO4, HCl and NaOH at 50 and 80°C for 120 min.
Chemical pretreatment resulted in partial degradation of the lignocellulosic material. In particular, after alkaline pretreatment of FS1 small amount of xylose was analyzed. The highest amount of soluble sugars was analyzed using ES1 after NaOH pretreatment. After acid pretreatment of ES1, xylose and increased amounts of arabinose (compared to 50°C), were determined at 80°C. Further research on the optimization of chemical pretreatment is still in progress. Soluble fermentable sugars such as arabinose, xylose from hemicellulose degradation and glucose from cellulose fracture are important for further enzymatic or microbial hydrolysis as well as anaerobic digestion.

4.4 Enzymatic hydrolysis of sweet sorghum stalks

Now a day research is going on accelerating biogas production from various substrates using addition of enzymes. Considerable differences in feedstock fluidity were also observed by the application of various hydrolytic enzymes such as cellulase, hemicellulase, xylanase, pectinase, lipase, amylase, glucosidase and protease. Whilst the activity of enzymes depend on characteristics of the substrate, reaction time, quantity of enzymes and reaction temperature.

4.4.1 Materials

For the enzymatic hydrolysis investigation fresh sorghum (FS1) was used. The chemical composition of FS1 is given in Table 4.2. Fresh sweet sorghum (FS1) mainly consisted of polysaccharides (22% cellulose, 12% hemicellulose). The total lignin content was 9% consisting mainly of acid-insoluble Klason lignin. The ash content of the total dry matter of sweet sorghum stalks was rather low (3.8%).

<table>
<thead>
<tr>
<th>Parameters(^a)</th>
<th>FS1</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.4 ± 0.01</td>
</tr>
<tr>
<td>Moisture (% wet weight)</td>
<td>74.0 ± 0.12</td>
</tr>
<tr>
<td>TS (% wet weight)</td>
<td>26.0 ± 0.03</td>
</tr>
<tr>
<td>VS (%TS)</td>
<td>96.2 ± 0.51</td>
</tr>
<tr>
<td>Ash (%TS)</td>
<td>3.8 ± 0.09</td>
</tr>
<tr>
<td>TOC (%TS)</td>
<td>57.0 ± 0.05</td>
</tr>
<tr>
<td>Total carbohydrates(^b) (%TS)</td>
<td>58.0 ± 0.91</td>
</tr>
<tr>
<td>Soluble carbohydrates(^b) (%TS)</td>
<td>28.0 ± 0.30</td>
</tr>
<tr>
<td>Cellulose (%TS)</td>
<td>22.0 ± 1.31</td>
</tr>
<tr>
<td>Hemicellulose (%TS)</td>
<td>12.0 ± 1.55</td>
</tr>
<tr>
<td>Lignin (%TS)</td>
<td>9.0 ± 2.01</td>
</tr>
<tr>
<td>Total nitrogen, TKN (%TS)</td>
<td>0.2 ± 0.01</td>
</tr>
<tr>
<td>Proteins (%TS)</td>
<td>1.2 ± 0.06</td>
</tr>
</tbody>
</table>

\(^a\) Mean values (± standard deviation); \(^b\) In equivalent glucose
4.4.2 Enzymatic hydrolysis procedure

For the enzymatic hydrolysis of FS1, two types of commercial enzymes purchased from Novozymes were employed, i.e. Cellic® CTec2 (Batch No. VCN1002) and Cellic® HTec2 (Batch No. VHN00001). Cellic® CTec2 is a blend of aggressive cellulases, high level of β-glucosidases and hemicellulose, whereas Cellic® HTec2 has endoxylanase with high specificity toward soluble hemicellulose. CTec2 contains a unique protein complex that is known to improve the performance of the enzyme and reduce the dosage required to access and convert the cellulose into fermentable sugars. Using HTec2 alone or in combination with CTec2, your plant can further optimize pretreatment by reducing chemical use and leaving HTec2 to complete the hemicellulose hydrolysis to fermentable sugars.

The enzymatic hydrolysis of FS1 was performed with combined levels of enzyme dosage (1.5–30% g CTec2/g cellulose and 0.05–0.8% g HTec2/g cellulose) at different pH (4.5-6.0), temperature (40-60°C) and incubation time (12-96 h). The enzyme dosages were chosen according to application sheet of Novozymes (http://bioenergy.novozymes.com). From the Novozyme Company, the suggested enzyme trial dosage levels for initial investigation of a substrate were 1.5%, 3.0%, 6.0%, and 30.0% w/w (g enzyme/g cellulose), whereas it was advised to combine Cellic® CTec2 and HTec2 to boost the cellulose hydrolysis. Prior to enzymatic hydrolysis the pH was adjusted using NaOH or HCl. A programmable shaking water-bath (Grant OLS200) was used in all experimental tests.

The glucose yields ($Y_G$) presented in this work were calculated using the following equation:

$$\% Y_G = \left( \frac{G_p - G_i}{G_i} \right) \times 100$$

(4.1)

where $G_i$ and $G_p$ are used to indicate glucose concentration (mg glucose/ g dry FS1) alone, measured by HPLC. Subscripts ‘i’ and ‘p’ indicate initial and produced (after hydrolysis) values, respectively.

The experimental design and optimization of enzymatic hydrolysis of FS1 was performed using the GAME.opt software (Link and Weuster-Botz, 2006). The software is able to handle high dimensional variable spaces and unknown interactions of design variables. In particular, GAME.opt enables users without expert knowledge to minimize the experimental effort (small population sizes and few generations). Design variables were processed by genetic algorithm (GA) operators (crossover points = 2 and mutation rate = 1%) throughout four generations. The optimization of FS1 enzymatic hydrolysis was carried out at constant initial solids load 8% (1.6 g dry FS1 substrate to 20 mL of water) and was targeted (maximized) against the total concentration of soluble monosugars (glucose and xylose expressed as equivalent glucose per g dry FS1).
the obtained results from GAME reached a maximum level of soluble monosugars (at
the 4th generation) another objective function was used, namely the cost or profit from
sugars production at the lab-scale estimated at the proposed process conditions by
GAME, in order to differentiate similar results and draw the final conclusion. In more
detail, the cost objective function used for calculation of total cost or profit was
Eq. (4.2):

\[ TC (\text{in } €) = M_G \cdot P_G + M_X \cdot P_X - V_{CTec2} \cdot C_{CTec2} - V_{HTec2} \cdot C_{HTec2} - El \cdot C_{el} \quad (4.2) \]

where \( TC \) represents the total cost or profit (in €) from the enzymatic treatment. \( M_G \) and
\( M_X \) denote the mass (in g) of glucose and xylose, respectively, obtained at the end of
treatment, while \( P_G \) and \( P_X \) are their average market prices (in €/g) considered as 0.0454
€/g and 0.0711 €/g, respectively (obtained from Sigma-Aldrich). \( V_{CTec2} \) denotes the
volume of CTec2 (mL) and \( V_{HTec2} \) the volume of HTec2 (mL) used, while \( C_{CTec2} \) and
\( C_{HTec2} \) their respective cost. Because of the fact that the price of CTec2 and HTec2 was
not available commercially by Novozymes, estimation was obtained based on the prices
from other common enzymes (such as Cellulase from Trichoderma reesei, ATCC
26921) used in enzymatic hydrolysis of sorghum (Sipos et al., 2009; Antonopoulou and
Lyberatos, 2013). For example, a price of 2.11 €/mL (obtained from Sigma-Aldrich)
was used for CTec2 and HTec2. ‘El’ represents the estimated electrical energy
consumed (in KWh) for the process, while \( C_{el} \) is the cost of electricity (0.056 €/KWh).

4.4.3 Results and Discussion

Table 4.3 illustrates the results obtained from the implementation of GAME
generations of proposed tests. The optimized parameter was the obtained concentration
of total soluble monosugars (glucose and xylose as equivalent glucose per g dry FS1).
Xylose is the dominant component of hemicellulose, so production of xylose was
observed through enzymatic hydrolysis due to hemicellulose degradation. On the other
hand, increase of glucose concentration as a result of cellulose destruction was noticed.
Since the obtained results of total sugars were, more or less, in the same range another
objective function was also used, i.e. the cost of sugars production at the lab-scale,
based on the proposed process conditions, in order to draw the final conclusion. For
total cost estimation, all costs (enzymes, electricity, etc.) versus the obtained income
from the produced sugars (glucose and xylose) were taken into account. Thus, based on
maximization of sugars production with simultaneous maximum profit, the optimum
enzymatic hydrolysis of FS1 was achieved using 3% CTec2 and 0.8% HTec2 for
contact time 12 h, at temperature 50°C and pH 5.50. Optimum enzymatic hydrolysis
resulted, in terms of \( Y_G \), in an increase of total soluble monosugars (glucose and xylose)
by 179.93% compared to soluble glucose in raw FS1 (28 g glucose/100 g dry FS1).
### Table 4-3: Enzymatic hydrolysis of FS1 (Proposed tests by GAME).

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>CTec2 (%)</th>
<th>HTec2 (%)</th>
<th>pH</th>
<th>T (°C)</th>
<th>t (h)</th>
<th>Glucose (%)</th>
<th>Xylose (%)</th>
<th>Sugar&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>Y&lt;sub&gt;c&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; (%)</th>
<th>Cost/Profit&lt;sup&gt;c&lt;/sup&gt; (£)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3.00</td>
<td>0.80</td>
<td>5.5</td>
<td>50</td>
<td>12</td>
<td>43.4</td>
<td>33.0</td>
<td>78.38</td>
<td>179.93</td>
<td>+2.54</td>
</tr>
<tr>
<td>2.</td>
<td>5.00</td>
<td>0.25</td>
<td>4.5</td>
<td>50</td>
<td>48</td>
<td>19.4</td>
<td>16.2</td>
<td>36.57</td>
<td>30.61</td>
<td>-0.72</td>
</tr>
<tr>
<td>3.</td>
<td>5.00</td>
<td>0.65</td>
<td>6.0</td>
<td>50</td>
<td>60</td>
<td>35.3</td>
<td>33.2</td>
<td>70.49</td>
<td>151.76</td>
<td>+1.02</td>
</tr>
<tr>
<td>4.</td>
<td>2.00</td>
<td>0.80</td>
<td>5.5</td>
<td>50</td>
<td>60</td>
<td>31.9</td>
<td>31.5</td>
<td>65.29</td>
<td>133.18</td>
<td>+2.12</td>
</tr>
<tr>
<td>5.</td>
<td>3.00</td>
<td>0.80</td>
<td>5.5</td>
<td>60</td>
<td>60</td>
<td>36.4</td>
<td>38.7</td>
<td>77.42</td>
<td>176.51</td>
<td>+2.25</td>
</tr>
<tr>
<td>6.</td>
<td>2.00</td>
<td>0.40</td>
<td>4.5</td>
<td>50</td>
<td>72</td>
<td>29.9</td>
<td>31.2</td>
<td>62.97</td>
<td>124.90</td>
<td>+2.13</td>
</tr>
<tr>
<td>7.</td>
<td>4.50</td>
<td>0.15</td>
<td>4.5</td>
<td>50</td>
<td>60</td>
<td>28.2</td>
<td>20.8</td>
<td>54.91</td>
<td>96.11</td>
<td>+0.58</td>
</tr>
<tr>
<td>8.</td>
<td>4.50</td>
<td>0.75</td>
<td>5.5</td>
<td>50</td>
<td>12</td>
<td>24.5</td>
<td>20.2</td>
<td>45.91</td>
<td>63.97</td>
<td>+0.02</td>
</tr>
<tr>
<td>9.</td>
<td>1.50</td>
<td>0.80</td>
<td>5.5</td>
<td>50</td>
<td>48</td>
<td>35.0</td>
<td>31.5</td>
<td>68.39</td>
<td>144.25</td>
<td>+2.57</td>
</tr>
<tr>
<td>10.</td>
<td>4.50</td>
<td>0.15</td>
<td>5.5</td>
<td>60</td>
<td>12</td>
<td>30.2</td>
<td>22.4</td>
<td>53.94</td>
<td>92.66</td>
<td>+0.71</td>
</tr>
<tr>
<td>11.</td>
<td>5.00</td>
<td>0.65</td>
<td>6.0</td>
<td>40</td>
<td>60</td>
<td>20.2</td>
<td>12.4</td>
<td>33.34</td>
<td>19.09</td>
<td>-1.10</td>
</tr>
<tr>
<td>12.</td>
<td>4.50</td>
<td>0.40</td>
<td>5.5</td>
<td>60</td>
<td>24</td>
<td>33.8</td>
<td>25.3</td>
<td>60.62</td>
<td>116.49</td>
<td>+0.87</td>
</tr>
<tr>
<td>13.</td>
<td>2.00</td>
<td>0.10</td>
<td>4.5</td>
<td>50</td>
<td>12</td>
<td>27.8</td>
<td>24.7</td>
<td>53.98</td>
<td>92.79</td>
<td>+2.04</td>
</tr>
<tr>
<td>14.</td>
<td>1.50</td>
<td>0.80</td>
<td>5.5</td>
<td>50</td>
<td>24</td>
<td>31.9</td>
<td>24.9</td>
<td>58.29</td>
<td>108.19</td>
<td>+2.07</td>
</tr>
<tr>
<td>15.</td>
<td>4.50</td>
<td>0.15</td>
<td>5.5</td>
<td>50</td>
<td>24</td>
<td>23.7</td>
<td>18.8</td>
<td>43.63</td>
<td>55.81</td>
<td>+0.10</td>
</tr>
</tbody>
</table>
Table 4-4: Continue

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>CTeC2 (%)</th>
<th>HTeC2 (%)</th>
<th>pH</th>
<th>T (°C)</th>
<th>t (h)</th>
<th>Glucose (%)</th>
<th>Xylose (%)</th>
<th>Sugars^a (%)</th>
<th>Yc^b (%)</th>
<th>Cost/Profit^c (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.</td>
<td>3.00</td>
<td>0.35</td>
<td>4.5</td>
<td>50</td>
<td>72</td>
<td>34.7</td>
<td>30.1</td>
<td>66.61</td>
<td>137.88</td>
<td>+1.81</td>
</tr>
<tr>
<td>17.</td>
<td>4.50</td>
<td>0.75</td>
<td>6.0</td>
<td>50</td>
<td>60</td>
<td>30.3</td>
<td>25.6</td>
<td>57.44</td>
<td>105.13</td>
<td>+0.42</td>
</tr>
<tr>
<td>18.</td>
<td>15.50</td>
<td>0.10</td>
<td>5.5</td>
<td>60</td>
<td>60</td>
<td>41.8</td>
<td>39.9</td>
<td>84.09</td>
<td>200.34</td>
<td>-3.14</td>
</tr>
<tr>
<td>19.</td>
<td>12.00</td>
<td>0.80</td>
<td>5.0</td>
<td>60</td>
<td>84</td>
<td>52.0</td>
<td>23.2</td>
<td>76.59</td>
<td>173.54</td>
<td>-2.77</td>
</tr>
<tr>
<td>20.</td>
<td>15.50</td>
<td>0.30</td>
<td>4.5</td>
<td>50</td>
<td>60</td>
<td>30.0</td>
<td>28.4</td>
<td>60.10</td>
<td>114.66</td>
<td>-4.51</td>
</tr>
<tr>
<td>21.</td>
<td>15.50</td>
<td>0.10</td>
<td>4.5</td>
<td>50</td>
<td>24</td>
<td>32.1</td>
<td>31.0</td>
<td>64.96</td>
<td>132.00</td>
<td>-3.91</td>
</tr>
<tr>
<td>22.</td>
<td>17.00</td>
<td>0.20</td>
<td>4.5</td>
<td>50</td>
<td>48</td>
<td>37.9</td>
<td>37.0</td>
<td>77.12</td>
<td>175.43</td>
<td>-4.11</td>
</tr>
<tr>
<td>23.</td>
<td>18.00</td>
<td>0.15</td>
<td>5.5</td>
<td>60</td>
<td>84</td>
<td>34.9</td>
<td>35.0</td>
<td>72.00</td>
<td>157.14</td>
<td>-5.26</td>
</tr>
<tr>
<td>24.</td>
<td>15.50</td>
<td>0.10</td>
<td>5.5</td>
<td>50</td>
<td>12</td>
<td>29.6</td>
<td>22.0</td>
<td>52.92</td>
<td>89.00</td>
<td>-4.64</td>
</tr>
<tr>
<td>25.</td>
<td>13.00</td>
<td>0.10</td>
<td>4.5</td>
<td>50</td>
<td>24</td>
<td>34.7</td>
<td>27.5</td>
<td>63.85</td>
<td>128.04</td>
<td>-2.85</td>
</tr>
<tr>
<td>26.</td>
<td>15.50</td>
<td>0.15</td>
<td>5.5</td>
<td>60</td>
<td>12</td>
<td>29.9</td>
<td>20.7</td>
<td>51.84</td>
<td>85.15</td>
<td>-4.77</td>
</tr>
<tr>
<td>27.</td>
<td>14.50</td>
<td>0.10</td>
<td>5.5</td>
<td>50</td>
<td>84</td>
<td>36.7</td>
<td>29.6</td>
<td>68.08</td>
<td>143.13</td>
<td>-3.67</td>
</tr>
<tr>
<td>28.</td>
<td>26.50</td>
<td>0.75</td>
<td>4.5</td>
<td>50</td>
<td>36</td>
<td>29.2</td>
<td>20.8</td>
<td>51.25</td>
<td>83.03</td>
<td>-10.54</td>
</tr>
<tr>
<td>29.</td>
<td>28.00</td>
<td>0.65</td>
<td>5.5</td>
<td>50</td>
<td>60</td>
<td>38.4</td>
<td>35.0</td>
<td>75.50</td>
<td>169.64</td>
<td>-9.86</td>
</tr>
</tbody>
</table>

^a: soluble monosugars (mg eq. glucose/100 g dry FS1) measured in HPLC;
^b: Yield of glucose equivalents estimated according to Eq. (4.1);
^c: Total cost estimated according to Eq. (4.2) (+, profit; -, cost)
Saini et al. (2013) determined the optimum conditions (substrate concentration 6%, w/v, time 48 h and enzyme loading of 22 FPU/g substrate) for enzymatic saccharification of sweet sorghum bagasse, whereas maximum saccharification yield of 51.21% was achieved. Moreover, Chen et al. (2009) studied the enzymatic digestibility of NaOH-pretreated corn stover and reached hydrolysis yield of 81.2% by 48 h at 8.0% substrate concentration and cellulase dosage of 20 FPU/g substrate.

Based on total cost calculations and sugars production, a correlation diagram was constructed using the results from all enzymatic tests (Fig. 4.4). As shown in Fig. 4.4, three different trendlines were observed, generated from three groups of CTec2 concentration namely 1.5% - 5%, 12% - 18% and 26.5% - 28%, respectively. Using low CTec2 concentration (1.5% - 5%) a trendline of $TC = 0.07695*S_p - 3.34531$ was obtained, where $TC$ presents the total cost or profit (in €) and $S_p$ presents the total sugars produced from the enzymatic hydrolysis. As you can see, increasing the concentration of CTec2 (12% - 18%), a decrease of total cost/profit was noticed due to enzyme cost according to the trendline $TC = 0.03959*S_p - 6.62141$. Moreover, higher concentration (26.5% - 28%) led to negative values of cost ($TC = 0.0277*S_p - 11.95575$). Regarding HTec2, it was independent in the concentration range (0.1% - 0.8%) was used in these proposed tests. Taking into account the aforementioned results, a satisfied sugars production can be achieved using low concentrations of enzymes. Further increase of enzymes concentration led to a high-cost process without equivalent increase of sugars.

Generally speaking, the majority of hydrolysate of hemicellulose in lignocellulosic waste is xylose, and there minor amounts of arabinose in the hydrolyzate of the bagasse (Taherzadeh and Karimi, 2008). The use of hydrolysate for biofuels production will take place with a mixed culture of microorganisms or genetically modified ones capable of using both C6-C5 sugars (e.g. Lisha and Sarkar, 2014).
4.5 Thermal-alkaline pretreatment of ensiled sorghum stalks for biogas production

The strategy proposed in this study consisted of thermal-alkaline hydrolysis of ensiled sorghum in order to prepare the material for the follow-up anaerobic digestion. The aim of this study was to hydrolyze the ensiled sorghum targeting to carbohydrates’ solubilization and removal of lignin that hinders the access of enzymes to cellulose, thus increasing significantly the porosity of substrate and facilitating its subsequent biochemical conversion to fermentable sugars.

The selection of alkaline treatment compared to acid one was based on previous results on chemical pretreatment of ES1 (Section 4.3.3). Moreover, it is well known that acid hydrolysis of hemicellulose is accompanied by production of inhibitory compounds for biogas production such as furfural and hydroxymethyl furfural (HMF) (Antonopoulou and Lyberatos, 2013). Moreover, alkaline pretreatments resulted efficient in altering the structure of lignin, solubilizing hemicellulose fraction and increasing efficiently the accessibility of cellulose by a swelling and a partial decrystallization of cellulose (McIntosh and Vancov, 2010). Alkali hydrolysis is a well-known method for the decomposition of lignin (Mosier et al., 2005) which can also be used for hemicellulose degradation. Numerous studies have also evaluated the use of alkaline pretreatment prior to enzymatic hydrolysis in order to increase further the sugar yields (Chen et al., 2009; McIntosh and Vancov, 2010). Although some studies suggested that only a combination of chemical pretreatment and enzymatic hydrolysis can enhance biofuels production (Michalska and Ledakowicz, 2013; Monlau et al., 2013), other research (Sambusiti et al., 2013a) has shown that alkaline pretreatment alone led to positive results, in terms of biogas production, without further step of enzymatic hydrolysis. Enzymatic saccharification following pretreatment is required for bioethanol production, but usually is not needed for anaerobic digestion because anaerobic microbes in the digester (e.g. Clostridium sp.) have their own hydrolytic enzyme system (Zheng et al., 2014). Moreover, the effect of enzymes in enhancing biogas production was minimal, and the cost of enzymes was high, whereas the retention time is relatively high compared to chemical pretreatments (Zheng et al., 2014). For these reasons, in the present study only a thermal-alkaline hydrolysis was selected, prior to anaerobic digestion.

4.5.1 Materials

For the thermal-chemical (alkaline) pretreatment investigation ensiled sorghum (ES3) was used. Its characteristics in terms of cultivation and harvesting and also the procedure of chopping and storage are described in Section 2.1.2-Table 2.1. The chemical composition of ES3 sorghum, after drying and milling, is given in Table 4.4. The ensiled sorghum (ES3) mainly consisted of polysaccharides (37.60% cellulose, 25.51% hemicellulose), whereas its total lignin content was 17.28%. These results are in
accordance with the typical composition of sweet sorghum reported by Panagiotopoulos et al. (2010). The ash content of the total dry matter of ensiled sorghum stalks was rather low (5.93%). During the ensiling procedure soluble carbohydrates are utilized by fermentative bacteria for the production of volatile fatty acids, lactic acid and ethanol, depending on the followed metabolic pathway. Thus, the amount of soluble carbohydrates of ES3 is low in contrast to the increased content of lactic acid.

Table 4-5: Chemical composition of ensiled (ES3) sweet sorghum.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ES3</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.10 ± 0.00</td>
</tr>
<tr>
<td>Moisture (% wet weight)</td>
<td>76.32 ± 0.10</td>
</tr>
<tr>
<td>TS (% wet weight)</td>
<td>23.73 ± 0.17</td>
</tr>
<tr>
<td>VS (%TS)</td>
<td>94.08 ± 3.15</td>
</tr>
<tr>
<td>Ash (%TS)</td>
<td>5.93 ± 3.15</td>
</tr>
<tr>
<td>TOC (%TS)</td>
<td>46.18 ± 0.00</td>
</tr>
<tr>
<td>Total carbohydratesb (%TS)</td>
<td>38.82 ± 1.29</td>
</tr>
<tr>
<td>Soluble carbohydratesb (%TS)</td>
<td>2.02 ± 0.06</td>
</tr>
<tr>
<td>Cellulose (%TS)</td>
<td>37.60 ± 5.37</td>
</tr>
<tr>
<td>Hemicellulose (%TS)</td>
<td>25.51 ± 3.66</td>
</tr>
<tr>
<td>Lignin (%TS)</td>
<td>24.38 ± 1.93</td>
</tr>
<tr>
<td>Total nitrogen, TKN (%TS)</td>
<td>0.96 ± 0.45</td>
</tr>
<tr>
<td>Proteins (%TS)</td>
<td>6.00 ± 2.81</td>
</tr>
<tr>
<td>Lactic acid (%TS)</td>
<td>4.28 ± 0.00</td>
</tr>
</tbody>
</table>

*a Mean values (± standard deviation); b In equivalent glucose

### 4.5.2 Pretreatment procedure

For alkaline pretreatment of ES3 the experimental parameters were the concentration of alkaline solution (0.5%NaOH-0.5%KOH and 1%NaOH-1%KOH), the temperature of treatment (25-80°C) and the treatment residence time (0.5-2 h). The concentration of alkaline solutions were chosen so that the concentrations of sodium and potassium in later mixtures for anaerobic digestion remained lower than 5.5 g Na⁺/L and 0.15 M K⁺, respectively, in order to avoid irrecoverable inhibition to methanogens (Chen et al., 2008). No higher temperature range was examined in order to avoid any traces of furfural compounds which are inhibitor compounds for the methane production process. Nevertheless, contrary to thermal and thermal-acid pretreatments, thermo-alkaline pretreatments are less responsible of furfural and 5-hydroxymethylfurfural (5-HMF) generation (Sambusiti et al., 2013b). Moreover, pretreatment conditions for anaerobic digestion could be milder than those for bioethanol production (Zheng et al., 2014).

Before the experiments, control experiments were performed (water instead of alkaline solution) at all different temperatures and residence times in order to evaluate
Pretreatment of sweet sorghum stalks for enhancing biofuels production

Chapter 4

93

the influence of thermal treatment in ES3 hydrolysis. All experiments were carried out at constant initial solids load 8% (3.2 g dry ES3 substrate to 40 mL of liquid alkaline solution). A programmable shaking water-bath (Grant OLS200) was used in all experimental tests.

The solubilization yields \(Y_G\) presented in this work were calculated using the following equation:

\[
\% Y_G = \left( \frac{S_p - S_i}{S_i} \right) \times 100
\]  

(4.3)

where \(S_i\) and \(S_p\) are used to indicate total soluble sugars (in equivalent glucose) concentration, measured using L-tryptophan. Subscripts ‘i’ and ‘p’ indicate initial and produced (after treatment) values, respectively.

4.5.3 Results and Discussion

Thermo-chemical pretreatment of the substrate aimed at increasing the content of soluble sugars of the sample in order to facilitate its subsequent anaerobic digestion. First of all, the impact of thermal treatment on soluble sugars production was tested. Table 4.5 shows the soluble carbohydrates production and also the solubilisation yield after increasing the temperature and contact time of treatment. An increase of solubilisation by 38.30% was observed when the ensiled sorghum (ES3) heat-treated at 80°C for 2 h. After that, thermal-alkaline pretreatment was tested using two different alkaline solutions (0.5%NaOH-0.5%KOH and 1%NaOH-1%KOH) at different temperature values and contact time.

Detailed results on the obtained soluble sugars, using as alkaline solution the mixture of 0.5%NaOH-0.5%KOH, are presented in Table 4.6. Treatment at 80°C exhibited the highest concentration of soluble carbohydrates. In particular, chemical pretreatment with 0.5%NaOH-0.5%KOH at 80°C for 2 h resulted in \(9.13 \pm 0.14\) g soluble carbohydrates/100 g dry ES3. A significant impact of temperature and contact time on solubilisation was observed (Table 4.6, Fig. 4.5(a)). A significant increase of sugar yield by 352.74% was observed, at 80°C and residence time 2 h. Moreover, Table 4.7 represents the obtained soluble carbohydrates after the alkaline pretreatment with the mixture of 1%NaOH-1%KOH. Treatment at 80°C exhibited the highest concentration of soluble carbohydrates (\(13.33 \pm 0.11\) g soluble carbohydrates/100 g dry ES3). However, the effect of temperature and contact time was negligible (Table 4.7, Fig. 4.5(b)) compared to the treatment with 0.5%NaOH-0.5%KOH as alkaline solution. The sugar yield obtained at 80°C and residence time 2 h was higher and equal to 561.13%, comparing with the initial soluble carbohydrates of the sorghum. An increase in alkaline solution dosage (from 0.5% to 1%) led to an increase of sugars solubilisation, whereas sugars yields were found to increase significantly with treatment.
temperature (Tables 4.6 and 4.7). The temperature appeared to have the greatest impact, particularly at 121°C, on cellulose and hemicellulose solubilisation of sorghum bicolor straw using dilute sulphuric acid pretreatment, as reported by Vancov and McIntosh (2012).

Table 4-6: Thermal treatment of ES3 (control experiments).

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>T (°C)</th>
<th>t (h)</th>
<th>Soluble carbohydrates&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>Y&lt;sub&gt;G&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>25</td>
<td>0.5</td>
<td>2.02 ± 0.06</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>25</td>
<td>1</td>
<td>2.18 ± 0.00</td>
<td>7.91 ± 0.00</td>
</tr>
<tr>
<td>3.</td>
<td>25</td>
<td>2</td>
<td>2.14 ± 0.08</td>
<td>6.36 ± 0.23</td>
</tr>
<tr>
<td>4.</td>
<td>37</td>
<td>0.5</td>
<td>2.05 ± 0.01</td>
<td>2.02 ± 0.01</td>
</tr>
<tr>
<td>5.</td>
<td>37</td>
<td>1</td>
<td>2.20 ± 0.04</td>
<td>9.15 ± 0.15</td>
</tr>
<tr>
<td>6.</td>
<td>37</td>
<td>2</td>
<td>2.22 ± 0.01</td>
<td>10.08 ± 0.04</td>
</tr>
<tr>
<td>7.</td>
<td>50</td>
<td>0.5</td>
<td>2.78 ± 0.09</td>
<td>14.74 ± 0.56</td>
</tr>
<tr>
<td>8.</td>
<td>50</td>
<td>1</td>
<td>2.48 ± 0.04</td>
<td>22.80 ± 0.33</td>
</tr>
<tr>
<td>9.</td>
<td>50</td>
<td>2</td>
<td>2.68 ± 0.13</td>
<td>33.03 ± 1.63</td>
</tr>
<tr>
<td>10.</td>
<td>80</td>
<td>0.5</td>
<td>2.74 ± 0.17</td>
<td>36.13 ± 2.21</td>
</tr>
<tr>
<td>11.</td>
<td>80</td>
<td>1</td>
<td>2.73 ± 0.15</td>
<td>35.51 ± 1.95</td>
</tr>
<tr>
<td>12.</td>
<td>80</td>
<td>2</td>
<td>2.79 ± 0.11</td>
<td>38.30 ± 1.45</td>
</tr>
</tbody>
</table>

<sup>a</sup>: soluble carbohydrates (g eq. glucose/100 g dry ES3) measured using L-tryptophan;
<sup>b</sup>: Yield of glucose equivalents estimated according to Eq. (4.3)

Table 4-7: Thermal-alkaline pretreatment of ES3 using 0.5%NaOH-0.5%KOH as alkaline solution.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>T (°C)</th>
<th>t (h)</th>
<th>Soluble carbohydrates&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>Y&lt;sub&gt;G&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>25</td>
<td>0.5</td>
<td>5.39 ± 0.08</td>
<td>167.61 ± 1.47</td>
</tr>
<tr>
<td>2.</td>
<td>25</td>
<td>1</td>
<td>5.50 ± 0.02</td>
<td>172.89 ± 0.56</td>
</tr>
<tr>
<td>3.</td>
<td>25</td>
<td>2</td>
<td>5.42 ± 0.08</td>
<td>168.85 ± 2.48</td>
</tr>
<tr>
<td>4.</td>
<td>37</td>
<td>0.5</td>
<td>5.44 ± 0.09</td>
<td>169.78 ± 2.76</td>
</tr>
<tr>
<td>5.</td>
<td>37</td>
<td>1</td>
<td>5.57 ± 0.13</td>
<td>176.30 ± 4.20</td>
</tr>
<tr>
<td>6.</td>
<td>37</td>
<td>2</td>
<td>6.50 ± 0.07</td>
<td>222.50 ± 2.42</td>
</tr>
<tr>
<td>7.</td>
<td>50</td>
<td>0.5</td>
<td>6.31 ± 0.02</td>
<td>213.20 ± 0.60</td>
</tr>
<tr>
<td>8.</td>
<td>50</td>
<td>1</td>
<td>6.33 ± 0.04</td>
<td>213.82 ± 1.20</td>
</tr>
<tr>
<td>9.</td>
<td>50</td>
<td>2</td>
<td>7.66 ± 0.37</td>
<td>280.18 ± 13.57</td>
</tr>
<tr>
<td>10.</td>
<td>80</td>
<td>0.5</td>
<td>8.16 ± 0.02</td>
<td>304.99 ± 0.66</td>
</tr>
<tr>
<td>11.</td>
<td>80</td>
<td>1</td>
<td>8.85 ± 0.11</td>
<td>339.10 ± 4.06</td>
</tr>
<tr>
<td>12.</td>
<td>80</td>
<td>2</td>
<td>9.13 ± 0.14</td>
<td>352.74 ± 5.47</td>
</tr>
</tbody>
</table>

<sup>a</sup>: soluble carbohydrates (g eq. glucose/100 g dry ES3) measured using L-tryptophan;
<sup>b</sup>: Yield of glucose equivalents estimated according to Eq. (4.3)
Pretreatment of sweet sorghum stalks for enhancing biofuels production

Figure 4-5: Soluble carbohydrates determination after thermal-alkaline treatment of ES3 with (a) 0.5%NaOH-0.5%KOH and (b) 1%NaOH-1%KOH at different temperatures and residence times.

Table 4-8: Thermal-alkaline pretreatment of ES3 using 1%NaOH-1%KOH as alkaline solution.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>T (°C)</th>
<th>t (h)</th>
<th>Soluble carbohydrates (%</th>
<th>YG (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>25</td>
<td>0.5</td>
<td>10.30 ± 0.46</td>
<td>411.04 ± 18.34</td>
</tr>
<tr>
<td>2.</td>
<td>25</td>
<td>1</td>
<td>11.23 ± 0.18</td>
<td>456.93 ± 7.19</td>
</tr>
<tr>
<td>3.</td>
<td>25</td>
<td>2</td>
<td>12.00 ± 0.11</td>
<td>495.39 ± 4.38</td>
</tr>
<tr>
<td>4.</td>
<td>37</td>
<td>0.5</td>
<td>10.66 ± 0.30</td>
<td>429.03 ± 12.09</td>
</tr>
<tr>
<td>5.</td>
<td>37</td>
<td>1</td>
<td>11.25 ± 0.32</td>
<td>458.17 ± 12.96</td>
</tr>
<tr>
<td>6.</td>
<td>37</td>
<td>2</td>
<td>12.09 ± 0.09</td>
<td>499.73 ± 3.65</td>
</tr>
<tr>
<td>7.</td>
<td>50</td>
<td>0.5</td>
<td>11.60 ± 0.28</td>
<td>475.54 ± 11.59</td>
</tr>
<tr>
<td>8.</td>
<td>50</td>
<td>1</td>
<td>11.93 ± 0.18</td>
<td>491.91 ± 7.29</td>
</tr>
<tr>
<td>9.</td>
<td>50</td>
<td>2</td>
<td>12.60 ± 0.28</td>
<td>525.16 ± 11.79</td>
</tr>
<tr>
<td>10.</td>
<td>80</td>
<td>0.5</td>
<td>12.65 ± 0.21</td>
<td>527.64 ± 8.85</td>
</tr>
<tr>
<td>11.</td>
<td>80</td>
<td>1</td>
<td>12.93 ± 0.11</td>
<td>541.28 ± 4.44</td>
</tr>
<tr>
<td>12.</td>
<td>80</td>
<td>2</td>
<td>13.33 ± 0.11</td>
<td>561.13 ± 4.47</td>
</tr>
</tbody>
</table>

a: soluble carbohydrates (g eq. glucose/100 g dry ES3) measured using L-tryptophan;
b: Yield of glucose equivalents estimated according to Eq. (4.3)

The aim of pretreatments was also to change raw material properties, remove or dissolve lignin and hemicellulose and reduce the crystallinity of cellulose. Dilute alkaline pretreatment of lignocellulose materials has been found to cause swelling, leading to an increase in internal surface area of the sweet sorghum bagasse and disruption of the lignin structure. Monlau et al. (2013), for example, observed an
increase of the accessible surface area from 1.55 to 2.55 m²/g TS by pretreating sunflower stalks at 170°C with 4% NaOH (w/w) for 1 h. Similarly, Gharpuray et al. (1983) reported an increase of the accessible surface area of wheat straw by 166% (from 0.64 to 1.7 m²/g TS) after pretreatment at 100°C with 10% NaOH (w/w) for 30 min.

Lignin-carbohydrate complexes remain the main obstacle for the use of lignocellulosic biomass. Their cleavage makes holocelluloses more accessible for enzymatic hydrolysis and microbial fermentation. Alkali hydrolysis is a well-known method for the decomposition of lignin (Mosier et al., 2005) which can also be used for hemicellulose degradation. As a result of that, the cellulose, which is the main source of saccharides, becomes more available to the enzymatic action and could be transformed into glucose—the monosaccharide most readily metabolized by fermentative microorganisms. Generally, decreased lignin content also leads to increased biogas yield. Fernandes et al. (2009) and Liew et al. (2012) indicated that the biodegradability of lignocellulosic biomass increased with decreasing lignin content, i.e., the higher the lignin content, the lower the biogas production.

As it could be seen from Figs. 4.6(a) and (b), high lignin removals of 36.87% and 42.33% were observed after thermo-alkaline pretreatment at 80°C and contact time 2 h with 0.5%NaOH-0.5%KOH and 1%NaOH-1%KOH solutions, respectively. Such results are in accordance with previous studies reporting that alkaline pretreatment is efficient to delignify and remove partly lignin and hemicellulose, whereas cellulose is preserved. For instance, Millett et al. (1976) reported that the NaOH pretreatment could decrease lignin content from 24% to 55%. Silverstein et al. (2007) also reported that 2% NaOH for 90 min at 121°C was the best pretreatment condition, resulting in 65% of delignification. Panagiotopoulos et al. (2010) examined the pretreatment of sweet sorghum bagasse with more aggressive alkali solution and found extensive delignification with approximately half of the lignin removed (up to 53.1%). Finally, Sambusiti et al. (2013b) was observed negligible reduction of lignin after an alkaline pretreatment performed at 40°C with 1% of NaOH using ensiled sorghum. However, at higher alkaline dosage (10% of NaOH) the lignin was removed up to 44%, whereas thermo-alkaline pretreatment at 100°C was found to be more effective.

Moreover, elevated temperatures enhanced significantly the lignin removal. The lignin removal obtained at 80°C for 2 h was 2.68 and 2.17 times higher than the one obtained at 25°C (with the same retention time) using alkaline solutions of 0.5%NaOH-0.5%KOH and 1%NaOH-1%KOH, respectively. Phummala et al. (2014) studied the effect of alkali solution dosage, temperature and retention time on delignification of wooden chopsticks waste and reported that the highest percentage of lignin removal (41%) was obtained with 2% NaOH at 100°C, correlated with the highest carbohydrate released (67 mg/g pretreated sample) in the hydrolysate.
4.6 Experimental ensiling procedure

Silage making (ensiling) of fresh crops is a necessary and common procedure to provide nutrient-rich fodder for animals throughout the year, especially where the growing period is short. The increasing use of energy crops and residues needed as raw materials to fulfill the requirements of bio-based transport fuels can also benefit from preservation of the raw material (Pakarinen et al., 2011). Ensiling is sometimes referred to as a pretreatment technology, but it has a limited effect on AD. Ensiling is predominantly carried out for storage reasons and not to increase the rate of biogas production (Kreuger et al., 2011). End-products of appropriate ensilage fermentation are mainly lactic acid, traces of ethanol and traces of volatile fatty acids (Miron et al., 2005). During a typical ensiling process, the water soluble carbohydrates in forage undergo a lactic acid fermentation (anaerobic) that lowers the pH and suppresses the growth of spoilage microorganisms (Egg et al., 1993).

4.6.1 Materials

To carry out laboratory ensiling tests, two different fresh sorghums were used as substrates (FS2 and FS3), in order to evaluate the performance of ensiling in two varieties of sweet sorghum. Their characteristics in terms of cultivation and harvesting and also the procedure of chopping and storage are described in Section 2.1.2-Table 2.1. The chemical composition of FS2 and FS3 sorghum, after drying and milling, is given in Table 4.8.
Table 4-9: Chemical composition of two fresh sorghums (FS2 and FS3). Values correspond to mean ± standard deviation of measurement performed in duplicate.

<table>
<thead>
<tr>
<th>Parametersa</th>
<th>FS2</th>
<th>FS3</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.60 ± 0.04</td>
<td>4.95 ± 0.08</td>
</tr>
<tr>
<td>Moisture (% wet weight)</td>
<td>76.70 ± 0.11</td>
<td>72.16 ± 0.25</td>
</tr>
<tr>
<td>TS (% wet weight)</td>
<td>23.30 ± 0.09</td>
<td>27.24 ± 0.14</td>
</tr>
<tr>
<td>VS (%TS)</td>
<td>94.50 ± 0.17</td>
<td>92.84 ± 0.03</td>
</tr>
<tr>
<td>Ash (%TS)</td>
<td>5.50 ± 0.03</td>
<td>7.16 ± 0.05</td>
</tr>
<tr>
<td>TOC (%TS)</td>
<td>59.80 ± 0.04</td>
<td>50.00 ± 0.38</td>
</tr>
<tr>
<td>Total carbohydratesb (%TS)</td>
<td>81.00 ± 0.33</td>
<td>65.00 ± 1.22</td>
</tr>
<tr>
<td>Soluble carbohydratesb (%TS)</td>
<td>49.00 ± 0.28</td>
<td>21.35 ± 0.93</td>
</tr>
<tr>
<td>Cellulose (%TS)</td>
<td>23.00 ± 0.41</td>
<td>38.25 ± 2.01</td>
</tr>
<tr>
<td>Hemicellulose (%TS)</td>
<td>11.00 ± 0.72</td>
<td>24.53 ± 1.54</td>
</tr>
<tr>
<td>Lignin (%TS)</td>
<td>8.00 ± 0.44</td>
<td>23.70 ± 4.12</td>
</tr>
<tr>
<td>Total nitrogen, TKN (%TS)</td>
<td>0.10 ± 0.01</td>
<td>0.50 ± 0.02</td>
</tr>
<tr>
<td>Proteins (%TS)</td>
<td>0.70 ± 0.06</td>
<td>3.12 ± 0.13</td>
</tr>
<tr>
<td>Lactic acid (%TS)</td>
<td>N.D</td>
<td>N.D</td>
</tr>
</tbody>
</table>

aN Mean values (± standard deviation); b In equivalent glucose; N.D: No Detected

4.6.2 Laboratory ensiling procedure

Freshly harvested sorghum stalks (amount of samples ca. 20 g) were immediately sealed in 160-mL serum bottles. The serum bottles were flushed for 5 min with nitrogen gas assured anaerobic conditions during the whole ensiling process and then sealed immediately using butyl rubber septum and aluminum crimp caps. Once sealed, the bottles were placed in constant temperature (20°C).

4.6.3 Results and Discussion

Fig. 4.7 illustrates soluble and total sugars degradation as well as fermentation products (both in solid and gaseous phase) evolved during ensiling of FS2 under ambient conditions. During the process, a decrease of soluble sugars by 85% was observed with a direct production of ethanol (89 mg/g dry FS2), lactic acid (15 mg/g dry FS2) and traces of volatile fatty acids (Fig. 4.7(b)). In particular, acetic acid (3.6 mg/g dry FS2) was the main acid detected, whereas low amounts (<0.5 mg/g dry FS2) of valeric, butyric and propionic acid were also determined. The pH value in the ensiled sorghum (end of the process) was 4.6, limiting thus any further microbial growth. Along with sugars degradation, a concomitant biogas production was measured with an overall yield of 234 mL and with H2 constituting 10% of total biogas (Fig. 4.7(c)). The stabilization of biogas production was monitored after 30 days of ensiling, indicating thus the practical ending of the process under the studied conditions.

On the other hand, Fig. 4.8 presents the performance of FS3 ensiling, in terms of sugars degradation and end-products evolution. A decrease of soluble sugars by 85.25%
was obtained, whereas no difference in insoluble sugars was observed (Fig. 4.8(a)). All soluble carbohydrates that vanished during ensiling were converted into lactic acid (48.5 mg/g dry FS3) (Fig. 4.8(b)). Ensiling of FS3 however, had different mode of FS2 fermentation resulting no production of ethanol, volatile fatty acids or gas products. Lactic acid accumulation led to pH reduction (from 4.95 to 3.47) which is a prerequisite for good conservation of the silage (Miron et al., 2005). The dry matter (DM) of ensiled sorghum was 26.6%, which is above the minimum DM content of 24.7% recommended by Castle and Watson (1973) to ensure successful ensiling. Similar ensiling characteristics were reported by Miron et al. (2006), who examined three different varieties of sorghum (BMR-101, Silobuster and FS-5) and observed lactic acid (31–68 mg/g dry matter), traces of VFA (11–22 mg/g dry matter) and ethanol (2–8 mg/g dry matter), while reducing silage pH level below 4.0.

**Figure 4-7**: Evolution of (a) total, soluble and insoluble sugars, (b) ethanol, lactic and volatile fatty acids (mg/g dry FS2) and (c) biogas (H₂/CO₂) during ensiling of FS2 under ambient conditions.
Chapter 4  Pretreatment of sweet sorghum stalks for enhancing biofuels production

![Graphs showing the evolution of total, soluble, and insoluble sugars as well as lactic acid during ensiling of FS3 under ambient conditions.](a) (b)

**Figure 4-8**: Evolution of (a) total, soluble and insoluble sugars and (b) lactic acid (mg/g dry FS3) during ensiling of FS3 under ambient conditions.

### 4.7 Biochemical Methane Potential

Batch anaerobic digestion tests were carried out in order to assess the biodegradability of fresh and ensiled sorghum (FS3 and ES3, respectively). Fig. 4.9 depicts the cumulative methane production of fresh and ensiled sorghum as a function of the digestion time. The calculated methane productions of the FS3 and ES3, after subtraction of the methane produced from the blank experiment, were $56.20 \pm 2.84$ and $55.44 \pm 2.78$ mL of CH$_4$, respectively. The methane yield obtained was $300.61 \pm 15.19$ mL CH$_{4@STP}$/g VS added for fresh sorghum (FS3) and $301.26 \pm 15.11$ mL CH$_{4@STP}$/g VS added for ensiled one (ES3). The results obtained are in close agreement with those obtained by Braun et al. (2010), who reported a range of methane yields (295-372 mL CH$_{4@STP}$/g VS) for sorghum plants. Moreover, no difference in methane yield was found between the two sorghums. As reported by Montgomery and Bochmann (2014), ensiling is predominantly carried out for storage reasons and not to increase the rate of biogas production. Although some studies show that ensiling increases the methane yield from certain crops (Pakarinen et al., 2011), other research (Kreuger et al., 2011) has shown that this is due to a very widespread and underreported calculation error and that ensiling actually has a minimal effect on methane yield.

Moreover, the methane potential was also measured using the ES3 after the two optimum chemical pretreatment conditions (alkaline treatment with 0.5%NaOH-0.5%KOH or 1%NaOH-1%KOH at 80°C for 2 h) applied in previous work (Section 4.5.3). Fig. 4.10 illustrates the cumulative methane productions of the two pretreated ensiled sorghums and also of blank experiment as a function of the experimental time.
Figure 4-9: Cumulative methane production during the BMP assay from (a) fresh sorghum (FS3) and (b) ensiled sorghum (ES3). Errors bars represent the standard deviation for the replicates.

The calculated methane production (after subtraction of the methane produced from the blank experiment) of the pretreated ES3 with 0.5%NaOH-0.5%KOH was 58.96 ± 4.47 mL of CH₄, whereas for pretreated ES3 with 1%NaOH-1%KOH was 60.65 ± 3.89 mL of CH₄. The methane yield obtained was 333.48 ± 25.28 mL CH₄@STP/g VS added, using ES3 pretreated with 0.5%NaOH-0.5%KOH, higher than untreated ES3. Increasing the alkaline dosage to 1%NaOH-1%KOH, a further increase of methane yield was observed (354.51 ± 22.74 mL CH₄@STP/g VS added). Sambusiti et al. (2013b) examined the effect of alkaline treatment (NaOH dosage of 1% and 10%) on methane production from ensiled sorghum forage and reported positive results based on improved methane yields, especially at the highest NaOH dosage. On the other hand, according to Antonopoulou and Lyberatos (2013), negligible effect or even decrease of methane yield was observed during alkaline and thermal-alkaline pretreatment of sweet sorghum. Sambusiti et al. (2013c) suggested that the impact on methane production of sodium hydroxide pretreatment depends on the type and variety of substrate used.

Moreover, the cumulative biomethane production profile from each experiment was fitted to a modified Gompertz bacterial growth model (Eq. 4.4).

\[
M(t) = P \exp \left\{- \exp \left[\frac{R_m e}{P} (\lambda - t) + 1 \right]\right\}
\]

(4.4)

where \(M(t)\) is the cumulative methane production (mL); \(P\) is the maximum methane production potential (mL); \(R_m\) is the maximum methane production rate (mL/d); \(\lambda\) is the lag-phase duration (d); \(t\) is the time (d) and \(e\) is \(\exp(1) = 2.71828\).
Chapter 4  Pretreatment of sweet sorghum stalks for enhancing biofuels production

**Figure 4-10:** Cumulative methane production during the BMP assay from ensiled sorghum (ES3) pretreated with (a) 0.5% NaOH-0.5% KOH and (b) 1% NaOH-1% KOH solution. Errors bars represent the standard deviation for the replicates.

Fig. 4.11 and Fig. 4.12 depict the methane production based on experimental data and the simulation generated using the fitted modified Gompertz model for raw sorghum (fresh and ensiled) and pretreated ensiled one, respectively. Comparing each set of experimental data with the relevant model simulation, the parameters of methane production potential (P), the maximum methane production rate (R_m) and lag-phase time (λ) were determined (Table 4.9).

**Figure 4-11:** Cumulative methane production (experimental data and modified Gompertz model simulation) during the BMP assay from a) fresh sorghum (FS3) and b) ensiled sorghum (ES3).
The modified Gompertz bacterial growth model was successful in interpreting the experimental production trend, as demonstrated by the high correlation coefficient ($R^2$) values (0.988-0.997), suggesting that such a simple model can be used in practice to describe the complex anaerobic degradation processes for those substrates, such as lignocellulosic ones, for which hydrolysis is the rate-limiting step, as reported by Angelidaki et al. (2009). The maximum methane potential (60.05 ± 0.45 mL) was presented at pretreated ES3 with 1%NaOH-1%KOH, whereas the lag-phase parameter ranged at similar values (12.37 to 16.32 days) for all experiments. An increase in the methane production rate was observed after the thermal-alkaline treatment of ES3, due to the reduction of lignin content, thus accelerating the disintegration and the hydrolysis, which are the limiting steps for lignocellulosic substrates. An increase in the methane production rate (from 3.73 to 5.37 mL/d) was also observed by increasing the alkaline dosage from 0.5% to 1%.

<table>
<thead>
<tr>
<th>Sorghum</th>
<th>$P$ (mL)</th>
<th>$R_m$ (mL/d)</th>
<th>$\lambda$ (d)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS3</td>
<td>53.26 ± 0.96</td>
<td>3.20 ± 0.34</td>
<td>12.50 ± 0.83</td>
<td>0.987</td>
</tr>
<tr>
<td>ES3</td>
<td>54.08 ± 0.82</td>
<td>3.00 ± 0.22</td>
<td>16.32 ± 0.71</td>
<td>0.993</td>
</tr>
<tr>
<td>Pretreated ES3 (0.5%NaOH-0.5%KOH)</td>
<td>58.14 ± 0.75</td>
<td>3.73 ± 0.28</td>
<td>13.56 ± 0.56</td>
<td>0.994</td>
</tr>
<tr>
<td>Pretreated ES3 (1%NaOH-1%KOH)</td>
<td>60.05 ± 0.45</td>
<td>5.37 ± 0.34</td>
<td>12.37 ± 0.34</td>
<td>0.997</td>
</tr>
</tbody>
</table>

Figure 4-12: Cumulative methane production (experimental data and modified Gompertz model simulation) during the BMP assay from a) fresh sorghum (FS3) and b) ensiled sorghum (ES3).
4.8 Conclusions

As an example of an energy crop residue, sweet sorghum was studied in this work. The complex structure of sorghum justifies the use of pretreatments in order to maximize the carbohydrate solubilization (saccharification) and increase the accessible surface area for the follow-up enzymatic hydrolysis and microbial fermentation. Firstly, various pretreatment methods, such as (thermal)-chemical through alkali (NaOH) or acid (HCl, H$_2$SO$_4$) at concentrations of 0.5 and 1.5% w/v addition or enzymatic through the addition of commercial enzymes, were applied in different varieties of sorghum. Using fresh sorghum (FS1) as a substrate, an increase of solubilization yield up to 60% was observed after acid pretreatment, whereas alkaline pretreatment led to a significant decrease in soluble carbohydrates. On the other hand, ensiled sorghum (ES1) presented an increased yield of glucose (540%) after alkaline pretreatment (0.5% NaOH) at 50°C, compared to the initial amount of glucose during composition analysis. Furthermore, enzymatic hydrolysis was performed with combined levels of enzyme dosage, different pH, temperature and incubation time. Based on maximization of sugars production (179.93%) with simultaneous maximum profit, the optimum enzymatic hydrolysis was achieved using 3% CTec2 and 0.8% HTec2 for contact time 12 h, temperature 50°C and pH 5.50. A satisfied sugars production can be achieved using low concentrations of enzymes, whereas further increase of enzymes concentration led to a high-cost process without equivalent increase of sugars. Finally, thermal-alkaline hydrolysis of ensiled sorghum was studied, using two different alkaline solutions, in order to prepare the material for the follow-up anaerobic digestion. Pretreatment with 0.5%NaOH-0.5%KOH solution at 80°C and contact time 2 h led to an increase of sugars by 352.74%, whereas lignin was removed by 36.87%. However, carbohydrates solubilization increased more (561.13%) after pretreatment at the same conditions with higher alkaline dosage (1%NaOH-1%KOH) resulting in 42.33% delignification. The latter pretreatments were used in BMP experiments in order to evaluate the performance on the methane yield. The pretreatment step did not cause inhibition of the anaerobic process because the amounts of Na$^+$ and K$^+$ remained lower than the concentrations causing inhibition. The alkali pretreatment led to an increase in methane yield of 17.68%, compared to untreated sorghum, whereas ensiling procedure did not change the methane production. Finally, laboratory ensiling tests were performed in two different varieties of sweet sorghum (FS2 and FS3) in order to examine the performance in terms of conversion profile of polysaccharides and water soluble carbohydrate content of sweet sorghum to fermentable end-products (i.e. ethanol, lactic acid) during the ensiling process. During FS2 ensiling at ambient anaerobic conditions, the amount of soluble sugars consumed was 85% with a simultaneous production of mainly ethanol (90 mg/g dry biomass) and lactic acid (15 mg/g dry biomass). The biogas release yield was measured at 234 mL. However, during FS3 ensiling led to different fermentable end-products without gaseous products. A decrease of soluble sugars by 85.25% was obtained, which were converted only into lactic acid (48.5 mg/g dry biomass).
4.9 References


Chapter 4
Pretreatment of sweet sorghum stalks for enhancing biofuels production


Sites
Chapter 5.

Biogas production from agro-industrial wastes through anaerobic co-digestion in a two-stage CSTR system.

5.1 Abstract

Co-digestion of organic waste streams is an innovative technology for the reduction of methane/greenhouse gas emissions. Different organic substrates are combined to generate a homogeneous mixture as input to the anaerobic reactor in order to increase process performance, realize a more efficient use of equipment and cost-sharing by processing multiple waste streams in a single facility. In this study, the potential of anaerobic digestion for the treatment of four different mixtures containing agro-industrial wastes, such as olive mill wastewater (OMW), cheese whey (CW) and/or liquid cow manure (LCM), using a two-stage process, has been evaluated by using two continuously stirred tank reactors (CSTRs) under mesophilic conditions (37°C) in order to separately monitor and control the processes of acidogenesis and methanogenesis. The mixtures examined in this study were OMW:CW:LCM (55:40:5, v/v/v), CW:LCM (90:10, v/v), OMW:CW (80:20, v/v) and OMW:LCM (20:80, v/v). The overall process was studied with a hydraulic retention time (HRT) of 19 days (3 d of acidogenesis and 16 d of methanogenesis). The maximum methane production rate (1.35 L CH₄/L_{R·d}) was obtained using the mixture of 55% OMW, 40% CW and 5% LCM with methane yield of 467.53 mL CH₄/g VS added. The average removal of soluble and total COD was 75.5% and 64%, respectively. Equally, a high methane production rate (1.33 L CH₄/L_{R·d}) was obtained using the mixture of 90% CW and 10% LCM with higher methane yield of 658.82 mL CH₄/g VS added compared to previous mixture. The soluble and total COD degradation was 85.2% and 79%, respectively. Using the mixture of 20% OMW with 80% LCM as substrate, 0.91 L CH₄/L_{R·d} was noticed with 50% removal of total COD. On the other hand, treating the mixture of 80% OMW and 20% CW an accumulation of volatile fatty acids was observed at HRTs of 16 and 20 d. For this reason, the HRT was increased to 30 d in order to stabilize the reactor performance, whereas the methane production rate reached 0.67 L CH₄/L_{R·d}. The methane yield obtained was 291.31 mL CH₄/g VS added with 55% removal of total COD.
5.2 Introduction

Olive mills, cheese factories and cow farms are agro-industries that represent a considerable share of the Mediterranean countries economy. Olive mills have a specific product that is seasonally produced and generate by-products such as olive mill wastewater (OMW) and olive cake that pose a serious environmental risk, especially in the Mediterranean, Aegean and Marmara regions accounting for approximately 95% of the worldwide production (FAO, 2007). OMW is becoming a serious environmental problem due to its high content of poly-phenolic compounds, suspended solids, volatile acids, polyalcohols and nitrogenous compounds and their resistance to biodegradation (Moreno et al., 1990; Ramos-Cormenzana et al., 1995). The high concentration of phenols in OMW, reaching up to 10 g/L (Borja et al., 1992), contributes to a high toxicity and antibacterial activity (Capasso et al., 1995). The values of chemical and biochemical oxygen demand (COD, BOD) range from 25 to 162 and from 9 to 100 g O₂/L, respectively (Tsonis, 1988). Cheese factories generate wastewaters of which whey is the most important waste stream produced with a high organic content (up to 70 g COD/L), which is highly biodegradable, and low alkalinity (50 meq/L) (Mawson, 1994). Cheese whey (CW) contains a significant amount of carbohydrates (4–5%), mainly lactose, proteins not exceeding 1%, fats at about 0.4–0.5%, lactic acid less than 1% and salts that may range from 1% up to 3% (Gelegenis et al., 2007a). Agricultural wastewaters, including animal manure, are another source of liquid waste. Cow farms produce animal manure, both in liquid and semi-liquid form, depending on the amount of fresh water used for daily operations. The potential pollutants from decomposing livestock manure include high value of BOD, pathogens, nutrients, methane, and ammonia emissions (US EPA, 2003), parameters that need to be controlled before disposal.

Anaerobic digestion is the natural breakdown pathway of organic materials into methane and carbon dioxide gas and fertilizer. This process takes place naturally or in an anaerobic digester and presents an attractive treatment solution for high strength wastewaters due to the operational economy and generation of biogas with pollution decreasing at the same time. Anaerobic digestion is considered as one of the best technologies for treating industrial wastewater with a high organic load, which also controls malodorous emissions and stabilizes the biomass prior to its agronomic use (Mata-Alvarez et al., 2000). Several studies have demonstrated that co-digestion of different organic wastes may lead to a distinct increase in methane yield and that individual waste streams could be combined as a substrate for more efficient treatment. Co-digestion of different types of organic by-products has been increasingly applied in order to improve plant profitability and overcome a number of problems such as nutrient imbalance, rapid acidification and presence of inhibiting compounds, among other factors (Monou et al., 2008; Cuetos et al., 2008; Martinez-Garcia et al., 2007; Azbar et al., 2008; Fountoulakis et al., 2008; Dareioti et al., 2009). Other benefits of co-digesting multiple waste streams include the improvement of nutrient balance and
Biogas production from agro-industrial wastes through anaerobic co-digestion in a two-stage CSTR system

Chapter 5

...digestion, equalization of particulate, floating, settling, acidifying, etc. wastes, through dilution by manure or sewage sludge, additional biogas production, possible gate fees for waste treatment, additional fertilizer (soil conditioner) reclamation and more efficient use of equipment as well as cost-sharing by processing multiple waste streams in a single facility.

Although many AD configurations have reported in the literature, either of the suspended or attached growth type (Rittmann and McCarty, 2001), a two-stage AD system has proved to be favorable for averting the imbalance between the processes of acidogenesis and methanogenesis, presenting at the same time excellent robustness and application of control and optimization of the overall AD process. Such a two-stage approach has been previously successfully applied to e.g. municipal solid wastes (Pavan et al., 2000), crop residues (Kalia et al., 1992), agroindustrial residues (Weiland, 1993), market wastes (Chanakya et al., 1992; Mata-Alvarez et al., 1993), food wastes (Shin et al., 2001; Lee et al., 1999) and OMW with CW and LCM (Dareioti et al., 2009). Physical separation of acidogenic and methanogenic bacteria takes place in this process into two interconnected reactors, maximizing their growth by maintaining optimum conditions in each tank for each particular group of bacteria. Conditions that favor the growth of acidogenic bacteria are acidic pH values with a residence time between 1 and 4 days, in contrast with methanogenic bacteria, that require much higher pH values (above neutral) and a residence time between 10 and 15 days, depending upon the wastewater characteristics (Antonopoulou et al., 2008a). The acidogenic bacteria will not thrive in the methane reactor as their growth substrate i.e. organic monomers (carbohydrates, amino acids etc.) are only available and mainly consumed in the acidogenic reactor, while methanogenic bacteria cannot thrive in the acidogenic reactor as the retention time is too short and the pH is too low (Rittmann and McCarty, 2001).

This article focuses on the anaerobic co-treatment of three representative types of agro-industrial wastewaters with a high organic content produced in Greece and other Mediterranean countries, as well: olive mill wastewater – OMW (production period October–March), cheese whey – CW (production period October-June) and liquid cow manure – LCM (production period whole year). The digestion of OMW alone as a sole substrate is not easy to be realized with satisfactory results because of the high concentration of phenols and long-chain fatty acids (Hamdi et al., 1992; Beccari et al., 1998). However, co-digesting OMW with LCM or CW as a sub layer would have the advantage that OMW will be effectively diluted as far as phenolic compounds and long-chain fatty acids concentrations are concerned. Moreover, a waste mixture is expected to be balanced in nitrogen and alkalinity, which are critical parameters for the stability of the anaerobic digestion (AD) process. The specific aim of the present work was to investigate, on a laboratory scale, the anaerobic co-digestion of these wastes in different mixtures, using a two-stage AD configuration.
5.3 Materials

Significant differences in the composition of the three wastewater streams presented in Table 5.1 were detected. OMW presented the higher organic content (131 g/L as total COD) compared to CW (72.1 g/L) and LCM (53.4 g/L). OMW and CW had low nitrogen content in contrast with LCM, while LCM had neutral pH and alkalinity in high levels. Phenols concentration in OMW was 6.8 g/L, lying in the range reported by Paraskeva and Diamadopoulos (2006), while their concentration in LCM and CW was extremely low.

According to Henze and Harremoes (1983) a ratio of COD/ N = 400/7 is required for a balanced carbon to nitrogen feed. Based on the physicochemical characteristics of the individual waste streams and their mixture, it is easily evidenced that mixing of OMW and/ or CW with LCM ensures a surplus in both nitrogen concentration and alkalinity. Moreover, OMW is widely regarded as a severe environmental problem because of its high organic content and especially due to the presence of phenolic compounds that are both antimicrobial and phytotoxic (Ramos-Cormenzana et al., 1995). Therefore, the dilution of OMW with CW and/ or LCM drastically reduced the inhibitory effect of contained polyphenols in OMW, whereas the organic content of the mixture still remained quite high for being converted to biogas (Dareioti and Kornaros, 2014).

Table 5-1: Chemical composition of each agro-industrial wastewater used in this study.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>OMW (g/L ± SD)</th>
<th>CW (g/L ± SD)</th>
<th>LCM (g/L ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-</td>
<td>5.00 ± 0.03</td>
<td>6.33 ± 0.12</td>
<td>7.70 ± 0.06</td>
</tr>
<tr>
<td>TSS</td>
<td>g/L</td>
<td>37.00 ± 1.30</td>
<td>9.00 ± 0.12</td>
<td>20.00 ± 0.90</td>
</tr>
<tr>
<td>VS</td>
<td>g/L</td>
<td>34.50 ± 2.50</td>
<td>8.07 ± 0.05</td>
<td>13.60 ± 0.80</td>
</tr>
<tr>
<td>TS</td>
<td>g/L</td>
<td>83.28 ± 7.40</td>
<td>63.80 ± 0.22</td>
<td>69.29 ± 1.31</td>
</tr>
<tr>
<td>VS</td>
<td>g/L</td>
<td>54.86 ± 4.50</td>
<td>49.62 ± 0.31</td>
<td>47.05 ± 0.69</td>
</tr>
<tr>
<td>VS</td>
<td>g/L</td>
<td>131.01 ± 6.90</td>
<td>72.12 ± 0.30</td>
<td>60.09 ± 1.06</td>
</tr>
<tr>
<td>TS</td>
<td>g/L</td>
<td>67.08 ± 0.80</td>
<td>53.51 ± 0.01</td>
<td>26.65 ± 0.19</td>
</tr>
<tr>
<td>Soluble COD</td>
<td>g/L</td>
<td>41.00 ± 1.80</td>
<td>36.00 ± 0.00</td>
<td>19.72 ± 0.24</td>
</tr>
<tr>
<td>BOD₅</td>
<td>g/L</td>
<td>26.15 ± 0.19</td>
<td>40.94 ± 0.84</td>
<td>13.72 ± 0.95</td>
</tr>
<tr>
<td>Total carbohydrates</td>
<td>g/L</td>
<td>21.65 ± 0.04</td>
<td>35.68 ± 0.40</td>
<td>0.96 ± 0.03</td>
</tr>
<tr>
<td>Soluble carbohydrates</td>
<td>g/L</td>
<td>6.80 ± 0.30</td>
<td>0.11 ± 0.00</td>
<td>0.90 ± 0.10</td>
</tr>
<tr>
<td>Total phenols</td>
<td>g/L</td>
<td>0.73 ± 0.01</td>
<td>0.92 ± 0.02</td>
<td>3.36 ± 0.00</td>
</tr>
<tr>
<td>Total nitrogen, TKN</td>
<td>g/L</td>
<td>0.10 ± 0.02</td>
<td>0.12 ± 0.00</td>
<td>1.54 ± 0.02</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>mg/L</td>
<td>350.30 ± 4.00</td>
<td>300.00 ± 0.90</td>
<td>660.00 ± 9.10</td>
</tr>
<tr>
<td>Soluble phosphorus</td>
<td>mg/L</td>
<td>210.00 ± 2.00</td>
<td>220.15 ± 1.48</td>
<td>20.30 ± 1.04</td>
</tr>
<tr>
<td>Oil and grease</td>
<td>g/L</td>
<td>9.85 ± 1.23</td>
<td>0.09 ± 0.00</td>
<td>3.24 ± 0.04</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>g CaCO₃/L</td>
<td>1.50 ± 0.01</td>
<td>0.80 ± 0.00</td>
<td>12.38 ± 0.32</td>
</tr>
</tbody>
</table>

* Mean values (± standard deviation); ① In equivalent glucose ; ② In equivalent syringic acid
5.4 Anaerobic mesophilic co-digestion of olive mill wastewater, cheese whey and liquid cow manure.

Experiments were carried out in a two-stage CSTR system. Two continuously anaerobic reactors (CSTRs) were operated, one used for the 1st stage of acidogenesis and the other one for the 2nd stage of methanogenesis. The experimental set-up is described in Section 2.2.1. A mixture of olive mill wastewater (OMW), cheese whey (CW) and liquid cow manure (LCM) at a ratio of 55:40:5 (v/v/v) was used in order to evaluate the performance in terms of biogas production, stability and COD reduction.

5.4.1 Acidogenic reactor

For start-up, the acidogenic reactor was filled up with 750 mL of feed, which consisted of 55%, 40% and 5% of OMW, CW and LCM respectively and was operated anaerobically at a batch mode for 72 h in order to activate the indigenous microflora. The operation was subsequently switched to continuous mode at the designated HRT (3 d). The reactor was operated under these conditions for 87 days. According to Henze and Harremoes (1983), a ratio of COD/N = 400/7 is required for a balanced carbon to nitrogen feed. Based on the physicochemical characteristics of the individual waste streams and their mixture, 0.26 g/L of urea (NH₂CONH₂) were added to the feed in order to ensure surplus in nitrogen concentration.

No SCOD removal (Fig. 5.1(a)) and solids hydrolysis (Fig. 5.1(b)) were observed for the 87 days of operation of the acidogenic reactor. A decrease in the effluent pH comparing with the influent (from 5.8 ± 0.16 to 4.1 ± 0.19) was noticed in the reactor (Fig. 5.3(b)) until the 87th day of operation. The reduction of the pH value was expected due to VFAs production by the acidogenic bacteria (Fig. 5.2(a) and (b)). For example the concentration of acetic acid increased from 732 ± 75 (mean value) to 3302 ± 245 mg/L.

![Figure 5-1: Concentration of (a) soluble COD (SCOD) and (b) total (TS) and volatile solids (VS) during acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v).](image-url)
The concentrations of other VFAs like propionic, butyric, isobutyric, valeric and isovaleric acid remained practically constant at about 164 ± 134 mg propionic acid/L and less than 50 mg/L (for each one of the other acids) throughout the operation of acidogenic reactor. It can be easily observed that VFAs concentration in the effluent increased linearly with the time of operation, mainly due to the linear increase of acetic acid concentration. This effect can be attributed to an increase in the activity and/or the concentration of the acidogenic bacteria. Biogas production in the acidogenic reactor was also observed (Fig. 5.3(a)). The rate over the entire experiment was oscillatory with an average of 0.46 ± 0.21 L/LR·d in which the percentage of hydrogen in the gas phase at the steady state was 27%. It should be noted that no methane was detected throughout the experimentation period. Phenol concentration decreased by 18% from 2.14 ± 0.10 to 1.75 ± 0.33 g/L, whereas the concentration of total carbohydrates decreased from 29.1 ± 1.8 to 16.2 ± 1.7 g/L (44.4% decrease) until the 87th day of operation.

**Figure 5-2:** Variation of volatile fatty acids (VFAs) in (a) the influent and (b) the effluent during acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v).

**Figure 5-3:** (a) Biogas production rate and (b) pH value during acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v).
5.4.2 Methanogenic reactor

The methanogenic reactor was seeded in the 6th day with 4 L anaerobic digested sludge taken from the municipal wastewater treatment plant of Patras (Greece). Following this step, the effluent of the acidogenic reactor was fed to the methanogenic according to the operating HRT (16 d). Alkalinity was kept in satisfactory levels by adding 14 g NaHCO3/L in the feed of the methanogenic reactor throughout the experimentation period. The OLR in the methanogenic reactor was 5.5 ± 0.36 kg total COD/m³·d (2.8 ± 0.17 kg soluble COD/m³·d).

A methane bioreactor was used for treating the acidified effluent of the first stage in order to assess the rate and extent of methanogenesis. The SCOD removal was 75.5% (mean value) (Fig. 5.4(a)), whereas the TCOD removal at the steady state was 64%. The removal of COD in conjunction with gas production in the anaerobic digester provided evidence of microbial activity, particularly methanogenic bacteria. A relatively short period of around 10 days was required to achieve the acclimatization and stable activity of methanogenic bacteria as measured by COD degradation and methane evolution. The TS removal remained around 41% (from 73.9 ± 6.2 to 43.5 ± 5.2 g/L). On the other hand, the removal efficiency in VS concentration for the examined mixture was 59% (from 46.2 ± 5.2 to 18.9 ± 2.1 g/L) (Fig. 5.4(b)). As shown in Fig. 5.5(a) the biogas production rate presented a high increase until the 30th day of operation from 0.72 (at the end of 1st day of operation) to 1.92 L/LR·d. Between the 31st and 87th day the rate was rather stable at 1.82 L/LR·d (mean value). The composition of methane in the biogas fluctuated between 59.7% and 79.3% with mean values of 73.5 ± 4.7%. The methane production rate at the steady state reached 1.35 ± 0.11 L CH₄/LR·d (Fig. 5.5(a)), while the methane yield in the reactor was 243 mL CH₄/g COD added or 467.53 mL CH₄/g VS added.

Figure 5-4: Concentration of (a) soluble COD (SCOD) and (b) total (TS) and volatile solids (VS) during methanogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v).
An increase in effluent pH was noticed in the reactor from 6.5 to 7.9 (Fig. 5.5(b)) until the 36th day of operation, although the influent pH was decreasing versus time from 6.8 to 5.4 due to VFAs production from the acidogenic reactor. This value which is at the desired (optimum) levels for anaerobic digestion remained stable at these levels for all the experimentation period. This stability could be attributed to the addition of NaHCO₃ in the influent at a concentration of 14 g NaHCO₃/L. The addition was taking place from the first day of the experimentation period. The total bicarbonate alkalinity after the addition of NaHCO₃ increased to 8800 mg of CaCO₃/L. The difference in the value of the pH between the effluent of the acidogenic reactor and influent of the methanogenic is due to this addition.

The influent of the methanogenic reactor was rich in VFAs, as anticipated due to the pretreatment achieved in the acidogenic reactor (Fig. 5.6(a) and (b)). During the first 20 days of the experiment an accumulation of propionic acid was observed in the effluent of the methanogenic reactor, which was surpassed successfully after the 40th day of operation. The mean value of total VFAs concentration in the effluent for the working scenario was 1767 ± 791 mg/L. It should be mentioned that the total VFAs concentration in the influent of the reactor was 4025 ± 1892 mg/L (mean value). The removal of total carbohydrates in glucose equivalents was 97% (data not shown) in the whole process (both reactors) since the concentration of carbohydrates in the influent was 29.1 ± 1.8 g/L and decreased to 0.8±0.2 g/L. Moreover, phenol concentration decreased by 53% from 1.75 ± 0.33 to 0.83 ± 0.19 g/L. The mean ammonium nitrogen concentration measured in the effluent of the methanogenic reactor was 0.18 ± 0.02 g/L verifying nitrogen availability for the anaerobic bacteria.

The high organic load and the presence of inhibitory compounds in the OMW mandate its mixing with other industrial wastewaters in order to digest them successfully and eliminate the environmental risks of no treatment at all.
A number of different types of anaerobic reactors and methods have been investigated for the treatment of OMW in which dilution, nutrient addition, and alkalinity adjustment were also required (Ursinos and Padilla, 1992; Sabbah et al., 2005). In a study (Angelidaki and Ahring, 1997), authors reported a biogas production of approximately 1250 mL/LR·d in an anaerobic co-digesting process treating OMW with manure at a ratio of 50:50 under thermophilic conditions with an organic loading rate of 7.8 kg COD/m³·d, which corresponds to a methane yield of 160 L CH₄/kg COD. They also showed that the amount of nitrogen needed to obtain a stable degradation of OMW can be provided by cattle manure, swine manure or piggery effluent during co-degradation. Other studies report that the co-digestion of OMW with piggery effluent using two identical upflow anaerobic filters, showed COD removal of about 76% (Marques et al., 1998; Marques, 2001). Other researchers (Fountoulakis et al., 2008) state that the co-digestion of OMW with slaughterhouse wastewaters and wine-grape residues results to a methane yield of 184 and 214 mL CH₄/g COD added, respectively. Our case proved to be more efficient than these studies although we didn’t use any pretreatment or high digestion temperature (thermophilic conditions). Other studies report that the co-digestion of OMW with piggery effluent using two identical upflow anaerobic filters, showed COD removal of about 76% (Marques et al., 1998; Marques, 2001). Other researchers (Fountoulakis et al., 2008) state that the co-digestion of OMW with slaughterhouse wastewaters and wine-grape residues results to a methane yield of 184 and 214 mL CH₄/g COD added, respectively. Our case proved to be more efficient than these studies although we didn’t use any pretreatment or high digestion temperature (thermophilic conditions). In a recent publication other researchers (Gelegenis et al., 2007) have shown a biogas production rate of 210 mL/LR·d digested diluted poultry manure and olive mill effluent at a ratio of 50:50 under mesophilic conditions and a hydraulic retention time of 20 d. Our methane yield rates were higher than the ones reported.

It should be also mentioned that in our case the presence of phenols (because of the OMW) did not seem to affect or inhibit, in any way, the process of anaerobic digestion. This result, however, is in contrast with other researchers (Beccari et al., 1998) who have stated that phenols and especially polyphenols are the most bio-recalcitrant compounds in OMW since only 20–30% of polyphenols was degraded in methanogenic conditions in their experiments and therefore identified them as being responsible for the inhibition of anaerobic process (Sorlini et al., 1986).
5.5 Anaerobic mesophilic co-digestion of cheese whey and liquid cow manure.

The composition of the previous feed was changed to 90% CW and 10% LCM in the same two-stage system (Section 2.2.1). This transition from the previous mixture of OMW+CW+LCM to CW+LCM alone is typical and anticipated in Greece and other Mediterranean countries due to the seasonal type of operation of these agro-industries. After the end of March the operation of olive-mills is ceased and only cheese making factories and dairy farms are still in operation. For example, a survey carried out in a Greek Ionian island (Kefalonia) identified 16 olive mills, 14 cheese making factories and 1 dairy farm producing 37, 26 and 3 m$^3$ of wastewaters respectively, on an average daily basis (Section 3.1-Table 3.2).

5.5.1 Acidogenic reactor

In this scenario no nitrogen source was added, since the 400/7 ratio of COD/N (Henze and Harremoes, 1983) in the feed was satisfied due to the nitrogen content of the LCM. The nitrogen availability for the anaerobic bacteria was also verified throughout the experimentation period by measuring the ammonium nitrogen concentration in the effluent from the methanogenic reactor.

No SCOD removal was observed for the 115 days of operation of the acidogenic reactor (Fig. 5.7(a)). However, in terms of solids hydrolysis, the reactor responded differently to this mixture, compared to the previous one (different substrate). Total and volatile solids concentration presented a minor decrease, 10.3% and 14.9% respectively, between influent and effluent due to hydrolysis (Fig. 5.7(b)). This can be attributed both to the insufficient retention time of waste in the acidogenic reactor and the different substrate composition in the feed. A decrease in the effluent pH comparing with the influent (from 6.7 ± 0.58 to 3.5 ± 0.27) was noticed in the reactor (Fig. 5.9(b)).

![Graph](a)

![Graph](b)

**Figure 5-7:** Concentration of (a) soluble COD (SCOD) and (b) total (TS) and volatile solids (VS) during acidogenesis of CW/LCM mixture (90/10, v/v).
The reduction of the pH value was expected due to VFAs production by the acidogenic bacteria (Fig. 5.8(a) and (b)). For example the concentration of acetic acid increased from $966 \pm 159$ (mean value) to $4913 \pm 118$ mg/L. The concentrations of other VFAs like propionic, butyric, isobutyric, valeric and isovaleric acid remained practically constant at about $194 \pm 40$ mg propionic acid/L and less than $50$ mg/L (for each one of the other acids) throughout the operation of acidogenic reactor. It can be easily observed that VFAs concentration in the effluent increased linearly with the time of operation, mainly due to the linear increase of acetic acid concentration. This effect can be attributed to an increase in the activity and/or the concentration of the acidogenic bacteria.

![Graph showing the variation of volatile fatty acids (VFAs) in (a) the influent and (b) the effluent during acidogenesis of CW/LCM mixture (90/10, v/v).](image1)

**Figure 5-8:** Variation of volatile fatty acids (VFAs) in (a) the influent and (b) the effluent during acidogenesis of CW/LCM mixture (90/10, v/v).

![Graph showing biogas production rate and pH value during acidogenesis of CW/LCM mixture (90/10, v/v).](image2)

**Figure 5-9:** (a) Biogas production rate and (b) pH value during acidogenesis of CW/LCM mixture (90/10, v/v).
Biogas production in the acidogenic reactor was also observed (Fig. 5.9(a)). No hydrogen production was observed which may be attributed to low pH and high VFAs concentration. The average rate of biogas production for this mixture was $0.66 \pm 0.19$ L/LR·d with a composition of ~90% in CO$_2$. It should be noted that no methane was detected throughout the experimentation period. Production of carbon dioxide without accompanying methane production in the hydrolytic/acidogenic stage is a sign of rapid fermentation (Hai-Lou et al., 2002). The concentration of total carbohydrates decreased from $33.1 \pm 3.2$ to $13.7 \pm 1.4$ g/L (58.6% decrease).

### 5.5.2 Methanogenic reactor

Following acidogenic step, the effluent of the acidogenic reactor was fed to the methanogenic according to the operating HRT (16 d). Alkalinity was kept in satisfactory levels by adding 14 g NaHCO$_3$/L in the feed of the methanogenic reactor throughout the experimentation period. The OLR in the methanogenic reactor was $4.5 \pm 0.30$ kg total COD/m$^3$·d ($3.5 \pm 0.17$ kg soluble COD/m$^3$·d).

A methane bioreactor was used for treating the acidified effluent of the first stage in order to assess the rate and extent of methanogenesis. The SCOD removal during the experimental period was 85.2% (mean value) (Fig. 5.10(a)), whereas the TCOD removal at the steady state was 79%. The removal of COD in conjunction with gas production in the anaerobic digester provided evidence of microbial activity, particularly methanogenic bacteria. The TS removal remained around 30% (from $61.2 \pm 5.5$ to $42.6 \pm 4.1$). On the other hand, the removal efficiency in VS concentration for the examined mixture was 56%, from $32.3 \pm 3.5$ to $14.3 \pm 4.7$ g/L (Fig. 5.10(b)). As shown in Fig. 5.11(a) the biogas production rate was $1.68$ L/LR·d until the 24th day of operation, while after this day an increase in the rate from 1.68 to 2.17 L/LR·d was observed.

![Figure 5-10: Concentration of (a) soluble COD (SCOD) and (b) total (TS) and volatile solids (VS) during methanogenesis of CW/LCM mixture (90/10, v/v).](image-url)
A temporary decrease of biogas production was noticed during the 55th and 68th day; however, 9 days later the biogas rate increased at much higher levels reaching 2.31 L/LR·d. The composition of methane in the biogas fluctuated between 59.8% and 66.9% with mean value of 63.8 ± 2.0%. The methane production rate at the steady state reached 1.33 ± 0.15 L CH₄/LR·d (Fig. 5.11(a)), whereas the methane yield in the reactor was 305 mL CH₄/g COD added or 658.82 mL CH₄/g VS added. The influent value of the pH was continuously decreasing during the experimentation period and stabilized at 4.7 on the 26th day, without affecting the effluent pH (Fig. 5.11(b)).

This stability could be attributed to the addition of NaHCO₃ in the influent at a concentration of 14 g NaHCO₃/L. The addition was taking place from the first day of the experimentation period. The total bicarbonate alkalinity after the addition of NaHCO₃ increased from 8800 (from the previous mixture) to 16750 mg of CaCO₃/L. The difference in the value of the pH between the effluent of the acidogenic reactor and influent of the methanogenic is due to this addition. The influent of the methanogenic reactor was rich in VFAs, as anticipated due to the pretreatment achieved in the acidogenic reactor (Fig. 5.12(a) and (b)). The mean value of total VFAs concentration in the effluent was 1281 ± 478 mg/L. The removal of total carbohydrates in glucose equivalents was 97% (data not shown) in the whole process (both reactors) since the concentration of carbohydrates in the influent was 33.1 ± 1.4 g/L and decreased to 1.1 ± 0.3 g/L. The mean ammonium nitrogen concentration measured in the effluent of the methanogenic reactor was 1.1 ± 0.08 g/L verifying nitrogen availability for the anaerobic bacteria. In a recent study (Martinez-Garcia et al., 2007) other researchers used an aerobic pretreatment stage with mycelium C. tropicalis in order to improve the process efficiency by reducing the components (contained in the OMW and CW) that...
Chapter 5 | Biogas production from agro-industrial wastes through anaerobic co-digestion in a two-stage CSTR system

are inhibitory to methanogenesis. The second stage of this process was mesophilic anaerobic digestion in which the maximum OLR was 3.0 kg COD/m³·d, achieving an average COD removal of 83% similar to our results. It should be mentioned that in this study (Martinez-Garcia et al., 2007) the start up period was much longer (3 months) in which feed, diluted with tap water was used as influent.

![Figure 5-12: Variation of volatile fatty acids (VFAs) in (a) the influent and (b) the effluent during methanogenesis of CW/LCM mixture (90/10, v/v).](image)

Another study (Antonopoulou et al., 2008b) in which treatment of cheese whey with a two stage process took place, reports a yield of 22.23 L of CH₄/L of treated influent which is very similar to our experimental results (21.28 L of CH₄/L of treated influent). Similar results were achieved by other researchers (Demirer et al., 2000) for the anaerobic treatment of cheese whey (23.40 L of CH₄/L of treated influent). The value of the pH in the methanogenic reactor was stabilized from the beginning of the process without any fluctuations at all, during the experimentation period, even though the influent pH was constantly decreasing at the same time. The stability of the system against the influent pH could be attributed to the excess of alkalinity that was produced during the anaerobic digestion process (increased from 8800 to 16750 mg of CaCO₃/L). This fact indicates that the addition of NaHCO₃ in the influent of our experimentation should be reconsidered or at least gradually reduced.
5.6 Anaerobic mesophilic co-digestion of olive mill wastewater and cheese whey.

The composition of the previous feed was changed from 90% CW and 10% LCM to 80% OMW and 20% CW in the same two-stage system (Section 2.2.1). This mixture is typical and anticipated in Greece and other Mediterranean countries. From November the operation of olive-mills is started and also cheese making factories are still in operation. For example, a survey carried out in an Achaia region identified the olive mills, cheese making factories and dairy farm producing 511, 112 and 5 m³ of wastewaters respectively, on an average daily basis (Section 3.1-Table 3.1).

5.6.1 Acidogenic reactor

In this scenario, 0.85 g/L of urea (NH₂CONH₂) were added to the feed in order to ensure surplus in nitrogen concentration. Moreover, NaHCO₃ was added (14 g/L) in the feed of the acidogenic reactor to keep alkalinity in satisfactory levels. In this scenario, NaHCO₃ was added to the first stage (acidogenesis) and not to the second one (methanogenesis) in order to evaluate the performance of acidogenesis in higher pH condition (~pH 5.0). It is acknowledged that low pH values result in inhibition of the hydrogenase activity, which is regarded to as a key factor explaining the influence of pH on fermentative hydrogen production (Khanal et al., 2004; Mohd Yasin et al., 2011).

No soluble COD (SCOD) removal was observed for the 250 days of operation of the acidogenic reactor (Fig. 5.13(a)), whereas fluctuations were observed may be due to the complexity of the feeding medium, microbial population shifts taking place during the extended period of operation. Furthermore, negligible solids hydrolysis was observed (Fig. 5.13(b)) between influent and effluent of the acidogenic reactor. The pH value in the feed was 7.21 due to the NaHCO₃ addition, whereas from 27th to 65th day of operation was 5.09 because of the fact that no addition of NaHCO₃ was occurred (Fig. 5.15(b)). A decrease in the effluent pH (4.86 ± 0.09) comparing with the influent was noticed in the reactor (Fig. 5.15(b)). The reduction of the pH value was expected due to VFAs production by the acidogenic bacteria (Fig. 5.14(a) and (b)). The VFAs concentration in the influent was very low, whereas in the effluent increased as a result of carbohydrates degradation, existed mainly in cheese whey. For instance, the concentration of acetic acid increased to 5606 ± 826 mg/L, whereas an increase of propionic acid was noticed (1899 ± 460 mg/L). Compared to the previous scenarios, a significant increase of butyric acid was observed, may be due to higher pH value. According to Guo et al. (2010), the metabolic pathways involving acetic and butyric acid production appear to be favored at pH ranging from 4.5 to 6.0. Some fluctuations occurred mainly the first 120 days of operation and an important reason is the instability of the effluent pH during those days (Fig. 5.15(b)). The concentrations of other VFAs like isobutyric, valeric and isovaleric acid remained practically constant, less than 700 mg/L throughout the operation of acidogenic reactor, except isobutyric acid that increased for a while (~75th day) but after a few days, the concentration decreased again
and stabilized (Fig. 5.14(b)). Biogas production in the acidogenic reactor was also observed (Fig. 5.15(a)). No hydrogen production was observed which may be attributed to low pH. Initially, the biogas production rate ranged in low levels, then increased to 6.87 L/LR·d (103rd day), whereas, afterwards, the rate decreased again and stabilized to 3.31 ± 0.37 L/LR·d. The concentration of total carbohydrates decreased from 28.9 ± 2.50 to 13.45 ± 1.33 g/L (53% degradation), whereas no reduction of phenol concentration was observed.

![Figure 5-13](image_url)  
**Figure 5-13:** Concentration of (a) soluble COD (SCOD) and (b) total (TS) and volatile solids (VS) during acidogenesis of OMW/CW mixture (80/20, v/v).

![Figure 5-14](image_url)  
**Figure 5-14:** Variation of volatile fatty acids (VFAs) in (a) the influent and (b) the effluent during acidogenesis of OMW/CW mixture (80/20, v/v).
5.6.2 Methanogenic reactor

Following acidogenic step, the effluent of the acidogenic reactor was fed to the methanogenic which was operated at three different HRTs (16, 20 and 30 d). The increase of HRT values, lowering thus the digester’s loading rate, became in order to stabilize the methanogenic performance. As shown in Fig. 5.17(a), the biogas production rate increased (up to 2.85 L/LR·d) until the 18\textsuperscript{th} day of operation as a result of mixing with the previous mixture (90\% CW with 10\% LCM, v/v). After this day, a decrease in the rate was noticed with simultaneous VFAs accumulation (Fig. 5.18(b)). Switching from HRT 16 d (OLR of 7.76 kg COD/m\textsuperscript{3} ·d) to the higher HRTs (20 and 30 d) and lower OLR (6.21 and 4.14 kg COD/m\textsuperscript{3} ·d, respectively), a stability was obtained. Finally, at HRT of 30 d, the biogas production rate ranged at 1.13 ± 0.16 L/LR·d (Fig. 5.17(a)), while the composition of methane in the biogas was 55\%. The methane production rate at the steady state reached 0.67 ± 0.07 L CH\textsubscript{4}/LR·d (Fig. 5.17(a)), whereas the methane yield in the reactor was 162 mL CH\textsubscript{4}/g COD added or 291.31 mL CH\textsubscript{4}/g VS added. The low methane yield may be correlated with the high percent of OMW in the mixture and as a result the high concentration of phenols, inhibitors for methanogenic microorganisms. According to Gelegenis et al. (2007b), co-digestion of OMW with other waste alleviates the effect of inhibitory factors. Kougi\textsuperscript{a}s et al. (2014) studied the influence of the waste ratio of OMW and swine manure in batch experiments and reported that the maximum methane production and VS removal occurred at 40\% OMW in the mixture. Further increase in OMW fraction caused instability of the anaerobic process.

Fig. 5.16(a) illustrates the evolution of soluble COD (SCOD) as a function of experimental time of methanogenic reactor. At HRTs of 16 and 20 d an increase of
effluent SCOD was noticed, whereas at HRT 30 d was stabilized at 42.60 ± 4.75 g/L. The SCOD removal during the experimental period was 51.3% (mean value), whereas the TCOD removal at the steady state was 55%. The TS removal remained around 34.45% (from 95.39 ± 3.84 to 70.95 ± 1.96). On the other hand, the removal efficiency in VS concentration for the examined mixture was 58.57%, from 69.17 ± 3.16 to 43.62 ± 1.22 g/L (Fig. 5.16(b)).

Figure 5-16: Concentration of (a) soluble COD (SCOD) and (b) total (TS) and volatile solids (VS) during methanogenesis of OMW/CW mixture (80/20, v/v).

Figure 5-17: (a) Biogas and methane production rates and (b) pH value during methanogenesis of OMW/CW mixture (80/20, v/v).
Compared to the influent pH fluctuations in the methanogenic reactor, effluent pH was stabilized at 7.47 ± 0.17, mainly at HRT 30 d (Fig. 5.17(b)). Although the addition of NaHCO₃ was at the first stage of acidogenesis, the total bicarbonate alkalinity was at satisfied levels (14850 mg of CaCO₃/L) in the second stage of methanogenesis. The influent of the methanogenic reactor was rich in VFAs (Fig. 5.18(a)), as anticipated due to the pretreatment achieved in the acidogenic reactor. As mentioned before, at HRTs 16 and 20 d an accumulation of VFAs was observed, mainly acetic and propionic acid, whereas increasing the HRT (30 d), a slight decrease of them was observed. In particular, acetic acid increased up to 5870 mg/L whereas propionic acid up to 4020 mg/L. The removal of total carbohydrates in glucose equivalents was 90% (data not shown) in the whole process (both reactors), whereas phenolics compounds decreased by 38.5%. The mean ammonium nitrogen concentration measured in the influent and the effluent of the methanogenic reactor and negligible difference between them was observed (2.25 g N-NH₃/L).

Figure 5-18: Variation of volatile fatty acids (VFAs) in (a) the influent and (b) the effluent during methanogenesis of OMW/CW mixture (80/20, v/v).
5.7 Anaerobic mesophilic co-digestion of olive mill wastewater and liquid cow manure.

Experiments were carried out in a two-stage CSTR system (Section 2.2.1), one reactor for acidogenesis and the other one for methanogenesis. A mixture of olive mill wastewater (OMW) and liquid cow manure (LCM) at a ratio of 20:80 (v/v) was used in order to evaluate the performance in terms of biogas production, stability and COD reduction.

5.7.1 Acidogenic reactor

For start-up, the acidogenic reactor was filled up with 750 mL of feed consisting of 20% and 80% (v/v) of OMW and LCM, respectively and was operated anaerobically at a batch mode for 72 h in order to activate the indigenous microflora. The reactor was subsequently switched to continuous mode at the designated HRT (3 d). The reactor was then operated using a feed consisting of a mixture of 80% LCM and 20% OMW for 82 days.

Fig. 5.19(a) presents the evolution of soluble COD (SCOD), whereas Fig. 5.19(b) shows the variation of influent and effluent TS and VS concentrations throughout the experimentation period. The concentrations of VFAs in both the influent and effluent reactor streams are shown in Fig. 5.20(a) and (b), respectively. The biogas production from the reactor and its methane content was monitored and their evolution with reactor operation is presented in Fig. 5.21(a). Its production rate over the entire experimentation period was oscillatory with an average of 0.77 L/LR·d, in which the percentage of methane in the gas phase at the steady state was 29%, with the rest being mainly CO₂, since methane and carbon dioxide accounted for more than 94% of biogas constituents.

![Figure 5-19: Concentration of (a) soluble COD (SCOD) and (b) total (TS) and volatile solids (VS) during acidogenesis of OMW/LCM mixture (20/80, v/v).](image-url)
Estimation of the average influent and effluent TCOD concentration revealed a slight decrease of 2.4% which, however, is consistent with the low biogas production rate shown in Fig. 5.21(a). The hydrolysis of particulate organics, from the one hand, along with the SCOD degradation resulted in the SCOD evolution illustrated in Fig. 5.19(a).

Particulate COD, calculated from the difference between total and soluble COD, presented a 4.1% decrease (comparing mean values in the influent and effluent streams), which is in agreement with the 3.9% estimated removal of VS in the same streams. The pH in the reactor presented a decrease, from 7.3 to 6.5 (Fig. 5.21(b)), which, in fact, was anticipated and can be attributed to VFAs production by the acidogenic bacteria (Fig. 5.20(b)). During the last 30 days of reactor operation, total VFAs demonstrated an 104.5% increase (in g VFAs/L as shown in Fig. 5.20(b)) or 87.0% (in g COD/L units) compared with the influent total VFAs concentration, comprising the 54.7% of SCOD in the reactor. In particular, the acetic acid concentration increased from 4.94 to 8.02 g/L (mean influent and effluent values, respectively). However, it was noticed that although the influent acetic acid concentration remained rather constant throughout the experimentation period, its concentration in the effluent presented a tendency to increase as a function of operating time.

This effect can be attributed to an increase of acidogenic bacteria concentration in the reactor. The concentration of phenolic compounds into the reactor was decreased during its 82 days of operation from 1.4 g/L (being the initial concentration into the influent) to 1.15 g/L, i.e. a reduction of 18%. Higher removal percentages of phenolic compounds (40.7%) have been reported by Rincón et al. (2009) during treatment of two-phase olive oil mill solid residues in the hydrolytic acidogenic reactor of their two-stage anaerobic digestion process when, however, their reactor was operated at a much higher OLR (12.9 kg COD/m³·d) and an HRT of 12.4 d.

Figure 5-20: Variation of volatile fatty acids (VFAs) in (a) the influent and (b) the effluent during acidogenesis of OMW/LCM mixture (20/80, v/v).
5.7.2 Methanogenic reactor

The methanogenic reactor was seeded in the 6th day, after the initial filling of the acidogenic reactor, with 4 L anaerobically digested sludge taken from the municipal wastewater treatment plant of Patras (Greece). Following this step the effluent of the acidogenic reactor was fed to the methanogenic one and its HRT was set at 16 d. Alkalinity was kept in satisfactory levels without any addition of external source in the feed of the methanogenic reactor due to the high value of LCM alkalinity. The OLR implemented was 3.63 kg COD/m³·d.

The soluble COD removal during the examined scenario was 63.2% (mean value) (Fig. 5.22(a)), while the total COD was removed at the steady state at approximately 50%. The removal of COD in conjunction with gas production in the methanogenic reactor provided evidence of effective microbial activity from methanogenic bacteria. The TS removal efficiency remained constant for the last 30 days of operation, about 25.6% from 65.4 (influent) to 48.6 g/L (effluent). On the other hand, the removal efficiency in VS concentration for the examined scenario was 34.2% from 45.8 to 30.2 g/L (Fig. 5.22(b)). The VS removal was thus lower than both total COD and soluble COD removal as was the case in the work of Rincón et al. (2009) when their reactor was operated at high OLRs. This can be explained because the content in volatile solids at the effluents takes into account both the soluble compounds, which are the easily biodegradable ones, and the insoluble compounds (suspended compounds) that must be hydrolyzed and transformed into soluble compounds and VFAs in order to be eliminated. For the same reason, the total COD removed (which expresses the sum of both soluble COD and particulate COD) was lower than the soluble COD removed. A relatively long period of around 50 days was required to achieve the acclimatization of
methanogenic bacteria to the specific feeding substrate and finally steady state conditions as measured by COD degradation, VFAs and biogas evolution. The stability in reactor’s performance was partially hindered because of the gradual increase of VFAs in the feeding stream. In fact, the influent of the methanogenic digester was rich in VFAs, as anticipated due to the pre-treatment of the wastewater mixture in the acidogenic reactor (Fig. 5.24(a)).

![Figure 5-22:](image)

However, the constant increase of acetic acid in the influent of methanogenic reactor caused an imbalance in the reactor after the first 20 days of operation. Both the total COD removal and biogas production (Fig. 5.23(a)) were seriously affected as clearly identified by the rapid increase of acetic acid in the reactor (Fig. 5.24(b)). However, the acclimatized methanogenic population proved able to cope successfully with this problem. Within a week (from the 36th to the 44th day), the biogas production rate increased to 2.01 L/LR·d, whereas, afterwards, the rate decreased again and stabilized to 1.31 L/LR·d. The composition of biogas in methane fluctuated between 66.5 and 70.8% and stabilized at 68.86 ± 1.11% for the last 30 days of operation. The methane production rate at the steady state reached 0.91 L CH₄/LR·d, whereas the methane yield was 317.90 mL CH₄/g VS added. Acetic acid (Fig. 5.24(b)), after exhibiting a maximum of 3640 mg/L on the 38th day, decreased to 780 mg/L at the steady state conditions with the mean concentration of total VFAs ranging about 945 mg/L. During the same period (last 30 days of operation) the total VFAs concentration in the influent of the methanogenic reactor was 13323 mg/L (mean value). A gradual increase in effluent pH from 7.7 to 8.0 was observed during the experimentation period (Fig. 5.23(b)), although the influent value was about 7.0 (mean value) for the 82 days of operation. The stability of the system against influent pH could be attributed to the high
alkalinity levels of LCM, which contributes 80% (v/v) to the influent feeding mixture. Moreover, alkalinity increased from 12 g CaCO₃/L (mean value, constant for the first 40 days of operation) to 18 g of CaCO₃/L, at the end of experimentation phase. Therefore, co-digestion of OMW with LCM proved to be a stable system with an increased resistance to acidification, as also reported by Angelidaki and Ahring (1997). The high organic load and the presence of inhibitory compounds in the OMW (phenolics compounds and long-chain fatty acids) mandate its mixing with other industrial wastewater(s) in order to digest them successfully and eliminate the environmental risks due to incomplete treatment or discharge of raw wastewater to water or ground receptors.

![Figure 5-23](image1.png)

**Figure 5-23:** (a) Biogas and methane production rates and (b) pH value during methanogenesis of OMW/LCM mixture (20/80, v/v).

![Figure 5-24](image2.png)

**Figure 5-24:** Variation of volatile fatty acids (VFAs) in (a) the influent and (b) the effluent during methanogenesis of OMW/LCM mixture (20/80, v/v).
A number of different types of anaerobic reactors and methods have been investigated for the treatment of OMW in which dilution, nutrient addition, and alkalinity adjustment was required (Ursinos and Padilla, 1992; Sabbah et al., 2005). In a recent study, other researchers (Martínez-García et al., 2009) used an aerobic pre-treatment stage with mycelium C. tropicalis in order to improve the process efficiency by reducing the phenolic compounds (contained in the OMW) that are inhibitory to methanogenesis. It should be mentioned that, in their study, a mixture of 75% OMW with 25% (v/v) pig slurry was fed to an anaerobic fixed-bed reactor and the start-up period was much longer (4.5 months) than ours for the acclimatization and growth of the methanogenic bacteria. After that long start-up period, the OLR was increased from 1.25 to 5 kg COD/m³·d during the last 30 days, resulting in subsequent increase in overall COD removal and biogas production, up to maximum values of 85% and 29 L biogas/LR·d, respectively. Methane content of the biogas produced from the anaerobic digestion ranged between 65% and 74%.

Other studies report that the co-digestion of OMW (up to 83% (v/v) of crude OMW) with piggery effluent using two identical upflow anaerobic filters resulted to COD removal of about 70–80% of the influent COD (20–60 kg COD/m³) producing 1–3 m³/m³·d of gas (65–75% CH₄) (Marques et al., 1998; Marques, 2001). In another study, Angelidaki and Ahring (1997) presented a biogas production of approximately 1.25 L/LR·d in an anaerobic co-digesting process treating OMW with manure at a ratio of 50:50 under thermophilic conditions (55°C) at an HRT of 13 d with an organic loading rate of 7.8 kg COD/m³·d, which corresponded to a methane yield of 160 mL CH₄/g COD fed to the reactor. They also showed that the amount of nitrogen needed to obtain a stable degradation of OMW could be provided by cattle manure, swine manure or piggery effluent during co-degradation. Other researchers (Fountoulakis et al., 2008) state that the co-digestion (50:50) of OMW with slaughterhouse wastewaters and wine-grape residues results to a methane yield of 170 and 163 mL CH₄/g COD added, respectively. In a recent publication, Gelegenis et al. (2007b) have shown a methane production rate of 0.99 L/LR·d by digesting diluted poultry manure and olive mill effluent at a ratio of 50:50 under mesophilic conditions and a hydraulic retention time of 18 d. Our case proved to be more efficient (250.9 mL CH₄/g COD fed to the system) than the studies of Angelidaki and Ahring (1997) and Fountoulakis et al. (2008), whereas is at the same efficiency level (0.91 L CH₄/LR·d) with the study reported by Gelegenis et al. (2007b). It should be also mentioned that in our case the presence of phenols (because of the OMW) not only did not affect or inhibit the process, but furthermore biodegradation of the phenolic content of the OMW + LCM mixture was observed. 27% (from 1.4 to 1.02 g/L) of the phenolic content was degraded in the overall process of our anaerobic system. Beccari et al. (1998) have stated that polyphenols are the most bio-recalcitrant compounds in OMW, since only 20–30% of polyphenols were degraded in methanogenic conditions which, in fact, is in agreement with our findings.
5.8 Conclusions

Based on the results of this study, it has been demonstrated that co-digestion of agro-industrial wastes, such as olive mill wastewater (OMW), cheese whey (CW) and liquid cow manure (LCM), in a two-stage mesophilic (37°C) acidogenic-methanogenic CSTR system is a sustainable and environmentally-attractive method to treat these wastes and moreover convert them efficiently from a burden to society to a useful resource. The biogas produced can be used for the generation of heat and/or electricity. Apart from this energy potential however, co-digestion results in liquid and solid effluents that are also valuable, as they retain most of their nutrient constituents (nitrogen, phosphorus, trace elements, etc.) and so they can be used as fertilizers and soil improvers. The use of LCM as co-substrate in the anaerobic digestion of OMW and/or CW exhibited many advantages:

- provides stability to the process and minimization of nutrients addition, due to nitrogen and alkalinity supply to the digested mixture,
- improvement of the methane yield when compared to anaerobic digestion of OMW alone by diluting its high phenolic content
- contributes to a stable year-round operation of an efficient anaerobic digestion plant able to provide a feasible solution to the seasonal problem of OMW or CW management.

Since OMW and CW are seasonally available, it may be also concluded that these substrates can be treated in existing facilities that already digest LCM. They can either be directly fed to a LCM digester or firstly acidified (to control odours and their degradation) and safely stored for quite a long period, in order to be appropriately treated in existing LCM-digesting plants. In any case, the addition rate of these wastes and its percentage in the mixture must not exceed critical values for their co-digestion.
5.9 References


Chapter 6.

Assessment of single vs. two-stage anaerobic digestion using liquid cow manure or cheese whey.

6.1 Abstract

Growing interest in processes that involve the conversion of biomass to renewable energy, such as anaerobic digestion, has stimulated research in this field and a considerable number of research projects have been developed to assess ideal digestion conditions for different substrates such as agro-industrial wastes. Anaerobic digestion is an attractive process for the decomposition of organic wastes via complex microbial processes and subsequent conversion of metabolic intermediates to hydrogen and methane. The present study focuses on the exploitation of liquid cow manure (LCM) and cheese whey (CW) wastewaters as a source for biogas production, using continuous stirred tank reactors (CSTRs) and it presents a comparison between single-stage and two-stage anaerobic digestion system. For LCM treatment, no significant differences were found in a two-stage system compared to single-one. LCM, as lignocellulosic material, can be anaerobically digested in a single-stage process with the greatest methane production rate of 0.67 L CH₄/Lₚ·d at an HRT of 16 d. On the other hand, using CW as mono-substrate the two-stage process was considered as a better treatment system for CW wastewater than single one. During the single-stage process operational problems were observed due to the limited buffering capacity of CW effluents. However, the two-stage anaerobic digestion of CW produced a stable methane production rate of 0.68 L CH₄/Lₚ·d or 13.7 L CH₄/L_feed. The total and soluble COD removal was 76% and 70.68%, respectively.
Chapter 6  |  Assessment of single vs. two-stage anaerobic digestion using liquid cow manure or cheese whey

6.2 Introduction

Agro-industries such as cheese factories and dairy farms represent a considerable share of the Mediterranean countries economy. However, agro-industries processing raw feedstock of meat, milk, cheese and so on generate large volumes of excess of wastewaters and large amounts of by-products. Inappropriate disposal of untreated wastewaters effluents arise considerable environmental problems.

In particular, the dairy industry is one of the main sources of industrial effluent generation in Europe (Demirel et al., 2005). This industry is based on the processing and manufacturing of raw milk into products such as cheese, yogurt, ice cream, butter etc. The traditional Greek name-protected “feta” cheese product is usually made from sheep or goats’ milk and feta cheese whey effluents (CW) reach 30 m³/day (according to regional feta cheese production data; Dareioti et al., 2009). A considerable number of cheese industries are scattered across Greek mainland and thus large amounts of untreated cheese whey wastewaters disposal have strong impact to the ecosystem. Worldwide, 40.7 × 10⁶ tons per year of cheese whey are produced (Prazeres et al., 2012). Cheese whey is a high strength wastewater, with associated high biological (BOD₅) in the range 27 – 60 g/L and chemical oxygen demand (COD) in the interval 50 - 102 g/L (Carvalho et al., 2013). The BOD₅/COD ratio is commonly higher than 0.5 constituting a substrate easily biodegradable by anaerobic or aerobic digestions (Prazeres et al., 2012). It contains a significant amount of carbohydrates (4–5%), mainly lactose (45-50 g/L), proteins (6-8 g/L), lipids (4-5 g/L) and mineral salts (8-10% of dried extract); mineral salts include NaCl and KCl (>50%), calcium salts and others (Gonzalez Siso, 1996). CW also contains appreciable quantities of lactic (0.5 g/L) and citric acid, non-protein nitrogen compounds (urea and uric acid) and B-group vitamins (Venetsaneas et al., 2009).

On the other hand, manure from dairy farms contains about 2 - 8% of total solid (TS) and is typical wastewater from animal agriculture. Worldwide, more than 55 million tons of animal wastes are produced every year for subsequent disposal (Liao et al., 2006). Dairy manure naturally contains microorganisms that aid in manure degradation, but the breakdown results in the release of many compounds, including volatile organic compounds (VOC), that can negatively impact the environment (Page et al., 2014). Liquid cow manure (LCM) is one of the most polluting agro-industrial wastewaters. Intensive dairy farming produces large amount of manure which, when not properly managed due to its high organic matter, nitrogen and phosphorous concentrations, can cause severe environmental problems such as eutrophication of water bodies (Carpenter et al., 1998), groundwater contamination (Hao and Chang, 2002), air pollution by volatilization of ammonia and others compounds (Ryden et al., 1987) and soil degradation when manure is applied in excess.

Biological processes such as anaerobic digestion offer sustainable methods to address the problems that may be caused by disposal of wastes. According to Mata-Alvarez (2000), anaerobic digestion will gain more attention in the future for ecological...
reasons in particularly less fugitive green house gas emissions and stabilized organic matter residue. The two-stage anaerobic treatment process has several advantages over the conventional single-stage process, since it permits the selection and the enrichment of different bacteria in each anaerobic digester and increases the stability of the whole process by controlling the acidification phase in the first digester and hence preventing the overloading and/or the inhibition of the methanogenic population in the second digester (Schievano et al., 2012; Nathao et al., 2013). Separation of acidogenesis and acetogenesis and methanogenesis in the two-stage system can recover both hydrogen and methane (Martinez-Garcia et al., 2007; Dareioti et al., 2009; Dareioti et al., 2010). Although, the single-stage system is generally more predominant than two-stage for the full scale application (Kavacik and Topaloglu, 2010), several studies demonstrated that the two-stage process achieved higher overall degradation efficiency (Nathao et al., 2013) and is more advantageous than the single-stage system for the treatment of the waste feedstocks containing a large fraction of recalcitrant organic matters such as cow manure and cheese whey. Notwithstanding, it remains unclear the contribution of two-stage system compared to the conventional one. According to our knowledge, such chemical and microbiological aspects have not yet been clarified and there is the need of deeper efforts in comparing single and two-stage anaerobic processes.

The scope of this study was to examine the valorization of liquid cow manure and cheese whey wastewater for maximization of biogas production and also the evaluation of performance efficiency of both single and two-stage anaerobic digester operation.
6.3 Materials

The raw wastewaters used in the present study were cheese whey (CW) and liquid cow manure (LCM) and were collected according to Section 2.1.1. The composition of each raw waste stream (CW and LCM) is summarized in Table 6.1. Significant differences in the composition of wastewater streams were detected. In particular, CW presented higher organic content (76.46 ± 1.99 g COD/L) compared to LCM (60.09 ± 1.06 g COD/L). CW was characterized by high organic load mainly due to carbohydrates (lactose) and low nitrogen content in contrast with LCM (3.36 ± 0.00 g/L). LCM has a buffering capacity as a consequence of neutral pH (7.70 ± 0.06) and alkalinity in high levels (12.38 ± 0.32 g CaCO3/L). It is important to take into consideration the fact that the alkalinity should be high enough to avoid the destabilization of the system originated by the possible accumulation of volatile fatty acids.

Table 6-1: Chemical composition of cheese whey (CW) and liquid cow manure (LCM).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>CW</th>
<th>LCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td>6.10 ± 0.03</td>
<td>7.70 ± 0.06</td>
</tr>
<tr>
<td>TS</td>
<td>g/L</td>
<td>64.67 ± 2.62</td>
<td>69.29 ± 1.31</td>
</tr>
<tr>
<td>VS</td>
<td>g/L</td>
<td>54.09 ± 2.37</td>
<td>47.05 ± 0.69</td>
</tr>
<tr>
<td>Total COD</td>
<td>g/L</td>
<td>76.46 ± 1.99</td>
<td>60.09 ± 1.06</td>
</tr>
<tr>
<td>Soluble COD</td>
<td>g/L</td>
<td>58.46 ± 2.52</td>
<td>26.65 ± 0.19</td>
</tr>
<tr>
<td>BOD5</td>
<td>g/L</td>
<td>36.00 ± 0.53</td>
<td>19.72 ± 0.24</td>
</tr>
<tr>
<td>Total carbohydrates</td>
<td>g/L</td>
<td>54.89 ± 1.84</td>
<td>13.72 ± 0.95</td>
</tr>
<tr>
<td>Soluble carbohydrates</td>
<td>g/L</td>
<td>29.20 ± 0.92</td>
<td>0.96 ± 0.03</td>
</tr>
<tr>
<td>Total nitrogen, TKN</td>
<td>g/L</td>
<td>0.73 ± 0.02</td>
<td>3.36 ± 0.00</td>
</tr>
<tr>
<td>Ammonium nitrogen</td>
<td>g/L</td>
<td>0.10 ± 0.01</td>
<td>1.54 ± 0.02</td>
</tr>
<tr>
<td>Proteins</td>
<td>g/L</td>
<td>4.56 ± 0.13</td>
<td>21.00 ± 0.00</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>g/L</td>
<td>0.32 ± 0.00</td>
<td>0.66 ± 0.01</td>
</tr>
<tr>
<td>Soluble phosphorus</td>
<td>g/L</td>
<td>0.20 ± 0.00</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>Oil and grease</td>
<td>g/L</td>
<td>0.09 ± 0.00</td>
<td>3.24 ± 0.04</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>g CaCO3/L</td>
<td>0.80 ± 0.00</td>
<td>12.38 ± 0.32</td>
</tr>
<tr>
<td>TVFAs</td>
<td>g/L</td>
<td>0.00 ± 0.00</td>
<td>10.41 ± 1.63</td>
</tr>
</tbody>
</table>

* Mean values (± standard deviation); *b In equivalent glucose

6.4 Results and Discussion

The experiments were performed consecutively as shown in Table 6.2. Firstly with the digestion of LCM in two-stage (E1) and then in a single-stage system (E2) and subsequently the methanogenic reactor was fed with CW and was operated in single-stage (E3) and then in a two-stage system (E4).
For the two-stage process prior to feeding with LCM the reactors were operated with 80% liquid cow manure and 20% olive mill wastewater, following previous experimental work (Section 5.7). Subsequently, the reactors were fed daily with 250 mL LCM/day (HRT 16 days). Over the course of the experiment, the operation of the systems changed from two-stage ($E_1$) to single-stage ($E_2$). After the operation of LCM treatment, the methanogenic reactor was fed with CW in order to evaluate the performance of single-stage process ($E_3$) and finally the system changed to two-stage operating the acidogenic reactor ($E_4$). For the two-stage process primary to the feed of CW the acidogenic anaerobic reactor was operated at a batch mode for 72 h activating the indigenous microflora and was subsequently switched to continuous mode, a method that has been widely used (Mariakakis et al., 2011).

Using CW as substrate, alkalinity was kept in satisfactory levels by adding 14 g NaHCO$_3$/L and 0.50 g NH$_2$CONH$_2$/L were added to the feed in order to ensure surplus in nitrogen concentration in the feed of the methanogenic reactor.

### Table 6-2: Operating conditions for CSTRs.

<table>
<thead>
<tr>
<th>Operating conditions</th>
<th>Liquid Cow Manure Treatment</th>
<th>Cheese Whey Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment $E_1$</td>
<td>Experiment $E_2$</td>
</tr>
<tr>
<td>System type</td>
<td>Two-stage</td>
<td>Single-stage</td>
</tr>
<tr>
<td>HRT (d)</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>Flow rate (mL/d)</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>OLR (kg VS/m$^3$·d)</td>
<td>16.27</td>
<td>3.05</td>
</tr>
<tr>
<td>OLR (kg COD/m$^3$·d)</td>
<td>20.33</td>
<td>3.81</td>
</tr>
<tr>
<td></td>
<td>Cheese Whey Treatment</td>
<td></td>
</tr>
<tr>
<td>System type</td>
<td>Single-Stage</td>
<td>Two-stage</td>
</tr>
<tr>
<td>Phase</td>
<td>Methane</td>
<td>Acid</td>
</tr>
<tr>
<td>HRT (d)</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Flow rate (mL/d)</td>
<td>200</td>
<td>150</td>
</tr>
<tr>
<td>OLR (kg VS/m$^3$·d)</td>
<td>2.36</td>
<td>1.57</td>
</tr>
<tr>
<td>OLR (kg COD/m$^3$·d)</td>
<td>3.83</td>
<td>2.55</td>
</tr>
</tbody>
</table>
6.4.1 Liquid cow manure treatment

Firstly, continuous operation in a two-stage system was performed (E1) with HRT of 19 d (3 and 16 d, respectively), using raw LCM as a substrate. The acidogenic reactor was operated only for a period of 16 days owing to LCM is characterized by negligible concentration of carbohydrates, so no variation in characteristics was observed. For example, the biogas production rate was very low and equal to 0.20 ± 0.06 L/LR·d containing 25% of methane. The pH value was maintained constant at 7.54 ± 0.20 as a result of no volatile fatty acids production. For this reason, it was considered insignificant the operation of a two-stage process for LCM treatment.

On the contrary, it has been previously reported better performance of a two-stage system compared with a single-stage one. For example, Nielsen et al. (2004) operated a laboratory-scale reactor to compare the performance of a two-stage system and the conventional single-stage system for the treatment of cow manure. In this case, the two-stage system was constructed using the first reactor operated at 68°C with an HRT of 3 d and the second reactor operating at 55°C with 12 d HRT. They found out that the two-stage system has a 6-8% higher methane yield than the conventional one. This could probably be explained by the degradation of biofibers as a consequence of the thermal pretreatment in the first reactor (68°C).

Subsequently, the system changed from two-stage (E1) to a single-stage system (E2) and the methanogenic reactor was started to feed with raw LCM at HRT of 16 d (influent flow rate of 250 mL/d). After 65 days of operation at HRT of 16 d, reaching steady-state conditions in the reactor, an HRT increase to 20 d was obtained in order to investigate a possible further hydrolysis of lignocellulosic materials. Fig. 6.1(a) shows the evolution of biogas and methane produced throughout the experimental period. The biogas production rate presented a decrease until 35th day of the operation and after that increased and stabilized to 1.01 ± 0.04 L/LR·d, whereas the methane production rate followed similar proceeding and finally at the steady-state condition reached to 0.67 ± 0.04 L CH₄/LR·d. The composition of methane in the biogas at steady-state condition was 66.60%, while H₂S, NH₃ and H₂ concentrations were detected (698, 79.28 and 142.25 ppm, respectively). Switching to the higher HRT of 20 d, the biogas and methane production rates were declined and stabilized to 0.81 ± 0.04 L/LR·d and 0.56 ± 0.04 L CH₄/LR·d, respectively. The composition of methane in the biogas in this HRT value was 68.96%, whereas the detected H₂S, NH₃ and H₂ concentrations remained at the same levels approximately (690, 96.5 and 21.7 ppm, respectively). A methane yield was determined (Table 6.3) on the basis of volatile solids added (expressed as mL CH₄/g VS added) and the COD added (expressed as mL CH₄/g COD added). Highest methane yield of 242.16 mL CH₄/g VS added, proportional to the amount of substrate added, was observed at HRT value of 20 d. According to Lababut et al. (2011), a similar methane yield of dairy manure was obtained (242.7 mL CH₄/g VS added), using the biochemical methane potential (BMP) assay. On the other hand, Ahring et al. (2001) conducted a study to investigate the influence of temperature (55
and 65°C) on the cattle manure treatment using CSTR reactors with methane yields of 202 and 165 mL CH₄/g VS added, respectively.

Table 6-3: Steady-state operational parameters for LCM treatment in a single-stage system.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HRT (d)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.85</td>
<td>7.85</td>
<td></td>
</tr>
<tr>
<td>Biogas (L/Rd)</td>
<td>1.01</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>CH₄ (L/Rd)</td>
<td>0.67</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>CH₄ (%)</td>
<td>66.60</td>
<td>68.96</td>
<td></td>
</tr>
<tr>
<td>Yield (mL CH₄/g VS added)</td>
<td>219.63</td>
<td>242.16</td>
<td></td>
</tr>
<tr>
<td>Yield (mL CH₄/g COD added)</td>
<td>175.74</td>
<td>183.61</td>
<td></td>
</tr>
<tr>
<td>TCOD removal (%)</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>SCOD removal (%)</td>
<td>49.89</td>
<td>48.16</td>
<td></td>
</tr>
<tr>
<td>TS removal (%)</td>
<td>19.92</td>
<td>11.32</td>
<td></td>
</tr>
<tr>
<td>VS removal (%)</td>
<td>30.30</td>
<td>19.57</td>
<td></td>
</tr>
</tbody>
</table>

The methane production is a result of volatile fatty acids and especially acetic acid conversion to methane. The LCM was characterized by high concentration of VFAs (10.41 ± 1.63 g/L) with approximately 7.45 ± 1.19 g acetic acid/L and 1.77 ± 0.21 g propionic acid /L. As shown in Fig. 6.1(b), the operation of the methanogenic reactor from the previous scenarios (Section 5.7) started with 0.67 g TVFAs/L, mainly due to acetic acid concentration (81.67% of TVFAs).
Chapter 6  Assessment of single vs. two-stage anaerobic digestion using liquid cow manure or cheese whey

Figure 6-1: Evolution of (a) biogas and methane production rates and (b) main volatile fatty acids concentration during LCM treatment in a single-stage process.

After the period of 20 days the stability in reactor’s performance was partially hindered because of an accumulation of acetic acid (up to 2.02 g/L) with a consequent decrease of biogas and methane production rates, respectively. However, the acclimatized methanogenic population proved able to cope successfully with this problem, which was overcame throughout the experiment (0.54 ± 0.14 g acetic acid/L). Simultaneously, the propionic acid concentration was gradually increased up to 1.68 ± 0.12 g/L (the influent concentration of propionic acid, approximately) and afterwards stabilized. This could be explained by the washout of the previous treatment (80% liquid cow manure, 20% olive mill wastewater), in which microbial population could convert the existing propionic acid to methane (Section 5.7).

Increase in pH value was observed from 7.64 ± 0.20 (influent) to 7.85 ± 0.10 (effluent) for both HRTs. The stability of the system could be attributed to the high alkalinity levels of LCM (12.38 ± 0.32 g CaCO₃/L). Moreover, the alkalinity in the reactor was increased to 22.77 ± 1.08 g CaCO₃/L and 25.12 ± 0.19 g CaCO₃/L for HRTs of 16 and 20 d, respectively. The evolution of soluble COD as a function of experimental time of methanogenic reactor is plotted in Fig. 6.2(a). The soluble COD removal during the experimental operation at HRT of 16 d was 49.89%, whereas at HRT of 20 d was 48.16% (mean values). The total COD removal for both HRTs at the steady state conditions was 20%. On the other hand, the removal efficiencies, in terms of TS and VS (Fig. 6.2(b)), were higher at HRT of 16 d and equal to 19.92% and 30.30% respectively, compared to HRT of 20 d (11.32% and 19.57%, respectively). The mean value of TKN concentration in the effluent was 5.18 g NH₃-N/L, whereas ammonia nitrogen mean concentration was 3.02 g NH₃-N/L for both HRT values.

Figure 6-2: Evolution of (a) soluble COD concentration and (b) total and volatile solids during LCM treatment in a single-stage process.
6.4.2 Cheese whey treatment

Cheese whey (CW) is a quite difficult substrate to treat anaerobically (especially in highly loaded reactors) because of its high organic content, low bicarbonate alkalinity (50 meq/L) tendency to acidify rapidly and granulation difficulties (Malaspina et al., 1996). However, the development of some technologies and systems of anaerobic digestion for the CW treatment, prove that it is worthy and valuable source of energy. Single-stage anaerobic digestion of cheese whey was operated at the beginning with HRT of 20 d \((E3)\) as a sequence of the previous single-stage operation of LCM \((E2)\). Biogas production rate was gradually augmented for 35 days of operation reaching 3.29 L/LR·d, whereas a decrease of biogas rate to 0.40 L/LR·d (mean value) was observed for the next few days (Fig. 6.3(a)). The change of HRT from 20 d to 30 d and 40 d resulted in the production of 0.62 and 0.53 L/LR·d, respectively. The highest methane production rate of 1.72 L CH\(_4\)/LR·d was observed on the 35\textsuperscript{th} day and stabilized to 0.24 L CH\(_4\)/LR·d. The composition of methane in the biogas on the 35\textsuperscript{th} day was 52.28\%. The reduction of methane production rate after the 34\textsuperscript{th} day was due to the inhibition of the methanogenic biomass by volatile fatty acids (VFAs) accumulation (Fig. 6.3(b)), suggesting the instability of the process.

The high concentration of total VFAs was mainly due to the high acetic acid concentration (up to 9.68 g/L), which is normally converted into methane. However, also propionic acid was increased (up to 5.4 g/L), leading to an inhibition of the system with a consequent reduction of the methane production. Weiland (2010) reported that acetic acid is usually present in higher concentration than other fatty acids, but propionic and butyric acids are more inhibitory effective to methanogens. The pH decreased to 6.46 after 68 days as a result of VFAs accumulation (data not shown). The alkalinity in the system was initially 24.05 g CaCO\(_3\)/L due to LCM treatment \((E2)\), then

![Figure 6-3: Evolution of (a) biogas and methane production rates and (b) main volatile fatty acids concentration during CW treatment in a single-stage process.](image)
decreased to 19.5 g CaCO$_3$/L on the 35$^{th}$ day and finally declined to 10.18 g CaCO$_3$/L. However, the buffering capacity of NaHCO$_3$ (addition of 14 g/L) was incapable to recover the process to the initial pH and proliferation of VFAs was observed. Accumulation of organic acids in the digester lowered the pH and caused inhibition to the point of depression of biogas production rate and methane yield. The tendency of CW effluent acidification was inevitable and nonreversible resulting into the decrease of methanogenic capacity thus the biogas and methane production is limited. Operating the digester without pH control resulted into the inhibition of methanogenic bacteria thus no process activation was observed despite the reset of pH at the initial value (Ghaly and Ramkumar, 1999).

The highest soluble COD removal was achieved until 40$^{th}$ day of the operation and was 94.01% (from 53.46 to 3.20 g/L). An effective microbial activity from methanogenic bacteria was achieved since COD was removed and gas production was observed. However, the effluent soluble COD was increased from 3.20 to 24.17 g/L after the 40$^{th}$ operation day (Fig. 6.4(a)). The removal efficiency of TS remained constant at 40.12% whereas for VS concentration for the examined scenario the removal efficiency was 48.09% (Fig. 6.4(b)). Finally, the influent concentration of total carbohydrates (31.6 g equivalent glucose/L) was decreased significantly, thus 94% of carbohydrates were consumed (data not shown).

![Figure 6-4: Evolution of (a) soluble COD concentration and (b) total and volatile solids during CW treatment in a single-stage process.](image_url)

Taking into account the aforementioned results, it is obvious that the contribution of LCM from the previous experiment ($E2$) was very crucial for biogas and methane productivity. Feeding the system with CW resulted in washout of LCM which kept the alkalinity in high levels the first days of operation. The proportion of CW and LCM the 34$^{th}$ day of operation gave the highest productivity. In our previous study, we reported the co-digestion of CW and LCM at a ratio of 90:10 (v/v) in a two-stage system with a
HRT of 19 d (Section 5.5; Dareioti et al., 2009), in which high removal of soluble COD (85.2%) was obtained. Bertin et al. (2013) investigated the optimal mix ratio of the two substrates in batch experiments and found that the methane yield improved (2.5 fold the value obtained by CM and 27 fold the value obtained by CW when used alone) using the mixture with ratio 50:50. In the latter case, acidification to low pH value was observed when the CW fraction was higher than 60%. Additionally, Kavacik and Topaloglu (2010) obtained the higher biogas (1.51 L/LR·d) from the co-digestion of cheese whey with dairy manure with methane content of 60% and suggested that co-digestion of these two wastes is advantageous than processing each one separately. According to Labatut et al. (2011), co-digestion of dairy manure with easily-degradable substrates (i.e. cheese whey) increases the specific methane yields when compared to manure-only digestion.

Because of the instability of the single-stage process using CW as a substrate, it was considered appropriate the investigation of the two-stage system (E4). The acidogenic reactor was operated at HRT of 3 d with an influent flow rate of 250 mL/d, which corresponds to an organic loading rate (OLR) of 19.12 g COD/d. The biogas production rate was 0.13 ± 0.01 L/LR·d with a composition of ~90% in CO₂ and 2-4% of H₂ (Fig. 6.5(a)). Fermentable carbohydrates in CW wastewater were easily and rapidly hydrolyzed and converted to simple sugars. As a consequence of carbohydrates fermentation, an increase of TVFAs concentration from 2.60 ± 0.37 to 7.74 ± 0.69 g/L (mean values of influent and effluent) was obtained (Fig. 6.5(b)). The main VFAs produced were acetic (4.20 ± 0.38 g/L) and propionic (2.52 ± 0.11 g/L) acids, whereas the other acids were detected at much lower concentrations (lower than 0.5 g/L). Previous research studies reported that acetic and propionic acids were the prevalent VFAs formed during the acid-phase anaerobic digestion of different substrates of primary sludge and CW wastewater (Elefsiniotis and Oldham, 1994; Ghary and Ramkumar, 1999). However, other researchers sustained that among the main microbial products measured included butyric acid (Yang et al., 2003; Venetsaneas et al., 2009). Simultaneous decrease in the pH value of the effluent from 6.14 to 3.51 was observed, which is regarded to as a key factor explaining the influence of pH on fermentative hydrogen production. It is acknowledged that anaerobic fermentative hydrogen production is suppressed by both low and high pH values because the pH is a crucial parameter for bioprocesses (Wang and Wan, 2009). It has been reported that maximum hydrogen yields are obtained when the pH of the culture medium is between 5 and 6 (Fang and Liu, 2002). According to Dareioti et al. (2014), low pH values result in inhibition of the hydrogenase activity, whereas controlled pH conditions also affect on the soluble end-products distribution. The metabolic products as well as the hydrogen production depended on the dominant microbial species contained in the whey and on the final pathway they followed under the prevailing conditions. CW characterization analysis exhibited low levels of protein concentration, thus negligible amounts of fermentation acid products (i.e. isobutyric, valeric and isovaleric acid) were expected. Such acids are largely associated with the fermentation of proteins (Elefsiniotis and
Oldham, 1994). The soluble COD was constant comparing mean values in the influent and effluent streams. However, the removal efficiency of TS and VS was 30.6% and 29.84%, respectively (Fig. 6.5(c)).

A methane bioreactor was used for treating the acidified effluent from the first stage in order to assess the rate and extent of cheese whey biodegradation in a two-stage system. The digester was operated at HRT of 20 days for about 69 days (which corresponded to 3.5 fold the HRTs), with an influent flow rate of 200 mL/d. In general, long HRT values (5–20 days) are used in studies on anaerobic treatment of undiluted cheese whey to overcome the potential instability problems and the failure of the process (Ergüder et al., 2001) because of its rapid acidification tendency. Fig. 6.6(a) shows the evolution of biogas and methane produced throughout the experimental period. The biogas production rate presented a high increase and stabilized to $1.47 \pm 0.05$ L/LR·d, whereas the methane production rate at the steady-state reached to $0.68 \pm 0.07$ L CH$_4$/L-R·d.
Figure 6-5: Evolution of (a) biogas production rate, (b) main volatile fatty acids concentration and (c) total and volatile solids concentration during acidogenesis of CW treatment in a two-stage process.

The composition of methane in the biogas at steady-state condition was 49.53%, whilst H₂S and H₂ concentrations were detected (545 and 159 ppm, respectively). Methane yields were estimated with regard to the methane productivity. Methane yield of 290.36 mL CH₄/g VS added was obtained in our study compared with obtained methane yield of 423.6 mL CH₄/g VS added reported by Labatut et al. (2011) in BMP study. According to the table values a yield of 13.7 L CH₄/L of influent CW was obtained, higher than Venetsanesas et al. (2009) reported (6.7 L CH₄/L of influent), for CW treatment in a two-stage system.

The influent of the methanogenic digester was rich in VFAs, as anticipated due to the pretreatment in the acidogenic reactor. During the first 20 days of the experiment an accumulation of acetic and propionic acid was observed in the methanogenic reactor (Fig. 6.6(b)), which however remained stable at 7.38 ± 0.08 g/L for acetic acid and 3.96 ± 0.13 g/L for propionic acid. Nevertheless, no inhibition of the reactor’s performance was detected. A gradual increase of the pH value from 6.34 ± 0.23 (influent value) to 7.31 ± 0.03 (effluent value) was observed during the methanogenic process by adding 14 g NaHCO₃/L in the influent. The total bicarbonate alkalinity after the addition of NaHCO₃ was 4.6 g CaCO₃/L in the influent, whereas was kept at acceptable levels of 13.6 g CaCO₃/L in the effluent. Venetsanesas et al. (2009) demonstrated that alkalinity adjustment with NaHCO₃ compared to NaOH in the feeding CW wastewater showed higher hydrogen production rate. Although inoculation of NaHCO₃ involves augmentation of CO₂ percentage through the bicarbonate conversion to gaseous CO₂, its non-phosphate-containing buffering capacity is recommended for manual pH correction (Ghary and Ramkumar, 1999).

Figure 6-6: Evolution of (a) biogas and methane production rates and (b) main volatile fatty acids concentration during methanogenesis of CW treatment in a two-stage process.
The total carbohydrate concentration in the effluent was consistently lower than $1.02 \pm 0.14$ g/L, corresponding to removal yield 95.3%. The total COD removal was 76% (from 76.46 to 18.35 g/L) whereas the soluble COD removal was 70.68% (from 61.05 to 17.90 g/L) (Fig. 6.7(a)). The removal of COD in conjunction with the biogas production in the methanogenic reactor provided evidence of effective microbial activity from methanogenic bacteria. Moreover, Fig. 6.7(b) presents the evolution of total and volatile solids throughout the experiment, whereas the TS removal efficiency was 32.64% and the removal efficiency in VS concentration was 59.55%.

![Graph](image)

**Figure 6-7:** Evolution of (a) soluble COD concentration and (b) total and volatile solids during methanogenesis of CW treatment in a two-stage process.

Patel et al. (1995) investigated anaerobic digestion of high strength CW with COD of 70 g/L, using an upflow fixed film reactor (UFFR) with various support media, obtaining a maximum COD removal of 81%. Gavala et al. (1999), using a laboratory scale UASB reactor for CW wastewaters (influent concentrations between 12 and 60 g COD/L), achieved a maximum COD removal efficiency of 98% at an HRT of 6 d with influent COD of 37 g/L. Saddoud et al. (2007) studied a system consisting of stirred acidogenic reactor followed by methanogenic reactor coupled with a membrane filtration system for the removal of soluble effluents and the preservation of solids. The average removal of COD was 98.5%, whereas the biogas production was higher than 10 times reactor volume.

The results, therefore, indicated that the two-stage process showed a better performance in treating cheese whey wastewater over the single-stage system in terms of biogas production at mesophilic conditions. The acidogenic pretreatment increases
both the methane content in the biogas and the methane production in the methanogenic reactor. This is in close agreement with Bertin et al. (2013), who also demonstrated the much higher efficiency of the two-stage systems than the single-stage one treating CW and CM in co-digestion. Additionally, Yang et al. (2003), comparing one and two phase thermophilic anaerobic digestion systems for CW treatment concluded that the two-phase process was more suitable for the management of CW wastewaters, whilst the COD removal in the two-phase process was 116% higher than this of the single-phase system.

6.5 Conclusions

Anaerobic digestion (AD) of liquid cow manure (LCM) and cheese whey (CW) may seem a complex task due to the high organic content. This study successfully demonstrated more efficient performance in two-stage than single-stage fermentation of CW. In conventional single (one phase) reactor, the acid forming and methane forming microorganisms are kept together and lead to instability and control problems. With the two-stage AD processes, these problems can be overcome and therefore this system should be considered as a better treatment system for CW wastewater. On the other hand, negligible difference between single and two-stage process with regard to LCM treatment. Nevertheless, with the AD process a percentage of pollution load remains in the treated effluent resulting in the necessity of subsequent processing.
6.6 References


Chapter 7.

Anaerobic co-digestion of agro-industrial wastewaters in a two-stage system.

7.1 Abstract

This study is focused on the anaerobic co-treatment of a mixture of agro-industrial liquid wastewaters, consisting of olive mill wastewater, cheese whey and liquid cow manure (with a ratio of 55:40:5, v/v/v). Firstly, batch experiments were performed under mesophilic conditions (37°C) at a range of pH from 4.5 to 7.5 in order to investigate the impact of pH on the production of bio-hydrogen and end-products from this mixture. The main end-products identified were acetic, propionic, butyric, lactic acid and ethanol. The highest hydrogen production yield was observed at pH 6.0 (0.642 mol H₂/mol equivalent glucose consumed), whereas the maximum VFAs concentration (i.e. 13.43 g/L) was measured at pH 6.5. The composition of acidified effluent in acetic and butyric acid was similar at pH 6.0 and 6.5, albeit an increase of propionic acid was observed in higher pH. Lactic acid was identified as a major metabolite which presented an intense accumulation (up to 11 g/L) before its further bioconversion to butyric acid and hydrogen. After finding the optimum pH, a two-stage anaerobic digestion system consisting of two continuously stirred tank reactors (CSTRs) operating at mesophilic conditions (37°C) were used to investigate the effect of hydraulic retention time (HRT) on hydrogen and methane production. The acidogenic reactor was fed with the same mixture (55% olive mill wastewater, 40% cheese whey and 5% liquid cow manure, v/v/v) and operated at five different HRTs (5, 3, 2, 1 and 0.75 d) aiming to evaluate hydrogen productivity and operational stability. The highest system efficiency was achieved at HRT 0.75 d with a maximum hydrogen production rate of 1.72 L/LR·d and hydrogen yield of 0.54 mol H₂/mol carbohydrates consumed. The methanogenic reactor was operated at HRTs of 20 and 25 d with better stability observed at HRT 25 d, whereas accumulation of volatile fatty acids took place at HRT 20 d. The methane production rate at the steady state of HRT 25 d reached 0.33 L CH₄/LR·d.
Chapter 7  Anaerobic co-digestion of agro-industrial wastewaters in a two-stage system

7.2 Introduction

Olive mills, cheese factories and cow farms are agro-industries that represent a considerable share of the worldwide economy with particular interest focused in the Mediterranean region. These industries generate millions of tons of wastewaters and large amounts of by-products, which are in many cases totally unexploited and thus dangerous for the environment. More specifically, approximately $5.4 \times 10^6$ m$^3$ of olive mill wastewater is produced annually worldwide (Basheer et al., 2007), while 180–190 $\times 10^6$ tons per year of cheese whey are generated (Mollea et al., 2013) and 55 $\times 10^6$ dry tons of animal manure are collected every year for subsequent disposal (Liao et al., 2006). Although technical solutions for their treatment do exist, their application is still lacking in these agro-industries due to their regional spatial distribution, their small to medium-size family character and also their seasonal nature.

The liquid by-product of olive oil production using the three-phase centrifugation process, i.e. olive mill wastewater (OMW), is recognized in the whole Mediterranean, Aegean and Marmara region as a severe environmental problem because of its high organic content and recalcitrance to biodegradation which is particularly due to the presence of phenolic compounds. The total concentration of phenols in OMW, which contribute to a high toxicity and antibacterial activity (Capasso et al., 1995) can reach up to 24 g/L (Borja et al., 2006; Paraskeva and Diamadopoulos, 2006). Its chemical oxygen demand (COD) and biological oxygen demand (BOD$_5$) range from 25 to 220 g O$_2$/L and 9 to 100 g O$_2$/L respectively (Paraskeva and Diamadopoulos, 2006), contributing to a significantly high bio-energy content. On the other hand, nitrogen, which is one of the essential nutrients required for anaerobic bioprocesses, is usually low in OMW, while the carbon-to-nitrogen-to-phosphorus ratio is around 100:1.77:0.94 (Azbar et al., 2008)

Cheese manufacturing industry generates large amounts of high strength wastewater, with associated high biological (BOD$_5$) and chemical oxygen demand (COD). Cheese whey (CW) is a by-product of cheese manufacturing which mainly contains a significant amount of carbohydrates (4–5%), mainly lactose (45–50 g/L), proteins (6–8 g/L), lipids (4–5 g/L) and mineral salts (8–10% of dried extract); mineral salts include NaCl and KCl (>50%), calcium salts and others. CW also contains appreciable quantities of lactic (0.5 g/L) and citric acid, non-protein nitrogen compounds and B-group vitamins (Venetsaneas et al., 2009). Despite the high carbohydrate content of CW which is suitable for biological processing, the anaerobic treatment of raw CW is quite problematic due to its low bicarbonate alkalinity (50 meq/L), high COD concentration (up to 70 g COD/L) and tendency for rapid acidification (Mawson, 1994; Prazeres et al., 2012).

Agricultural wastewaters, including liquid animal manure (LCM), are characterized by high organic content with high amounts of total solids, ammonia and pathogens (Rico et al., 2011). Insufficient or uncontrolled handling and disposal of such highly
polluting agro-wastes encounter imminent danger to environment and thus to public health.

Multiple waste streams of organic substrates can be anaerobically co-digested to generate a homogeneous mixture increasing both process and equipment performance. Co-digestion of different types of organic by-products such as agro-industrial wastewaters has been increasingly applied in order to enhance the biogas production and overcome a number of problems such as nutrient imbalance, rapid acidification and presence of inhibiting compounds, among other factors (Paraskeva and Diamadopoulos, 2006; Martinez-Garcia et al., 2007; Azbar et al., 2008; Dareioti et al., 2009; Dareioti et al., 2010; Frigon et al., 2012). Two-stage anaerobic digestion (AD) for integrated bio-hydrogen and bio-methane production from organic materials has been reported to promise higher process efficiency and energy recoveries as compared to traditional one-stage AD (Schievano et al., 2012). Moreover, the first stage of acidogenesis may be optimized so that bio-hydrogen generation takes place, whereas at the second stage of methanogenesis, methane evolves. Optimum environmental and operational conditions for each microbial community (i.e. acidogens and methanogens) may be achieved in such a separated two-reactor system resulting in the production of significant amounts of gaseous products (hydrogen and methane) in the overall anaerobic two-stage system.

If correctly operated, the first stage of these systems can achieve several steps including hydrolysis, acidification, and hydrogen gas production. The performance of an acidogenic reactor is of paramount importance especially during the two-phase anaerobic stabilization of wastes, since the acid reactor should provide the most appropriate substrate for the subsequent methanogenic one. Since many different types of bacteria are involved in the fermentation of organic wastes, anaerobic acidification of agro-industrial wastes may produce volatile fatty acids (VFAs), alcohols, H₂, CO₂ and other intermediate products (i.e. lactic acid). For sustainable bio-hydrogen production the feedstock has to meet certain criteria. For example, carbohydrate-rich feed stocks produced from sustainable resources in large quantities are of paramount importance, since they can be easily fermented favoring thus energy recovery, require minimum pretreatment and are of low cost. Different substrates such as solid wastes and food industry wastewaters can be easily fermented to produce hydrogen (Kapdan and Kargi, 2006) although pure carbohydrates (e.g. glucose, sucrose) have been most commonly used (Wang and Wan, 2009).

Enhancing biological production of hydrogen gas is of great interest nowadays, because it is a promising alternative to fossil fuels due to its clean and high-energy yield. However, very little information is available even on pilot-scale (Table 7.1) and practically none on full-scale. A pilot-scale study is critical to testify the productivity before a new biotechnological process is put into full-scale operation. In larger than lab-scale, the use of mixed microbial cultures is a cost-effective and promising approach to achieve bio-hydrogen production.
Table 7-1: Bio-hydrogen production in pilot-scale plants using various substrates.

<table>
<thead>
<tr>
<th>Reactor type</th>
<th>Working volume (m²)</th>
<th>Substrate</th>
<th>pH</th>
<th>T (°C)</th>
<th>HRT</th>
<th>OLR</th>
<th>Optimal index (value)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous stirred tank reactor</td>
<td>0.2</td>
<td>food waste</td>
<td>5.7</td>
<td>55°C</td>
<td>3.3 d</td>
<td>16.3 kg TVS/m³-d</td>
<td>Specific H₂ production (66.7 L H₂/kg TVS_{in})</td>
<td>Cavinato et al. (2012)</td>
</tr>
<tr>
<td>(CSTR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gas production rate (2.6 m³/m²-d)</td>
<td></td>
</tr>
<tr>
<td>Plug-flow reactor</td>
<td>0.15</td>
<td>kitchen waste</td>
<td>5.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Specific H₂ production (72 mL H₂/g VSS_{in})</td>
<td>Jayalakshmi et al. (2009)</td>
</tr>
<tr>
<td>Hydrolytic Upflow Sludge Bed (HUSB)</td>
<td>25.5</td>
<td>domestic wastewater</td>
<td>7.07</td>
<td>21 – 14°C</td>
<td>0.5-3.2 kg COD/m³-d</td>
<td>-</td>
<td>-</td>
<td>Álvarez et al. (2008)</td>
</tr>
<tr>
<td>Continuous flow bioreactor</td>
<td>0.1</td>
<td>brewery grains</td>
<td>6.0</td>
<td>40°C</td>
<td>3 d</td>
<td>32 L/day (3%)</td>
<td>H₂ production (20 L H₂/d)</td>
<td>Chou et al. (2008)</td>
</tr>
<tr>
<td>Continuous-flow stirred tank reactor (CSTR)</td>
<td>0.2</td>
<td>mixture of pulverized garbage/ shredded paper</td>
<td>5.8-6.0</td>
<td>60°C</td>
<td>1.2 d</td>
<td>97 kg COD/m³-d</td>
<td>H₂ production rate (5.4 m³/m²-d)</td>
<td>Ueno et al. (2007)</td>
</tr>
<tr>
<td>Continuous flow anaerobic fermentation reactor</td>
<td>1.48</td>
<td>molasses</td>
<td>4.5</td>
<td>35°C</td>
<td>4.11 h</td>
<td>68.21 kg COD/m³-d</td>
<td>H₂ production rate (5.57 m³/m²-d)</td>
<td>Ren et al. (2006)</td>
</tr>
<tr>
<td>Batch</td>
<td>3</td>
<td>organic municipal solid wastes</td>
<td>4.5</td>
<td>14–22°C</td>
<td>4.5 d</td>
<td>40 kg VS/m²·d</td>
<td>Yield (g SCOD/g VS_{in})</td>
<td>Bolzonella et al. (2005)</td>
</tr>
<tr>
<td>Leaching-bed reactors</td>
<td>0.05</td>
<td>food waste</td>
<td>6.2</td>
<td>37°C</td>
<td>0.22 d</td>
<td>-</td>
<td>Cumulative H₂ production (742.7 mL H₂)</td>
<td>Han et al. (2005)</td>
</tr>
</tbody>
</table>
Anaerobic co-digestion of agro-industrial wastewaters in a two-stage system

Chapter 7

Not only hydrogen gas itself is a beneficial energy source but also VFAs can be used further for methane production by methanogenesis. The reactions involved in biogenic hydrogen production are rapid and can be effectively used for treating large quantities of organic wastes. The behavior of acidogens in a two-phase process plays a primary role in producing major substrates, such as short-chain organic acids and hydrogen. A series of operating parameters including pH, temperature and hydraulic retention time are known to influence the performance of fermentation and the formation of intermediate fermentative products such as hydrogen, organic acids and ammonia (Wang and Wan, 2009). Among these factors, pH has been found to be crucial to the distribution of acidogenic products (Ren et al., 1997; Chen et al., 2002). Although a substantial number of studies have been conducted on the optimal pH range for fermentative hydrogen production, the results are often inconsistent due to differences in substrate and seed type and other operating conditions adopted (Luo et al., 2010; Wu et al., 2010). Furthermore, many studies have been conducted to look into the effect of initial pH on fermentative hydrogen production, whereas the importance of pH control has rarely been investigated (Wang and Wan, 2009). It is acknowledged, for example, that low pH values result in inhibition of the hydrogenase activity, which is regarded as a key factor explaining the influence of pH on fermentative hydrogen production (Khanal et al., 2004; Mohd Yasin et al., 2011). The metabolic pathways involving acetic and butyric acid production appear to be favored at pH ranging from 4.5 to 6.0, while neutral or higher pH are believed to promote ethanol and propionate production (Guo et al., 2010). Meanwhile, the reported optimal pH values for different substrates differed substantially from 4.0 to 6.5, but for each specific situation, the optimal pH range was quite narrow (usually within 0.5). For instance, an optimal pH value of 6.0 was obtained using cheese whey (Ferchichi et al., 2005), food wastes (Jiang et al., 2013) and kitchen wastes (Zhang et al., 2013), a lower pH value of 5.5 was considered as optimum using glucose (Fang and Liu, 2002), whereas the initial pH of 4.5 gave the highest specific hydrogen production potential when sucrose and starch was used as substrate (Khanal et al., 2004).

Furthermore, the hydraulic retention time (HRT) is reported as one of the most important parameters significantly affecting microbial ecology and characteristics in CSTR operational systems and must be optimally controlled for each waste mixture fed in such a two-stage system. Literature is still lacking such information for two-stage systems fed with agro-industrial waste mixtures. Dinsdale et al. (2000), for example, studied the mesophilic anaerobic co-digestion of a mixture of activated sludge, fruit and vegetable waste in a two-stage system consisting of an acidogenic continuous stirred tank reactor (CSTR) and a methanogenic inclined tubular digester operated at 30°C. Their system achieved stable anaerobic digestion at an overall system loading rate of 5.7 g VS/L·d, overall HRT of 13 d (3 day acidogenic HRT, 10 day methanogenic HRT), with 40% VS destruction and a system biogas yield of 0.37 m³/kg VS added.
This study is focused on the anaerobic co-treatment of a mixture of agro-industrial liquid wastewaters with a high organic content, consisting of 55% olive mill wastewater, 40% cheese whey and 5% liquid cow manure, in a two-stage continuous anaerobic system. The efficiency of this waste mixture for biogas production has been demonstrated by the authors in a previous study (Dareioti et al., 2009). In the particular mixture, dilution of OMW with CW and LCM drastically reduced the inhibitory effect of contained polyphenols in OMW, whereas the organic content of the mixture still remained quite high for being converted to biogas. Moreover, the lack of nitrogen in OMW and CW was balanced by the high nitrogen content of LCM. The specific aim of the present work was to study the effect of pH on hydrogen production and also the effect of HRT on hydrogen and methane productivity and stability in the acidogenic and methanogenic reactor respectively comprising of the overall system. First of all, our purpose was to determine experimentally the optimum pH value and identify the main metabolic pathways followed for both hydrogen and volatile fatty acids production in the acidogenic reactor of a two-stage anaerobic co-digestion system. The pH values tested ranged from 4.5 to 7.5 with 0.5 increment and were maintained constant throughout the process. Finally, a two-stage anaerobic digestion system consisting of two continuously stirred tank reactors (CSTRs) operating at mesophilic conditions (37°C) were used to investigate the effect of hydraulic retention time (HRT) on hydrogen and methane production.
7.3 Materials and Methods

7.3.1 Agro-industrial wastewaters

The raw wastewaters used in the present study included olive-mill wastewater (OMW), cheese whey (CW) and liquid cow manure (LCM) were collected and stored according to Section 2.1.1. The composition of each raw waste stream (OMW, CW and LCM) as well as their mixture (55% OMW, 40% CW and 5% LCM, v/v/v) is summarized in Table 7.2. After homogenization of the waste mixture used in each experiment, duplicate samples were received and measured. Throughout the experimentation period samples were also taken periodically and measured in duplicate from the separate waste streams (OMW, CW, LCM), in order to verify their integrity and constant characteristics. Significant differences in the composition of wastewater streams were detected. In particular, OMW presented the highest organic content (140 g/L as total COD and 56.01 g/L as TOC), compared to CW (75 g COD/L and 31.74 g TOC/L) and LCM (62.50 g COD/L and 24.19 g TOC/L), and phenols concentration (6.60 g/L) lying however in the range reported by Paraskeva and Diamadopoulos (2006). CW was characterized by its high content of carbohydrates (lactose). OMW and CW had low nitrogen content in contrast with LCM, while LCM had neutral pH and alkalinity in high levels. Mixing of OMW with CW and LCM ensured sufficient levels of both nitrogen and alkalinity in the mixture, whereas the presence of phenolics, which are known antimicrobial and phytotoxic compounds (Capasso et al., 1995), were reduced because of OMW’s ‘dilution’ with CW and LCM (as shown in Table 7.2). Normally, phenolics recovery from OMW is pursued by researchers and companies around the globe due to their high commercial value, and their degradation, especially when concentrated up to 7 g/L, as in our case, would not be desirable. Nevertheless, their dilution through co-digestion may represent an economically feasible alternative in conditions where the capital expenditures (capex) for phenolics recovery increases the overall plant capex unproportionally.

7.3.2 Bacterial growth model

The cumulative bio-hydrogen production profile from each batch experiment was fitted to a modified Gompertz bacterial growth model (Eq. 7.1) using OriginPro version 8. This equation has been widely used to model gas production data (Mohd Yasin et al., 2011).

\[
H = P \exp \left\{ - \exp \left[ \frac{R_m e}{P} (\lambda - t) + 1 \right] \right\}
\]

(7.1)
where $H$ is the cumulative hydrogen production (mL); $P$ is the maximum hydrogen production potential (mL); $R_m$ is the maximum hydrogen production rate (mL/h); $\lambda$ is the lag-phase duration (h); $t$ is the time (h) and $e$ is $\exp(1) = 2.71828$.

The specific hydrogen production potential (SHPP) was obtained by dividing $P$ with the substrate COD applied (Khanal et al., 2004). The maximum specific hydrogen production rate (SHPR$_m$) was determined by dividing $R_m$ by the volatile suspended solids (VSS) added. The hydrogen conversion efficiency for different pH values was compared based on SHPP and SHPR$_m$.

### Table 7-2: Chemical composition of each agro-industrial wastewater and mixture used in this study.

<table>
<thead>
<tr>
<th>Parameters$^a$</th>
<th>Units</th>
<th>OMW</th>
<th>CW</th>
<th>LCM</th>
<th>Waste Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-</td>
<td>5.02 ± 0.15</td>
<td>5.69 ± 0.32</td>
<td>7.26 ± 0.18</td>
<td>5.55 ± 0.04</td>
</tr>
<tr>
<td>TSS</td>
<td>g/L</td>
<td>52.20 ± 0.78</td>
<td>13.87 ± 0.12</td>
<td>48.60 ± 0.24</td>
<td>35.73 ± 0.81</td>
</tr>
<tr>
<td>VSS</td>
<td>g/L</td>
<td>49.93 ± 1.31</td>
<td>12.37 ± 0.05</td>
<td>35.80 ± 0.53</td>
<td>33.00 ± 0.78</td>
</tr>
<tr>
<td>TS</td>
<td>g/L</td>
<td>82.53 ± 1.12</td>
<td>68.96 ± 0.42</td>
<td>52.40 ± 0.93</td>
<td>73.52 ± 0.34</td>
</tr>
<tr>
<td>VS</td>
<td>g/L</td>
<td>65.84 ± 0.42</td>
<td>57.85 ± 0.33</td>
<td>35.83 ± 0.64</td>
<td>63.52 ± 0.23</td>
</tr>
<tr>
<td>Total COD</td>
<td>g/L</td>
<td>140.00 ± 0.00</td>
<td>75.00 ± 0.00</td>
<td>62.50 ± 2.12</td>
<td>95.00 ± 0.00</td>
</tr>
<tr>
<td>Soluble COD</td>
<td>g/L</td>
<td>66.37 ± 0.18</td>
<td>66.73 ± 0.11</td>
<td>23.02 ± 0.26</td>
<td>58.70 ± 0.49</td>
</tr>
<tr>
<td>TOC</td>
<td>g/L</td>
<td>56.01 ± 1.24</td>
<td>31.74 ± 0.70</td>
<td>24.19 ± 0.16</td>
<td>40.69 ± 0.17</td>
</tr>
<tr>
<td>BOD$_5$</td>
<td>g/L</td>
<td>12.50 ± 0.71</td>
<td>32.00 ± 0.00</td>
<td>17.00 ± 1.41</td>
<td>19.50 ± 0.71</td>
</tr>
<tr>
<td>Total carbohydrates$^b$</td>
<td>g/L</td>
<td>30.33 ± 0.95</td>
<td>53.55 ± 1.34</td>
<td>8.12 ± 0.31</td>
<td>36.88 ± 0.74</td>
</tr>
<tr>
<td>Soluble carbohydrates$^b$</td>
<td>g/L</td>
<td>24.88 ± 0.67</td>
<td>48.75 ± 0.49</td>
<td>1.20 ± 0.01</td>
<td>31.90 ± 0.35</td>
</tr>
<tr>
<td>Total phenols$^c$</td>
<td>g/L</td>
<td>6.60 ± 0.00</td>
<td>0.13 ± 0.00</td>
<td>1.25 ± 0.01</td>
<td>3.88 ± 0.04</td>
</tr>
<tr>
<td>Total nitrogen, TKN</td>
<td>g/L</td>
<td>0.81 ± 0.04</td>
<td>0.86 ± 0.03</td>
<td>3.36 ± 0.00</td>
<td>0.98 ± 0.04</td>
</tr>
<tr>
<td>Ammonium nitrogen</td>
<td>g/L</td>
<td>0.12 ± 0.00</td>
<td>0.10 ± 0.00</td>
<td>2.39 ± 0.02</td>
<td>0.20 ± 0.00</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>mg/L</td>
<td>480.40 ± 2.05</td>
<td>446.53 ± 4.00</td>
<td>863.50 ± 22.49</td>
<td>501.20 ± 16.18</td>
</tr>
<tr>
<td>Soluble phosphorus</td>
<td>mg/L</td>
<td>309.70 ± 3.04</td>
<td>258.20 ± 1.91</td>
<td>21.68 ± 0.04</td>
<td>287.43 ± 11.70</td>
</tr>
<tr>
<td>Oil and grease</td>
<td>g/L</td>
<td>12.76 ± 1.31</td>
<td>1.62 ± 0.02</td>
<td>2.87 ± 1.02</td>
<td>8.06 ± 1.11</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>g CaCO$_3$/L</td>
<td>0.80 ± 0.07</td>
<td>0.50 ± 0.00</td>
<td>12.38 ± 0.32</td>
<td>1.38 ± 0.00</td>
</tr>
<tr>
<td>Total VFAs</td>
<td>g/L</td>
<td>0.23 ± 0.00</td>
<td>0.04 ± 0.00</td>
<td>7.24 ± 0.002</td>
<td>0.43 ± 0.01</td>
</tr>
</tbody>
</table>

$^a$ Mean values (± standard deviation); $^b$ In equivalent glucose; $^c$ In equivalent syringic acid
7.4 Effect of pH on the anaerobic acidogenesis of agro-industrial wastewaters for maximization of bio-hydrogen production: A lab-scale evaluation using batch tests.

7.4.1 Effect of pH

Batch experiments were carried out using a mixture of agro-industrial wastewaters at a ratio of 55% OMW, 40% CW and 5% LCM in order to assess the effect of pH on hydrogen production and distribution of products. All experiments were conducted in the same reactor configuration (Section 2.2.2) using acclimatized anaerobic inoculum, which was obtained from an anaerobic acidogenic CSTR digester fed daily with the same mixture of OMW, CW and LCM (with a ratio of 55% OMW, 40% CW and 5% LCM (v/v/v)) and operated at steady-state conditions at hydraulic retention time (HRT) of 3 days and organic loading rate (OLR) of 16 kg VS/m³·d under mesophilic conditions (37°C). The working volume of the fermentor was adjusted to 400 mL. The amount of anaerobic sludge used as inoculum was 60 mL (15% v/v of working volume), while the remaining consisted of the tested waste mixture. The pH values tested were 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5. The pH of the mixed liquor was kept constant throughout the course of the experiment via automatic control (using a HACH PID-controller) by adding NaOH or HCl solution (6 N) via respective peristaltic pumps. The quantity of produced biogas and its composition was monitored throughout the course of each experiment. At regular intervals samples were collected for analysis, i.e. determination of carbohydrates, VFAs, alcohols, lactic acid, phenol, TS, VS, TOC and SOC.

Fig. 7.1 illustrates the quantity of biogas and hydrogen produced at STP conditions, the total sugar consumption and the main metabolic end-products, as function of pH. In all pH values tested, the biogas was mainly composed of hydrogen and carbon dioxide, whereas the mixed liquor was composed of VFA, lactic acid and ethanol. As shown in Fig. 7.1(a) the biogas and hydrogen production were pH dependent. The maximum production of hydrogen (603.7 mL) was obtained at pH 6.0, whereas lowering the pH value below 5.0 resulted to almost negligible hydrogen productivity. During all fermentation experiments, no methane production was detected indicating that only acidogenesis was active, even though some of the adopted pH conditions (higher than pH 7.0) may have been suitable for methanogenesis. The consumption of total carbohydrates, measured as equivalent glucose, (Fig. 7.1(b)) was high in all tested pH values with the maximum degradation (85.4%) observed at pH 6.0. Similarly, soluble carbohydrates consumption was even higher (94.5%) at pH 6.0 despite their simultaneous production due to hydrolysis of total carbohydrates. Ferchichi et al. (2005) observed equally high sugar consumption (97%) studying hydrogen production from cheese whey at different initial pH values ranging from 5.0 to 9.0, suggesting that the microorganisms’ ability to consume sugars did not alter within this initial pH range.
Degradation of glucose under anaerobic conditions is accompanied by production of hydrogen and various metabolic products, mainly volatile fatty acids (i.e. acetic, propionic, and butyric acids), lactic acid and alcohols (ethanol), depending on the microbial species present and the prevailing conditions. The analysis of metabolic products provides useful information on the evolution of the process and can be used to explain the observed hydrogen generation yields. In the present study, the course of soluble metabolites’ concentration was monitored during the process. A mixture of acetic, propionic, butyric and lactic acid was measured as abundant metabolites which are characteristic of clostridia fermentation. The highest total VFAs concentration was detected at pH 6.5 (13.43 g/L) but this was due to the concentration of propionic acid (Fig. 7.1(c)), which was observed to increase with increasing pH (from 0.09 to 4.02 g/L), whereas concentrations of acetic and butyric acid were practically the same in both pH values of 6.0 and 6.5 (1.1 g/L and 8.6 g/L, respectively). Horiuchi et al. (2002) also studied the effect of changes in operating pH (ranging from 5.0 to 8.0) on organic acid production in continuous reactors. Their reactors were inoculated with anaerobic digester sludge and fed with a glucose–yeast extract medium. A switch from butyric acid to propionic acid production, as the pH increased, was attributed to a change in the dominant microbial population during the transition period of around 120 h, rather than a metabolic pathway change within the same bacterial population, which would be expected to occur more quickly. It was previously reported by Zhang et al. (2013) that acetic acid was main product at pH 5.0 whereas butyric acid was dominant at pH 6.0, during anaerobic acidogenesis of kitchen wastes. In our experiments (Fig. 7.1(c)), ethanol concentration was higher (1230 mg/L) at the lowest value of pH (pH 4.5) which is in agreement with the study of Ren et al. (1997) that suggested a pH value of about 4.5 as optimum for maximizing the production of ethanol. Besides ethanol, limited amounts (<400 mg/L) of other volatile fatty acids (i.e. i-butyric, valeric, i-valeric and caproic) were detected in all pH values tested in this study. Table 7.3 presents additional results, including organic carbon, phenols and solids’ degradation, obtained during the tests carried out in this work. Apart from the highest carbohydrates’ degradation, the maximum removal of total and soluble organic carbon (i.e. 21% and 38%, respectively) and total and volatile solids (i.e. 22% and 40%, respectively) occurred at pH 6.0. Similarly, Zhang et al. (2013) observed also the greatest degree of hydrolysis and acidogenesis accompanied with the maximum VFA concentration when the pH value was controlled at 6.0. However, negligible phenolics’ depletion was observed at pH values lower than 6.0 (Table 7.3), whereas an increase in their degradation was observed as pH value increased (29.03% degradation at pH 7.5).

Fig. 7.2 presents the acidogenenic experiment of the mixture at pH 6.0, whereas the experimental results obtained in different pH values can be found in Appendix A (Fig. A.1–Fig. A.6). In particular, the consumption of total and soluble sugars is associated with the rate of production of major products during the whole experiment course. The degradation of carbohydrates (Fig. 6.2(a)) contributed to an increase of the concentration of acetic acid, lactic acid and ethanol (Fig. 7.2(b)).
Significant accumulation of butyric acid (approximately 9 g butyric acid/L) was observed after a period of 40 h, mainly as a result of lactic acid degradation, which was also accompanied by simultaneous decrease in acetic acid and production of hydrogen. Furthermore, propionic acid was found to be produced in appreciable amounts only at later fermentation stages (i.e., when lactic acid depletion was observed). Matsumoto and Nishimura (2007) found that a mixed substrate of acetic and lactic acid enhanced hydrogen production by strain *Clostridium diolis* JPCC H-3. It was also observed that 1 mol of acetic acid reacted with 2 mol of lactic acid and produced 1 mol of H₂, 2 mol of CO₂, and 1.5 mol of butyric acid.

![Figure 7-1](image-url)

*Figure 7-1:* (a) Biogas and hydrogen evolution at STP conditions, (b) consumption of total carbohydrates and (c) main metabolic products, as a function of different pH values tested.
Table 7-3: Net hydrogen yield and organic carbon and solids’ degradation during acidogenesis of agro-industrial mixture at different pH values.

<table>
<thead>
<tr>
<th>pH</th>
<th>Yield (mol H₂/mol equiv.glucose)</th>
<th>TOC removal (%)</th>
<th>SOC removal (%)</th>
<th>TS removal (%)</th>
<th>VS removal (%)</th>
<th>Phenols removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>0.005</td>
<td>15.27</td>
<td>9.94</td>
<td>6.63</td>
<td>18.83</td>
<td>-</td>
</tr>
<tr>
<td>5.0</td>
<td>0.003</td>
<td>11.55</td>
<td>6.06</td>
<td>1.81</td>
<td>13.27</td>
<td>-</td>
</tr>
<tr>
<td>5.5</td>
<td>0.380</td>
<td>16.07</td>
<td>27.38</td>
<td>13.65</td>
<td>29.64</td>
<td>-</td>
</tr>
<tr>
<td>6.0</td>
<td>0.642</td>
<td>21.06</td>
<td>38.18</td>
<td>22.40</td>
<td>40.10</td>
<td>-</td>
</tr>
<tr>
<td>6.5</td>
<td>0.607</td>
<td>20.35</td>
<td>36.37</td>
<td>15.46</td>
<td>33.95</td>
<td>11.11</td>
</tr>
<tr>
<td>7.0</td>
<td>0.495</td>
<td>18.77</td>
<td>23.13</td>
<td>9.86</td>
<td>33.32</td>
<td>24.73</td>
</tr>
<tr>
<td>7.5</td>
<td>0.158</td>
<td>19.38</td>
<td>22.71</td>
<td>3.61</td>
<td>27.22</td>
<td>29.03</td>
</tr>
</tbody>
</table>

Fig. 7.3(a) depicts the hydrogen yield (moles of hydrogen produced per mole equivalent glucose of total consumed carbohydrates) at each pH value tested. The maximum hydrogen yield was observed at pH 6.0 and was equal to 0.642 mol H₂/mol equivalent glucose consumed. According to literature, the optimum hydrogen yield should be achieved with acetic acid as the fermentation end-product (theoretical yield of 4 mol of H₂/mol of glucose). However, in our case no hydrogen seemed to be produced along with acetic acid in pH 6.0 (Fig. 7.2(b and c)), whereas hydrogen productivity seemed to be closely related to butyric acid production and lactic acid degradation. We consider that hydrogen production decrease in both lower and higher pH values than 6.0 is mainly due to enzymatic inhibition and not simultaneous consumption of produced hydrogen, since no methane was detected in all experiments (even at pH higher than 7.0). In addition, the yield of TVFAs is also a good indicator for pH optimization. As shown in Fig. 7.3(b) the greatest TVFAs yield was 0.417 g carbon in TVFAs/g carbon in substrate (0.264 g carbon in TVFAs/g VS_fed) at pH 6.0 compared with 0.05 and 0.228 g carbon in TVFAs/g carbon in substrate at pH values of 4.5 and 7.5, respectively. Pham et al. (2013) observed a maximal TVFAs yield of 0.371 g carbon in TVFAs/g carbon in substrate at 6.2 g alginate/L and initial pH 7.6. Moreover, Jiang et al. (2013) emphasized that TVFAs yield is very important, as it shows how much substrate is converted into VFAs and observed the optimum pH of 6.0 with highest TVFAs yield of 0.316 g TVFAs/g VS_fed, the highest among all pH conditions tested (5.0, 6.0, 7.0 and uncontrolled pH). Our results indicate that pH is a very important factor because of the limitation of hydrogen production outside a narrow pH range. Anaerobic fermentation
of food waste at thermophilic conditions was suitable for bio-hydrogen production at controlled pH 5.5 with a yield of 79 mmol H$_2$/L-medium/d (Mohd Yasin et al., 2011). In the latter case, total bacteria quantification analysis was carried out at different pH values (5.0, 5.5 and 6.0) and showed that 92% of the total bacteria belonged to *Clostridium* sp. at controlled pH 5.5.

![Graph](image)

**Figure 7-2:** (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of OMW / CW / LCM mixture (55 / 40 / 5, v/v/v), at pH 6.0.

A range of optimum pH values has been reported in the literature for fermentation of carbohydrates by mixed bacterial cultures. Van Ginkel et al. (2001), for example, studied the effect of varying pH (4.5–7.5) and demonstrated that the highest hydrogen production rate (74.7 mL H$_2$/L·h) occurred at pH 5.5 and substrate concentration of 7.5 g COD/L. Another study using glucose at similar range of pH values from 4.0 to 7.0
Chapter 7  Anaerobic co-digestion of agro-industrial wastewaters in a two-stage system

has been presented by Fang and Liu (2002) who suggested that the maximum yield of 2.1 ± 0.1 mol H₂/mol glucose was observed at pH 5.5. Davila-Vazquez et al. (2008) studied the effect of the initial pH (3.88–8.12) using glucose, lactose and cheese whey powder as substrates, with the highest hydrogen yield being observed from glucose and lactose at an initial pH 7.5 and from cheese whey powder at an initial pH value of 6.0.

It should also be noted that a comparison of the pH effects on hydrogen production reported in the present study and those documented in the literature is complicated by the fact that most of the latter report the results of runs where only the initial pH was adjusted (Ferchichi et al., 2005; Davila-Vazquez et al., 2008) without any further control along the process. In addition, we believe that the pH value during the process is very crucial for the main metabolic products evolution.

![Graph of yield vs. pH](image)

**Figure 7-3:** (a) Net hydrogen yield and (b) TVFAs produced at each pH value tested.

### 7.4.2 Kinetic analysis

Lactic acid was identified as a major intermediate soluble product in all fermentation batches since it was firstly produced and subsequently metabolized during the process at a greater or lower extent depending on the applied pH. For example, in pH 6.0, lactic acid after exhibiting an intense accumulation phase for about 34 h started to degrade until it was slightly detected at the end of the batch test (Fig. 7.2(b)). More specifically, accumulation of lactic acid was observed at both low and high pH values due to kinetic limitation in the reactions converting lactic acid to butyric acid and hydrogen. Lactic acid bacteria, including species of the *Lactobacillus* genus, are naturally found in CW as a result of the cheese making process. *Lactobacilli* produce lactic acid as the major fermentation product from sugars (Stiles and Holzapfel, 1997). They belong to the *Firmicutes* phylum of bacteria and have a high acid tolerance, surviving pH values of
5.0 and lower. However, the appearance of lactic acid has also been observed with other carbohydrate-rich substrates like potato waste (Parawira et al., 2004) and garbage (Akao et al., 2007). According to Castelló et al. (2009), *Lactobacilli* are capable of producing lactic acid from lactose via three metabolic pathways, i.e. the homofermentative (Eq. (7.2)), the heterofermentative (Eq. (7.3)) and the bifidum pathway (Eq. (7.4)):

**Homofermentative pathway**
\[
C_6H_{12}O_6 \rightarrow 2\text{CH}_3\text{CH(OH)}\text{COOH} \tag{7.2}
\]

**Heterofermentative pathway**
\[
C_6H_{12}O_6 \rightarrow \text{CH}_3\text{CH(OH)}\text{COOH} + \text{CH}_3\text{CH}_2\text{OH} + \text{CO}_2 \tag{7.3}
\]

**Bifidum pathway**
\[
2C_6H_{12}O_6 \rightarrow 3\text{CH}_3\text{COOH} + 2\text{CH}_3\text{CH(OH)}\text{COOH} \tag{7.4}
\]

In addition, ethanol can be produced due to alcoholic fermentation (Eq. (7.5)):

**Ethanol production**
\[
C_6H_{12}O_6 \rightarrow 2\text{CH}_3\text{CH}_2\text{OH} + 2\text{CO}_2 \tag{7.5}
\]

In general, hydrogen yields vary proportionally to the final metabolic products. Lactic acid and ethanol production are not accompanied by hydrogen generation (Antonopoulou et al., 2008). However, it is well-known that production of acetic and butyric acid favors the production of hydrogen with the fermentation of glucose to acetic acid giving the highest theoretical yield of 4 mol of H\textsubscript{2}/mol of glucose (Eq. (7.6)) and the conversion to butyric acid resulting in 2 mol of H\textsubscript{2}/mol of glucose (Eq. (7.7)):

\[
C_6H_{12}O_6 + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH} + 2\text{CO}_2 + 4\text{H}_2 \tag{7.6}
\]

\[
C_6H_{12}O_6 \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{CO}_2 + 2\text{H}_2 \tag{7.7}
\]

On the other hand, the production of other metabolic products is accompanied by negative or zero hydrogen yield which results in lower overall hydrogen yield (mol H\textsubscript{2}/mol sugars consumed). Vavilin et al. (1995), for example, gave the overall equation for the production of propionic acid from glucose, showing that this involves the consumption of hydrogen (Eq. (7.8)) implicating that the production of propionic acid should be avoided. Vavilin et al. (1995) stated also that the limiting substrate for butyric acid production is glucose, whereas for propionic acid production is hydrogen.

\[
C_6H_{12}O_6 + 2\text{H}_2 \rightarrow 2\text{CH}_3\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \tag{7.8}
\]
However, the transformation of lactic acid into propionic and acetic acid may occur with no hydrogen production. *Clostridium propionicum* is one of the species having such an ability following the reaction shown in Eq. (7.9) (Baghchehsaraee et al., 2009). In such a case, glucose can be firstly converted to lactic acid and finally to propionic and acetic acid, following various alternative pathways without producing any hydrogen.

\[
3\text{CH}_3\text{CH(OH)COOH} \rightarrow 2\text{CH}_3\text{CH}_2\text{COOH} + \text{CH}_3\text{COOH} + \text{CO}_2 + \text{H}_2\text{O} \quad (7.9)
\]

Alternatively, lactic acid may also decompose to butyric acid with simultaneous hydrogen production (Eq. (7.10); Alais, 1984).

\[
2\text{CH}_3\text{CH(OH)COOH} \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{CO}_2 + 2\text{H}_2 \quad (7.10)
\]

Sträuber et al. (2012) reported a direct correlation of lactic acid degradation and butyric acid production during the steps of hydrolysis and acidogenesis using maize silage as a model substrate. The intense hydrogen production phase they observed was mainly associated with the production of butyric acid and the main gaseous products that evolved were hydrogen and carbon dioxide which are in agreement with Eq. (10). Baghchehsaraee et al. (2009) reported that when lactic acid was used as a sole substrate for hydrogen production, propionic and acetic acid was produced. More hydrogen was produced using a mixed substrate with starch and lactic acid, whereas the latter consumed resulted in increased butyric acid.

According to Bhat and Barker (1947), butyric acid may be also formed by a condensation of two moles of acetic acid with two moles of hydrogen (Eq. (7.11)).

\[
2\text{CH}_3\text{COOH} + 2\text{H}_2 \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \quad (7.11)
\]

Taking into account the aforementioned transformation pathways (Eqs. (7.2), (7.3), (7.4), (7.9), (7.10), and (7.11)), our experimental data obtained at the optimum pH (6.0) were used to calculate mass balances in order to estimate the pathways that result to hydrogen production under these conditions (Table 7.4). The main calculations were carried out after the peak detection of lactic acid (34th h) and during its further consumption with simultaneous hydrogen production. Because of the complexity of microbial populations present in the mixed culture used in our experiment, a combination of fermentative pathways was assumed to take place initially converting glucose to lactic acid, acetic acid and ethanol (Eqs. (7.2)–(7.5)) during the lactic acid accumulation phase (0–34 h). The contribution of each pathway (Eqs. (7.2)–(7.5)) to the production of intermediates was impossible to be clarified at this point. Therefore, the continued degradation of carbohydrates after the 34th h till the end of experiment was utilized to estimate proportionally the extra lactic and acetic acid production assuming that reactions Eqs. (7.2)–(7.4) were still functional and that the overall lactic and acetic acid.
Acid production yields remained constant throughout the experimental test (Table 7.4, React. (1)). The propionic acid formation was attributed to lactic acid degradation with simultaneous acetic acid production, according to Eq. (7.9) (Table 7.4, React. (2)). The rest of lactic acid was decomposed to butyric acid as well as hydrogen and carbon dioxide, according to Eq. (7.10) (Table 7.4, React. (3)). However, a significant experimental observation was that the butyric acid mass produced was higher than the mass calculated to be produced from lactic acid degradation. Moreover, a decrease in the acetic acid mass was observed even though it was produced simultaneously with propionic acid (Eq. (7.9)). As a result, an additional calculation was done, assuming that acetate depletion goes along with the corresponding hydrogen towards butyric acid production (Table 7.4, React. (4)). As shown in Table 7.4 (React. (3)), hydrogen production is associated exclusively with butyric acid production whereas the molar ratio of hydrogen/butyric acid was estimated to be 0.944 (mol/mol). According to Table 7.4 a slight absolute error of 5.7% was calculated in the mass balances of acetic acid and hydrogen which however can be partially attributed to experimental errors and also to biomass production which was not encountered in all calculations presented in Table 7.4. Taking into account the low biomass productivity expected under anaerobic conditions (Rittmann and McCarty, 2001) we consider that the postulated reaction pathway presented in Table 7.4 can sufficiently describe hydrogen and VFAs productivity under the optimum conditions of pH 6.0.

Based on these calculations and assumptions, i.e. hydrogen production is associated exclusively with butyric acid production, a correlation diagram was constructed using the hydrogen and butyric acid production from all batch tests (Fig. 7.4). A well-correlated relationship between hydrogen and butyric acid production was realized which can be attributed to Reactions (3,4) (Table 7.4). As shown in Fig. 7.4, hydrogen production appears to be proportional with butyric acid production by a factor of 0.914 ($r^2 = 0.987$). At low pH values 4.5 and 5.0 hydrogen productivity was negligible due to lactic acid kinetic limitation, whereas the fermentation at high pH (7.5) presented a different transformation pathway, compared with the other pH tests, with the highest ratio of hydrogen/butyric acid of 2.03, which indicates the dominance of Reaction (3) against Reaction (4) (Table 7.4).

Fig. 7.5(a) depicts the hydrogen production based on our experimental data and the simulations generated using the fitted modified Gompertz model. The correlation coefficient ($r^2$) ranged between 0.870 and 0.999. Comparing each set of experimental data with the relevant model simulation, the parameters of hydrogen production potential (P), the maximum hydrogen production rate ($R_m$), and lag-phase time (k) were determined (Table 7.5). The P values increased as pH increased from 4.5 to 6.0, whereas $R_m$ values increased as pH increased from 4.5 to 5.5. The P and $R_m$ values peaked at pH of 6.0 and 5.5 respectively and decreased as pH increased to 7.5. The optimal pH for hydrogen production was found to be in the range of 6.0. This is in close agreement with a previous study by Lin et al. (2011) where pH 6.0 was deemed as the optimum initial pH for hydrogen production.
**Table 7-4:** Mass balances for hydrogen production at the optimum pH 6.0.

<table>
<thead>
<tr>
<th>Reactions</th>
<th>Calculated values (mmoles)</th>
<th>Literature Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactic</td>
<td>Butyric</td>
</tr>
<tr>
<td>1. $\text{C}_4\text{H}_8\text{O}_6 \rightarrow \text{CH}_3\text{CH}({\text{OH}})\text{COOH}, \text{CH}_3\text{COOH}$ (Eq. 7.2-7.4)</td>
<td>+15.51</td>
<td>-</td>
</tr>
<tr>
<td>2. $\text{CH}_3\text{CH}({\text{OH}})\text{COOH} \rightarrow 2\text{CH}_3\text{CH}_2\text{COOH}+\text{H}_2\text{COOH}+\text{CO}_2+\text{H}_2\text{O}$ (Eq. 7.9)</td>
<td>-8.16</td>
<td>-</td>
</tr>
<tr>
<td>3. $2\text{CH}_3\text{CH}({\text{OH}})\text{COOH} \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}+2\text{CO}_2+2\text{H}_2$ (Eq. 7.10)</td>
<td>-39.60</td>
<td>+19.80</td>
</tr>
<tr>
<td>4. $2\text{CH}_3\text{COOH}+2\text{H}_2 \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}+2\text{H}_2\text{O}$ (Eq. 7.11)</td>
<td>-</td>
<td>+7.10</td>
</tr>
</tbody>
</table>

**Net calculated values**

<table>
<thead>
<tr>
<th>Lactic</th>
<th>Butyric</th>
<th>Acetic</th>
<th>Propionic</th>
<th>H₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>-32.25</td>
<td>+26.90</td>
<td>-7.37</td>
<td>+5.44</td>
<td>+25.40</td>
</tr>
</tbody>
</table>

**Experimental values**

<table>
<thead>
<tr>
<th>Lactic</th>
<th>Butyric</th>
<th>Acetic</th>
<th>Propionic</th>
<th>H₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>-32.24</td>
<td>+26.90</td>
<td>-6.97</td>
<td>+5.44</td>
<td>+26.95</td>
</tr>
</tbody>
</table>

**Error (%)**

<table>
<thead>
<tr>
<th>Lactic</th>
<th>Butyric</th>
<th>Acetic</th>
<th>Propionic</th>
<th>H₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03</td>
<td>0.00</td>
<td>5.74</td>
<td>0.00</td>
<td>5.75</td>
</tr>
</tbody>
</table>

+, production; -, consumption.
The bioreactor with pH 5.5 had the longest lag time of 91.46 h. The lag-phase decreased when pH increased from 5.5 to 7.5, whereas at pH values of 4.5 and 5.0 the lag-phase was not measurable due to the limited hydrogen productivity. Fig. 7.5(b) and (c) present the variation in SHPP and SHPR\textsubscript{m} along with operating pH values, respectively. The SHPP peaked at 15.11 mL H\textsubscript{2}/g COD demonstrating a clear optimum at pH 6.0. However, the peak value of SHPR\textsubscript{m} was 2.88 mL H\textsubscript{2}/g VSS·h at pH 5.5.

![Figure 7-4: Correlation between the hydrogen production and the produced butyric acid in all pH values tested. Labels indicate the controlled pH value.](image)

**Table 7-5:** Kinetic parameters of hydrogen production estimated using the modified Gompertz equation.

<table>
<thead>
<tr>
<th>pH</th>
<th>P (mL)</th>
<th>R\textsubscript{m} (mL/h)</th>
<th>λ (h)</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>0.86</td>
<td>0.01</td>
<td>-</td>
<td>0.870</td>
</tr>
<tr>
<td>5.0</td>
<td>0.97</td>
<td>0.01</td>
<td>-</td>
<td>0.875</td>
</tr>
<tr>
<td>5.5</td>
<td>347.36</td>
<td>56.80</td>
<td>91.46</td>
<td>0.999</td>
</tr>
<tr>
<td>6.0</td>
<td>561.58</td>
<td>20.51</td>
<td>38.96</td>
<td>0.984</td>
</tr>
<tr>
<td>6.5</td>
<td>536.03</td>
<td>26.16</td>
<td>26.10</td>
<td>0.990</td>
</tr>
<tr>
<td>7.0</td>
<td>417.95</td>
<td>22.24</td>
<td>41.40</td>
<td>0.988</td>
</tr>
<tr>
<td>7.5</td>
<td>128.97</td>
<td>5.45</td>
<td>20.19</td>
<td>0.997</td>
</tr>
</tbody>
</table>
Figure 7-5: (a) Cumulative hydrogen production (experimental data and modified Gompertz model simulation) during batch acidogenesis of OMW / CW / LCM mixture (55 / 40 / 5, v/v/v), (b) specific hydrogen production potential (SHPP) and (c) maximum specific hydrogen production rate (SHPRm) at the pH values tested in this study.
7.5 Effect of hydraulic retention time (HRT) on the anaerobic co-digestion of agro-industrial wastes in a two-stage CSTR system

The experiments were carried out in two CSTR reactors, one used for acidogenesis and the other one for methanogenesis. The bioreactors’ setup is briefly described in Section 2.2.1. Experiments were conducted successively to determine the optimum HRT for maximum hydrogen and methane production. Since the feeding mixture composition was kept constant throughout the experimentation period, the organic loading rate in each reactor was increased by decreasing the applied HRT. The acidogenic reactor was thus operated at five different HRTs of 5, 3, 2, 1 and 0.75 d, which were equivalent to OLRs of 19.00, 31.73, 47.50, 95.00, and 126.67 kg COD/m³·d, respectively. The effluent from the acidogenic reactor operated at HRTs of 2 and 1 d, exhibiting more or less similar characteristics, in terms of total VFAs, TS, VS, etc., was used for feeding the methanogenic reactor which was operated at two different HRTs, 20 and 25 d, equivalent to OLRs of 4.21 and 3.37 kg COD/m³·d, respectively. The methanogenic reactor’s feeding characteristics were thus maintained relatively constant throughout the testing period enabling a clear identification of the effect of HRT on the reactor’s performance. Table 7.6 presents the experimental loading conditions in the system at various HRTs.

The pH in the acidogenic reactor was kept constant at pH 6.0, based on the results of our previous work (Dareioti et al., 2014), throughout the experimentation phase via automatic control (using a Hach PID-controller) by the addition of a solution mixture of NaOH/KOH (3N/3N) via a peristaltic pump. Operating the acidogenic reactor at pH 6.0 could facilitate the proliferation of acidogenic bacteria (Dareioti et al., 2014), whereas the methanogenic reactor was operated at non-controlled pH 7.78 ± 0.20 (HRT 20 d) and pH 7.68 ± 0.18 (HRT 25 d).

Table 7.6: Operating conditions for the acidogenic and methanogenic CSTR.

<table>
<thead>
<tr>
<th>Operating conditions</th>
<th>Acidogenic Reactor</th>
<th>Methanogenic Reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HRT (d)</strong></td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Flow rate (mL/d)</td>
<td>100</td>
<td>167</td>
</tr>
<tr>
<td>OLR (kg VS/m³·d)</td>
<td>12.70</td>
<td>21.17</td>
</tr>
<tr>
<td>OLR (kg COD/m³·d)</td>
<td>19.00</td>
<td>31.75</td>
</tr>
</tbody>
</table>
7.5.1 Effect of HRT in the acidogenic reactor

The first phase of continuous operation aimed at further selection of H₂-producing bacteria in the biomass by application of high HRT and low OLR, which were stepwise increased during the experiment. For the start-up of the system the acidogenic reactor was filled up with 500 mL of the waste mixture (55% OMW, 40% CW and 5% LCM) and was operated anaerobically in batch mode for 48 h in order to enrich the biomass in H₂-producing microorganisms, a method that has been widely used (e.g. Mariakakis et al., 2011). After reactor start-up, the initial HRT was set at 5 d for a period of 62 days. HRT was then decreased to 3 d, 2 d, 1 d and 0.75 d for a period of 101, 97, 126 and 19 days, respectively, reaching steady-state conditions in the reactor. The characteristics of the acidogenic reactor at each HRT regarding gas production, under steady state operation, are shown in Table 7.7. At the beginning of each phase where HRT was decreased, there was a corresponding increase in biogas production but the system recovered and adapted to the new conditions shortly (Fig. 7.6(a)). This is probably attributed to the fact that the microorganisms are facing a sudden stepwise increase of organic loading, they overproduce hydrogen due to this change and afterwards they re-adjust their operation based on the newly applied conditions slowing down their metabolic operations and thus adapting to the new environment resulting from the HRT shifting. The biogas produced from the acidogenic reactor consisted exclusively of hydrogen and carbon dioxide and was free of methane, despite the use of LCM in the feeding mixture. The complete absence of methane could be attributed to a number of reasons with the most valid ones being the use at a very low percentage (5% v/v) of a rather recalcitrant to biomethanization substrate (LCM), fermented under acidic conditions. At low HRT.

![Figure 7-6: (a) Biogas and hydrogen production and (b) total and soluble carbohydrates concentration during acidogenesis for each HRT tested.](image-url)
Initially, the biogas production rate at HRT of 5 d was fluctuating with a mean value of 0.36 L/LR·d containing 11.69% of hydrogen at the steady state (with the rest being mainly CO₂). Fluctuation of the biogas produced may be due to the complexity of the feeding medium, microbial population shifts taking place during the extended period of operation as well as because of the presence of various organic and inorganic materials in the feed inhibiting treatment performance (Torkian et al., 2003). With the decrease of HRT to 0.75 d, biogas and hydrogen production rates increased up to 4.90 and 1.72 L/LR·d respectively, with the highest hydrogen content of 34.45% being observed also at 0.75 d HRT. Hydrogen yield (calculated as mol H₂/mol carbohydrate consumed) is a good indicator of the microbial populations’ effectiveness for hydrogen production and represents the capability of microorganisms to convert carbohydrates into hydrogen gas. It has been widely reported that the hydrogen yield increased with decreasing HRT. For instance, Scoma et al. (2013) investigated the anaerobic acidogenic process of dephenolized OMW, using Packed Bed Biofilm Reactor (PBBRs) filled with ceramic cubes, and observed a significant production of a hydrogen-rich biogas when shorter HRTs (7, 5, 3 and 1 d) were applied, with their maximum hydrogen productivity (0.146 L/LR·d) obtained at HRT of 1 d operating, however, at a much lower OLR (38.8 kg COD/m³·d). Under such conditions (i.e. low OLR), low hydrogen productivity is expected since the hydrogen-producing bacteria are receiving lower amounts of reducing equivalents and thus producing lower amount of hydrogen per reactor volume. Table 7.7 shows that the maximum hydrogen yield (0.54 mol H₂/mol carbohydrate consumed) was achieved at HRT 0.75 d, whereas the hydrogen yield was very low at higher HRT (0.10 mol H₂/mol carbohydrate consumed). This inverse relationship between the HRT and the daily hydrogen production in acidogenic anaerobic digester has been corroborated by a lot of authors (e.g. Romero Aguilar et al., 2013). This fact may be due to that H₂-producing bacteria (Clostridium sp.) are being selected among the different populations of microorganisms involved on the anaerobic digestion at low HRTs (Romero Aguilar et al., 2013). According to Chen et al. (2009), the dominant microbial population can be selected by the corresponding suitable substrate concentration. Another explanation could possibly be the existence of homoacetogenism in longer HRTs, which has been predicted in many H₂-producing systems (Hussy et al., 2003; Kim et al., 2006) and was found to play an important role in limiting hydrogen production at various HRTs (Arooj et al., 2008). In order to verify and evaluate the existence of H₂-consuming bacteria, the acidogenic reactor was switched from continuous to batch mode at the end of HRT 3 d (163rd day of operation) for 28 hours. As shown in Fig. 7.7, a hydrogen percentage of 5.57% was detected (overall consumption of 53.3%) at the end of the 28th hour, whereas the significant decrease of hydrogen percentage (47.8%) was observed in the first 14 h (from 11.93% to 6.23%). On the other hand, the short HRT (0.75 d) had a negative effect on the consumption of total and soluble carbohydrates (Fig. 7.6(b)), which was associated with the main end products obtained. For all HRTs studied, apart from HRT 0.75 d, carbohydrates removal was over 80% and 92% for total and soluble ones, respectively.
This is consistent with that reported by Fang and Yu (2000) who presented that dairy wastewater acidogenesis was not affected by HRT changes, whereas the same and even higher consumption (over 90%) was supported by Badiei et al. (2011) who examined the effect of HRT on hydrogen production using palm oil mill effluent. However, the drop in carbohydrates consumption to 64 and 72% (total and soluble, respectively) suggests kinetic limitation of the acidogenic population to consume carbohydrates due to low retention time of microorganisms. Total and soluble carbohydrates were quantitatively converted to volatile fatty acids and lactic acid, according to the calculations made using the mass balances reported by Dareioti et al. (2014).

Figure 7-7: Consumption of hydrogen in batch mode at HRT of 3 d.

Significant production of volatile fatty acids (i.e. acetic, propionic, butyric) and lactic acid was observed at all tested HRTs (Fig. 7.8(a)) and thus they were the dominant soluble end-products in the reactor's effluent. Lactic acid, as intermediate metabolic product of acidogenesis, was not detected at the highest HRT of 5 d, whereas it was gradually increased when lower HRTs were applied. Isobutyric and ethanol were also measured in concentrations, though, less than 1500 ppm. As shown in Fig. 7.8(a) and Table 7.7, the highest amount of total VFAs (approximately 21 g/L) was produced at HRT of 2 and 1 d, whereas their relative presence was affected by decreasing the HRT at 0.75 d. The complex and fluctuating end-product distribution of this reactor is likely attributed to the complex nature of the wastewater mixture tested. A decrease in acids concentration was observed during the HRT shifting from 2 to 1 d (Fig. 7.8(a)) due to temporary failure of the feeding peristaltic pump which caused unstable feeding conditions. However, after a few days, acids concentration increased again and stabilized while the system returned closely to its previous conditions. The major portion of the substrate was consumed producing VFAs and lactic acid, as shown in Fig. 7.8(a). A removal of 15% of total and soluble organic carbon in the acidogenic
reactor for all HRTs was observed (data not shown). Maximization of soluble organic matter and VFAs concentration was crucial for the following methanogenic process. In our previous works, operating acidogenic reactors at fixed HRT of 3 d without pH control, no COD removal was observed using the same mixture (Dareioti et al., 2009), or a slight decrease of 2.4% of total COD was evident during co-digestion of 20% OMW and 80% LCM (Dareioti et al., 2010), which is consistent with the low hydrogen production rate reported under these conditions. Table 7.7 shows the solids hydrolysis between influent and effluent for each HRT tested with the maximum VS removal (31.23%) obtained at the highest HRT (5 d). Fig. 7.8(b) depicts the TS and VS concentration in the acidogenic reactor effluent, which remained practically constant at 62.97 ± 4.79 g TS/L and 40.23 ± 2.69 g VS/L, respectively, throughout the experimentation period (in all HRTs tested). However, at the lowest HRT of 0.75 d a tendency for TS and VS concentration increase as a function of operating time was realized.

Figure 7-8: (a) Evolution of main volatile fatty acids and lactic acid and (b) total (TS) and volatile solids (VS) concentration during acidogenesis for each HRT tested.

The highest phenols removal (15.36%) was observed at HRT 5 d, demonstrating that the biomass used as inoculum was already well adapted against the presence of phenolics compounds. However, the subsequent decrease of HRT in the reactor affected seriously the phenols degradation ability of the system since a 3-5 fold decrease was realized, as shown in Table 7.7. Higher removal percentages of phenolics compounds (40.7%) have been reported by Rincón et al. (2009) during treatment of two-phase olive oil mill solid residues in the hydrolytic-acidogenic reactor of their two-stage anaerobic digestion process when, however, their reactor was operated at a much higher OLR.
Chapter 7 | Anaerobic co-digestion of agro-industrial wastewaters in a two-stage system

(12.9 g COD/L·d) and an HRT of 12.4 d. Gonçalves et al. (2012) operated two different reactors with raw OMW at concentrations from 5 to 48 g COD/L and HRT between 10 and 5 d and proved that the intermittent feeding improved the removal of phenolics compounds reaching the remarkable removal efficiencies of 60% and 81%.

Table 7-7: Performance of the acidogenic CSTR under steady-state conditions for each HRT tested.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HRT (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Biogas (L/LR·d)</td>
<td>0.36</td>
</tr>
<tr>
<td>H₂ (L/LR·d)</td>
<td>0.06</td>
</tr>
<tr>
<td>H₂ (%)</td>
<td>11.69</td>
</tr>
<tr>
<td>Yield (mol H₂/mol carboh. consumed)</td>
<td>0.11</td>
</tr>
<tr>
<td>TVFA (g/L)</td>
<td>20.01</td>
</tr>
<tr>
<td>Total carbohydrates removal (%)</td>
<td>80.24</td>
</tr>
<tr>
<td>Soluble carbohydrates removal (%)</td>
<td>92.04</td>
</tr>
<tr>
<td>TS removal (%)</td>
<td>5.87</td>
</tr>
<tr>
<td>VS removal (%)</td>
<td>31.23</td>
</tr>
<tr>
<td>Phenols removal (%)</td>
<td>15.36</td>
</tr>
</tbody>
</table>

7.5.2 Effect of HRT in the methanogenic reactor

The methanogenic reactor was filled up with anaerobic digested sludge taken from the municipal wastewater treatment plant of Patras (Greece) and subsequently was fed with acidified effluent of the first stage, at the HRT of 20 d. In order to assess the rate and extent of methanogenesis at two different hydraulic retention times, i.e. 20 and 25 d, a methanogenic CSTR was used for treating the acidified effluent from the acidogenic reactor operating at HRTs of 2 and 1 d having almost constant characteristics in terms of TVFAs, TS, VS etc. In this way, the best compromise between hydrogen and VFAs production was also obtained. In integrated sequential biorefineries (e.g. Kaparaju et al., 2009), this makes perfectly sense, as reducing the efficiency of a process is desirable if beneficial for the following steps and the biorefinery as a whole.

Table 7.8 presents the experimental results obtained at steady-state conditions for the different HRTs, including pH, biogas and methane production rates, methane percentage and methane yields. The influent of the methanogenic reactor was rich in VFAs, as anticipated due to the pretreatment of the wastewater mixture in the
acidogenic reactor (Fig. 7.8(a)). The pH in the methanogenic reactor remained practically constant at 7.78 ± 0.2 and 7.68 ± 0.18 for HRT 20 and 25 d, respectively.

During the operation at HRT of 20 d (OLR 2.01 kg VS/m³·d), the process became unstable and VFA accumulation (mainly acetic acid) was noticed in the effluent of the methanogenic reactor, which was surpassed successfully after the increase of HRT to 25 d, lowering thus the digester’s loading rate (Fig. 7.9(a)). These results are in agreement with the study of Salminen and Rintala (2002), where the digestion of poultry slaughterhouse wastes at similar OLR (2.1 kg VS/m³·d) was followed by gradual accumulation of TVFA and partially depressed methane yield. The inhibition was also reversible, at least to a certain extent, by lowering the digester’s loading rate. Such a behavior is anticipated in the presence of one or more inhibiting substances which depress the methanogenic activity, causing reversible inhibition on the methanogenic stage and TVFA accumulation, either because they are constituents of the feeding substrate (as in our case, i.e. phenolic substances contained in OMW) or due to their production as result of substrate degradation (increased ammonia and long-chain fatty acids concentration in the case of Salminen and Rintala, 2002).

As shown in Fig. 7.9(b) the biogas production rate increased until the 60th day of operation up to 0.97 L/L·R·d, whereas, after that, the rate decreased and stabilized at 0.84 ± 0.08 L/L·R·d (mean value). Switching to the higher HRT (25 d) and lower OLR (1.61 kg VS/m³·d) at the 83rd day, a decrease in the volume of gas produced was evident which then stabilized at 0.50 ± 0.07 L/L·R·d. The composition of methane in the biogas fluctuated between 51.37 to 69.61% at the HRT of 20 d and 61.04 to 69.75% at HRT 25 d with mean values of 59.43 ± 3.83% and 65.43 ± 2.77%, respectively. The methane production rate at the steady state reached 0.50 ± 0.06 and 0.33 ± 0.05 L CH₄/L·R·d for the two tested HRTs.

![Figure 7-9](image_url)  
**Figure 7-9:** (a) Variation of main volatile fatty acids (VFA) and (b) biogas and methane evolution during methanogenesis for each HRT tested.
In our previous study (Dareioti et al., 2009), using the same mixture in a two-stage system under uncontrolled pH acidogenic conditions, a significant higher methane production rate of 1.35 ± 0.11 L CH₄/LR·d was obtained at HRT of 16 d. One explanation could be inhibition of methanogenesis by higher influent phenols concentration in the current study (3.96 g/L) compared to 1.75 g/L in the influent of our previous study (Dareioti et al., 2009). Inhibition of methanogens in the present study could be also attributed to the increased concentration of sodium and potassium in the influent due to pH control during acidogenesis. Koutrouli et al. (2009) studied the effect of HRT in a two-stage process from two-phase olive mill waste (water diluted 1:4) and observed that the methane yield increased as the HRT increased (0.16 L CH₄/kg COD added at HRT of 20 d). However, methane productivity reached the maximum value of 1.13 ± 0.08 L/LR·d at HRT 10 d, whereas the reactor failed at lower HRT tested (HRT 5 d). Kavacik and Topaloglu (2010) obtained the highest biogas production (1.51 L/LR·d) at HRT 5 d from the co-digestion of 50% cheese whey with 50% dairy manure (diluted 1:1) with a methane content of 60% and suggested that co-digestion of these two wastes is more advantageous than processing each one separately. Biogas production rate of 1.25 L/LR·d and overall 93% COD reduction has been reported by Martinez-Garcia et al. (2007) co-digesting a mixture of OMW (75%) and CW (25%) (v/v) in a two-stage system (aerobic followed by anaerobic treatment) operated in OLR of 3.0 kg COD/LR·d.

The methane yield determined from our experimental data at both HRTs on the basis of volatile solids added (expressed as mL CH₄/g VS added) and the COD removed (expressed as mL CH₄/g COD removed) is shown in Table 7.8. Methane yields 250.75 and 203.11 mL CH₄/g VS added were obtained at HRTs of 20 and 25 d, respectively. On the other hand, a higher methane yield, estimated on the basis of the amount of substrate consumed, was observed at HRT 25 d (316.08 mL CH₄/g COD removed) compared to HRT 20 d (294.37 mL CH₄/g COD removed). Considering the theoretical methane production of 350 mL of methane per gram of COD removed and considering virtually negligible biomass growth and cell maintenance, our results proved an elevated effectiveness of the methanogenic stage for converting the particular agro-industrial waste mixture to methane under the tested operating conditions. Although accumulation of VFA and instability was observed at HRT 20 d, the methane productivity was higher albeit the lower methane yield coefficient. These values are slightly higher than those obtained in previous studies of anaerobic digestion of two-phase olive mill solid residue (Rincón et al., 2008). In the latter study 244 mL CH₄/g COD removed was achieved at HRT 17 d and OLR of 9.2 g COD/LR·d. Bayr et al. (2012) studied the co-digestion of rendering and slaughterhouse wastes and obtained a methane potential of 720 mL CH₄/g VS added in OLR of 1.0 and 1.5 kg VS/m³·d (HRT 50 d), in comparison to 262-572 mL CH₄/g VS added using the different substrates separately. Fig. 7.10(a) illustrates the evolution of COD, organic carbon (total and soluble) and TVFA (expressed in units of COD), as a function of experimental time of methanogenic reactor. At the HRT of 20 d (OLR of 4.21 kg COD/m³·d) the soluble
and total organic carbon in the effluent of the methanogenic reactor increased as a function of operation, as a result of acetic acid accumulation. However, the soluble and total organic carbon removal in the reactor was 47.44% and 44.64% (mean values), respectively, at the high HRT value (HRT 25 d). The TS removal remained constant (26.32%) for both HRTs tested, i.e. from 62.97 (influent) to 46.39 g/L (effluent), whereas, the VS removal was 44.74%, i.e. from 40.23 to 22.23 g/L (Fig. 7.10(b)). The degradation of total carbohydrates in glucose equivalents was 94.46% in both HRTs, whereas the phenol concentration decreased by 35.10%. A high removal of phenolics compounds has been reported by Martinez-Garcia et al. (2007) co-digesting a mixture of OMW (75%) with low phenolics content (0.7 g/L) and CW (25%) (v/v) in a two-stage system (aerobic followed by anaerobic treatment). The high performance presented in the above study could be also attributed to the aerobic pre-treatment used, in order to biodegrade the already low and diluted (with CW) phenolics content of the mixture (by 54%) prior to anaerobic digestion.

**Figure 7-10:** (a) Total COD (TCOD), soluble COD (SCOD), total organic carbon (TOC), soluble organic carbon (SOC) and TVFA (expressed in units of COD) evolution and (b) total (TS) and volatile solid (VS) concentration during methanogenesis for each HRT tested.
Table 7-8: Steady-state characteristics of the methanogenic CSTR for each HRT tested.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HRT (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>pH</td>
<td>7.78</td>
</tr>
<tr>
<td>Biogas (L/LR·d)</td>
<td>0.84</td>
</tr>
<tr>
<td>CH4 (L/LR·d)</td>
<td>0.50</td>
</tr>
<tr>
<td>CH4 (%)</td>
<td>59.43</td>
</tr>
<tr>
<td>Yield CH4 (mL CH4/g VS added)</td>
<td>250.75</td>
</tr>
<tr>
<td>Yield CH4 (mL CH4/g COD consumed)</td>
<td>294.37</td>
</tr>
<tr>
<td>TOC removal (%)</td>
<td>51.74</td>
</tr>
<tr>
<td>SOC removal (%)</td>
<td>51.97</td>
</tr>
<tr>
<td>Total carbohydrates removal (%)</td>
<td>94.55</td>
</tr>
<tr>
<td>TS removal (%)</td>
<td>26.11</td>
</tr>
<tr>
<td>VS removal (%)</td>
<td>47.51</td>
</tr>
<tr>
<td>Phenols removal (%)</td>
<td>33.11</td>
</tr>
</tbody>
</table>

7.6 Biochemical methane potential

Biochemical Methane Potential (BMP) of the mixture was also studied according to batch assay (Section 2.2.3). In Fig. 7.11 the cumulative methane production as a function of the digestion time, is presented. The calculated methane production of the mixture, after subtraction of the methane produced from the blank experiment, was 98.78 ± 4.25 mL of CH4. The potential value was 472.41 ± 20.32 mL CH4@STP/g VS added. The obtained methane potential is higher than the respective value obtained from CSTR experiment in this study (250.75 mL CH4/g VS added at HRT of 20 d), probably due to the phenols concentration (toxicity) in the system. Nevertheless, the methane potential obtained from BMP assay was very similar with this obtained from the two-stage CSTR system (467.53 mL CH4/g VS added) and described at Section 5.4.2, treating the same mixture without controlled pH in acidogenesis. It is most likely due to the concentrations of sodium and potassium in the system, in order to keep the pH value, in the acidogenic reactor, stable and equal to 6.0. It is well-known that high concentration of sodium (5.5 g Na+/L) or potassium (0.15 M K+) lead to strong inhibition of methanogenesis (Chen et al., 2008)
Moreover, the cumulative biomethane production profile was fitted to a modified Gompertz bacterial growth model. The equation used (Eq. (7.12)) is a modified form from Eq. (7.1).

\[
M(t) = P \exp \left\{ - \exp \left[ \frac{R_m e}{P} (\lambda - t) + 1 \right] \right\}
\]  

(7.12)

where \( M(t) \) is the cumulative methane production (mL); \( P \) is the maximum methane production potential (mL); \( R_m \) is the maximum methane production rate (mL/d); \( \lambda \) is the lag-phase duration (d); \( t \) is the time (d) and \( e \) is \( \exp(1) = 2.71828 \).

**Fig. 7.12** depicts the methane production based on experimental data and the simulation generated using the fitted modified Gompertz model. The correlation coefficient (\( R^2 \)) was 0.998, whereas the methane production potential (\( P \)) was 97.39 ± 0.69 mL, the maximum methane production rate (\( R_m \)) was 5.85 ± 0.26 mL/d and finally the lag-phase (\( \lambda \)) was 11.18 ± 0.35 d.

**Figure 7-11:** Cumulative methane production during the BMP assay from the mixture (55% OMW, 40% CW and 5% LCM) and blank sample. Errors bars represent the standard deviation for the replicates.
7.7 Conclusions

In this study, bio-hydrogen was efficiently produced and maximized by properly adjusting the operating pH during the acidogenesis stage of OMW, CW and LCM co-digestion with a ratio of 55:40:5 (v/v/v). The maximum TVFAs yield and hydrogen productivity was observed at pH 6.0 with a hydrogen yield of 0.642 mol H₂/mol equivalent glucose consumed. Hydrogen production was correlated to the evolution of butyric acid with a molar ratio of 0.91 (moles of hydrogen/mole of butyric acid) at all pH values tested except in pH 7.5 at which the highest ratio (2.03) was observed. It has also been demonstrated that co-digestion of OMW, CW and LCM (55:40:5, v/v/v) in a two-stage mesophilic (37°C) CSTR system is a sustainable and environmentally attractive method. The highest hydrogen production rate (1.72 L/Lₚ·d) and hydrogen yield of 0.54 mol H₂/mol carbohydrates consumed was achieved when the acidogenic reactor was operated at HRT 0.75 d, whereas at higher HRTs vast amounts of VFAs could be produced accompanied by lower hydrogen productivity. Stable methanogenesis could be obtained operating the digester at non-controlled pH 7.8 and HRT 25 d producing 0.33 L CH₄/Lₚ·d with a high methane yield of 316.08 mL CH₄/g COD consumed.
7.8 References


Anaerobic co-digestion of agro-industrial wastewaters in a two-stage system


8.1 Abstract

This study is focused on the anaerobic co-digestion using agro-industrial liquid wastewaters mixed with sweet sorghum stalks (i.e. 55% sorghum, 40% cheese whey and 5% liquid cow manure). Firstly, batch acidogenic experiments were performed to investigate the effect of controlled pH (5.0, 5.5, 6.0, 6.5) on the production of bio-hydrogen and volatile fatty acids. According to the obtained results, the maximum hydrogen yield of 0.52 mol H₂/mol equiv. glucose was measured at pH 5.5, whereas the highest degradation of carbohydrates and hydrogen productivity was observed at pH 6.5. The use of heat-treated anaerobic sludge had positive impact on bio-hydrogen production exhibiting a yield of 1.09 mol H₂/mol equiv. glucose. On the other hand, usage of ensiled sorghum, instead of fresh one, led to lower hydrogen yield. In all experiments, the main fermentation end-products were volatile fatty acids (i.e. acetic, propionic, butyric) ethanol and lactic acid. Then, the effect of hydraulic retention time (HRT) on hydrogen and methane production, using a two-stage anaerobic process, was investigated. Two continuously stirred tank reactors (CSTRs) were used under mesophilic conditions (37°C) in order to enhance acidogenesis and methanogenesis. A mixture of pretreated ensiled sorghum, cheese whey and liquid cow manure (55:40:5, v/v/v) was used. The acidogenic reactor was operated at six different HRTs of 5, 3, 2, 1, 0.75 and 0.5 d, under controlled pH 5.5, whereas the methanogenic reactor was operated at three HRTs of 24, 16 and 12 d. The maximum H₂ productivity (2.14 L/Lₚ·d) and maximum H₂ yield (0.70 mol H₂/mol carbohydrates consumed) were observed at 0.5 d HRT. On the other hand, the maximum CH₄ production rate of 0.90 L/Lₚ·d was achieved at HRT of 16 d, whereas at lower HRT the process appeared to be inhibited and/or overloaded.
8.2 Introduction

Renewable energy sources have received great interest from the international community during the last decades. Biomass is one of the oldest and the most promising energy sources. Organic wastes such as animal wastes, wastewaters with high organic content, energy crops, agricultural and agro-industrial residues can be used for the production of power, heat and biofuels (Claassen et al., 1999). Among agro-industrial wastewaters, cheese whey and liquid cow manure can be used for the production of biogas.

Cheese manufacturing industry generates large amounts of high strength wastewater, with associated high biological (BOD₅) and chemical oxygen demand (COD) with the BOD₅/COD ratio being commonly higher than 0.5 (Prazeres et al., 2012). Cheese whey (CW) is a by-product of cheese manufacturing which mainly contains a significant amount of carbohydrates (4–5%), mainly lactose (45-50 g/L), proteins (6-8 g/L), lipids (4-5 g/L) and mineral salts (8-10% of dried extract); mineral salts include NaCl and KCl (>50%), calcium salts and others. CW also contains appreciable quantities of lactic (0.5 g/L) and citric acid and B-group vitamins (Venetsaneas et al., 2009; Prazeres et al., 2012). Hence, this substrate is easily amenable to bioconversions (Prazeres et al., 2012). However, despite its high carbohydrate content, the anaerobic treatment of raw CW is quite problematic due to its low bicarbonate alkalinity (50 meq/L), high COD concentration (up to 70 g COD/L) and tendency to rapid acidification (Prazeres et al., 2012).

Liquid cow manure (LCM) is one of the most polluting agro-industrial wastes. The amounts of liquid and semi-liquid animal manure produced in dairy farms depend on the amount of fresh water used in daily operations. Large amounts of dairy manure are usually poorly managed, while decomposing livestock manure is considered to be a targeted environmental pollutant due to its high organic matter, nutrients (i.e. nitrogen and phosphorous concentrations), methane and ammonia emissions and pathogens (Ryden et al., 1987; Carpenter et al., 1998).

Sorghum is a C₄, heat- and drought- tolerant highly productive crop, with a high photosynthetic efficiency. It can be considered as replacement to corn since it requires less water and exhibits better yields than corn in hotter and drier areas. Sorghum is one of several plant species that has been identified as a promising “energy crop” and has thus generated great interest as a feedstock for bioethanol (Li et al., 2010) and biogas production (Ntaikou et al., 2008; Sambusiti et al., 2013b), due to its high yields and biodegradability. Sorghum is a quite diverse species but generally falls into four categories, including grain sorghum, forage sorghum, sudangrass and sudangrass hybrids sorghum. Its biomass, as lignocellulosic grass, is composed mainly of cellulose, hemicelluloses and lignin, while its fraction of soluble sugars is rich in glucose and sucrose (Panagiotopoulos et al., 2010). Fermentable soluble sugars are the primary source for bioethanol and biogas production. Normally, cellulose and hemicelluloses are degradable by anaerobic microorganisms; nevertheless, their association with lignin,
which acts as a physical barrier, limits their degradation. These limitations can be overcome by pretreatment methods, which break down the linkage between polysaccharides and lignin thus making cellulose and hemicellulose more accessible to hydrolytic enzymes during anaerobic digestion (Mosier et al., 2005). Utilization of sorghum stalks during a year-round operation of an anaerobic digester requires a cost-effective and non-destructive, if possible, method of preservation and storage of harvested sorghum stalks for extended periods of time. To this end, sorghum ensiling is the preferred long-term preservation method.

Multiple streams of organic substrates can be anaerobically co-digested to generate a homogeneous mixture increasing both process and equipment performance. The two-stage anaerobic treatment process has several advantages over the conventional single-stage one, since it permits the selection and enrichment of different bacteria in each digester. It increases thus the stability of the whole process by controlling the acidification phase in the first digester and hence preventing overloading and/or inhibition of the methanogenic population in the second digester (Nathao et al., 2013). At the first stage (acidogenesis) generation of biological hydrogen occurs whereas, at the second stage (methanogenesis), methane evolves. Optimum environmental and operational conditions for each microbial community may be achieved in such a separated two-reactor system resulting in the production of significant amounts of gaseous high-energy end-products (CH₄ and/or H₂). A series of operational parameters including pH (Davila-Vazquez et al., 2008; Dareioti et al., 2014), temperature (Bayr et al., 2012), reactor configuration (Nasir et al., 2012), organic loading rate (Mariakakis et al., 2011) and hydraulic retention time (Rincón et al., 2008) have been investigated in the literature due to their effect on biogas productivity.

It is known that under fermentative conditions sugar degradation is accompanied by the production of hydrogen and metabolic products, mainly volatile fatty acids (i.e. acetic, propionic, butyric acid), lactic acid and ethanol. The biochemical pathway followed and consequently the production of hydrogen and fermentation end-products are highly dependent on the conditions of the process, such as pH (Fang and Liu, 2002; Ferchichi et al., 2005; Dareioti et al., 2014) among others. The pH dependency during acidogenesis by mixed microbial populations is strongly related to hydrogen production and selective acid production for subsequent methanogenic phase of anaerobic digestion. A number of studies point to pH value of 5.0 to 6.0 for maximum hydrogen yields (Lay, 2000; Van Ginkel et al., 2001; Fang and Liu, 2002). Lay (2000), for example, reported maximum hydrogen evolution efficiency at pH 5.2 and HRT 17 h using starch as substrate, while alcohol production was favored below pH 4.1, probably due to low hydrogenase activity at low pH values. On the other hand, Ren et al. (1997) stated that the operating pH must be maintained at about pH 4.5 to avoid propionic fermentation stimulating ethanol fermentation. However, the optimum pH, in terms of maximized volatile fatty acids production, for hydrolysis and acidogenesis of kitchen wastes was 7.0, while the lactic acid concentration was relatively low considering that it may be inhibiting the subsequent methanogenic phase (Zhang et al., 2005).
Optimization of operational conditions (i.e. pH, temperature) for hydrogen fermenters results in high hydrogen yields by simultaneously preventing methanogens and acetogens from hydrogen consumption. Observations and theoretical considerations reported in the literature suggest that homoacetogenesis and propionic acid production would occur predominantly at pH 5.0 or higher. Homoacetogenesis is unlikely to occur at pH 5.0 or lower due to the fact that production of acetic acid acts as an uncoupler under such conditions (Rogers and Gottschalk, 1993). Accumulation of propionic acid at pH 5.0-6.0 with a mixed culture resulted into limited hydrogen production, thus operation at pH 4.5 was recommended (Ren et al., 1997). The production of methane is strongly related to hydrogen-consumption, under acidic conditions (Hwang et al., 2004). In the latter case, hydrogen-utilizing methanogens were active at pH 5.0, resulting into negative pressure in the headspace of the reactor. According to Lay (2000), heat-treated inocula may also change the microbial community during acidogenesis by developing hydrogen-producing bacteria (i.e. *Clostridium* sp.). However, limited studies have assessed heat-treated versus non heat-treated inocula under same batch experimental conditions (Kraemer and Bagley, 2007). Biohydrogen production is inhibited as hydrogen partial pressure increases (Ruzicka, 1996). Operating bioreactors at low hydrogen partial pressure, e.g. by sparging with nitrogen gas, may result into hydrogen stripping from the liquid samples of mixed cultures (Mizuno et al., 2000; Hussy et al., 2003).

Furthermore, hydraulic retention time (HRT) has been reported as one of the most important parameters significantly affecting microbial ecology in CSTR digesters and must be thus optimized for the particular feedstock fermented in the digester. There are many reports in the literature on the continuous anaerobic digestion of cheese whey and liquid cow manure either as mono-substrates (Ghaly, 1996), or being co-digested (Kavacik and Topaloglu, 2010) or, even more, co-digested with other substrates (Dareioti et al., 2009). Notwithstanding, continuous anaerobic digestion experiments using sorghum as substrate, are time-consuming and complex, so methane productivity testing in the literature is generally based on the results of batch tests (Sambusiti et al., 2012; Antonopoulou and Lyberatos, 2013). The commonly used batch tests, although valuable for establishing methane production potentials under specific conditions may fail to truly predict full-scale anaerobic reactors performance, due to their dependency on inoculum type, the substrate to inoculum ratio, and the batch nature of the test itself. Therefore, in order to monitor possible inhibition effects due to addition of chemicals and evaluate the anaerobic digestion performance in terms of biogas production, tests with continuous reactors are needed in order to confirm and quantify the effect on anaerobic digestion of a specific substrate and/or specific operational conditions (Carrère et al., 2010). According to our knowledge, lignocellulosic substrates treatment and especially co-digestion of them with other substrates have been poorly studied with continuous anaerobic reactors. For instance, co-digestion of manures, energy crops and agro-wastes, was studied by Giuliano et al. (2013) using pilot-scale CSTRs, and proved its viability at all operating conditions tested. One experience on biogas production from
steam pretreated and enzymatically hydrolyzed wheat straw was also conducted at pilot-scale (Nkemka and Murto, 2012). Recently, some authors reported experiences of anaerobic continuous laboratory-scale reactors treating the hydrolysate fraction (syrup) of sweet sorghum (Antonopoulou et al., 2008; Saraphirom and Reungsang, 2011), wheat straw (Kaparaju et al., 2009) and oat straw (Gomez-Tovar et al., 2012). For instance, Antonopoulou et al. (2008) reported the production of hydrogen and methane from the hydrolysate of sweet sorghum in a continuous stirred tank reactor (CSTR). The hydrolysate was obtained by aqueous extraction at 30°C and was used as substrate for the production of hydrogen; the effluent of the hydrogen-producing reactor was used to produce methane in a CSTR. Furthermore, only few studies are yet available treating raw sorghum on anaerobic digestion in continuous anaerobic reactors (Jerger et al., 1987; Sambusiti et al., 2013b) and especially in a two-stage system for hydrogen and methane production, respectively. Sambusiti et al. (2013b), for example, studied the effect of pretreated ensiled sorghum forage in single-stage anaerobic digestion and showed an increase of 25% on methane production in comparison with untreated sorghum.

The aim of this study was to investigate the effect of pH during anaerobic acidogenesis of an agro-waste mixture consisting of cheese whey (CW) and liquid cow manure (LCM) with sweet sorghum stalks (FS2) on hydrogen production and end-products (i.e. volatile fatty acids, lactic acid, ethanol) distribution. The pH values tested ranged from 5.0 to 6.5 with 0.5 increment and were maintained constant throughout the fermentation process. This study also determined experimentally the influence of heat-treated sludge, organic loading of initial substrate and the role of ensiled sorghum on the hydrogen yield. Another significant investigation was to study the anaerobic co-treatment of a mixture of pretreated ensiled sorghum (ES3), cheese whey (CW) and liquid cow manure (LCM) (in a ratio 55:40:5, v/v/v) in a two-stage continuous anaerobic process. More specifically, our aim was to study the effect of HRT, as one of the most critical operating parameters, on hydrogen and methane productivity and also the contribution of ensiled sorghum which replaced olive mill wastewater (OMW) in the same mixture previously studied by Dareioti and Kornaros (2014). Replacement of OMW by ES3 simulates the operation of a decentralized AD plant fed with regional agro-wastes which lacks OMW due to seasonal unavailability.
8.3 The influence of pH and relative factors on acidogenic hydrogen production from a mixture of sweet sorghum stalks, cheese whey and cow manure

Anaerobic batch experiments were carried out using a mixture of agro-wastes at a ratio of 55% FS2, 40% CW and 5% LCM (v/v/v) in order to assess the effect of pH on biohydrogen production and the composition of anaerobic acidogenic end-products. All experiments were conducted in the same reactor configuration (Section 2.2.2) using acclimatized anaerobic culture seed sludge, which was obtained from a lab-scale anaerobic acidogenic reactor (CSTR), that was being fed a mixture of 55% OMW, 40% CW and 5% LCM (v/v/v). Prior to batch experiments, centrifugation (4500 rpm) of the acclimatized anaerobic culture seed sludge used as inoculum was performed to remove the soluble part of the sludge. The working volume of the fermentor was adjusted to 900 mL. The amount of anaerobic sludge used as inoculum was 20% (v/v) of the working volume, while the remaining consisted of the tested waste mixture. The pH of the mixed liquor was kept constant throughout the course of the experiment via automatic control (using a HACH PID-controller) by adding drops of NaOH or HCl solution (6 N). The pH of agro-waste mixture was controlled at 5.0, 5.5, 6.0 and 6.5. At regular intervals samples were withdrawn for composition analysis, i.e. determination of carbohydrates, lactic acid, volatile fatty acids, alcohols (i.e. ethanol), TS, VS, TOC and SOC. Qualitative and quantitative composition analysis of produced biogas was performed throughout the course of each experiment.

When the optimum pH value was selected, based on the obtained results, additional anaerobic batch experiments were carried out to assess and compare their performance in terms of bio-hydrogen yield and acidified end-products. The following experiments were thus carried out using: a) heat-treated enriched anaerobic sludge (100°C for 20 min) instead of the acclimatized anaerobic culture seed sludge (to assess the effect of inoculum), b) half initial mixed substrate concentration, i.e. 17.15 g total carbohydrates/L (to assess the effect of initial organic loading) and c) pretreated ensiled sorghum (ES2), instead of fresh one (FS2). The ES2 pretreatment was performed at constant temperature of 80°C for 120 min with the addition of alkaline solution 1.0% NaOH and 1.0% KOH (w/w) based on previous study (Section 4.5).

8.3.1 Materials

The substrates used in the present study were two typical agro-industrial wastewaters, namely cheese whey (CW) and liquid cow manure (LCM), and an energy crop, i.e. fresh and ensiled sorghum stalks (FS2, ES2). The raw wastewaters used were collected and stored according to Section 2.1.1, whereas the characteristics of each type of sorghum were presented in Section 2.1.2. Table 8.1 represents the average values measured during the charaterization of each wastewater, whereas the chemical composition of fresh and ensiled sweet sorghums, after drying and milling is given in Table 8.2.
Table 8-1: Chemical composition of cheese whey (CW) and liquid cow manure (LCM).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>CW</th>
<th>LCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-</td>
<td>6.17 ± 0.03</td>
<td>7.26 ± 0.18</td>
</tr>
<tr>
<td>TSS</td>
<td>g/L</td>
<td>38.00 ± 0.11</td>
<td>48.60 ± 0.24</td>
</tr>
<tr>
<td>VSS</td>
<td>g/L</td>
<td>34.60 ± 0.14</td>
<td>35.80 ± 0.53</td>
</tr>
<tr>
<td>TS</td>
<td>g/L</td>
<td>69.40 ± 0.34</td>
<td>52.40 ± 0.93</td>
</tr>
<tr>
<td>VS</td>
<td>g/L</td>
<td>60.17 ± 0.25</td>
<td>35.83 ± 0.64</td>
</tr>
<tr>
<td>Total COD</td>
<td>g/L</td>
<td>75.00 ± 0.29</td>
<td>62.50 ± 2.12</td>
</tr>
<tr>
<td>Soluble COD</td>
<td>g/L</td>
<td>66.73 ± 0.17</td>
<td>23.02 ± 0.26</td>
</tr>
<tr>
<td>TOC</td>
<td>g/L</td>
<td>31.45 ± 0.64</td>
<td>24.19 ± 0.02</td>
</tr>
<tr>
<td>Total carbohydrates(^b)</td>
<td>g/L</td>
<td>64.40 ± 0.90</td>
<td>8.12 ± 0.31</td>
</tr>
<tr>
<td>Soluble carbohydrates(^b)</td>
<td>g/L</td>
<td>62.80 ± 1.04</td>
<td>1.20 ± 0.01</td>
</tr>
<tr>
<td>Total nitrogen, TKN</td>
<td>g/L</td>
<td>0.84 ± 0.08</td>
<td>3.36 ± 0.00</td>
</tr>
<tr>
<td>Ammonium nitrogen</td>
<td>g/L</td>
<td>0.10 ± 0.02</td>
<td>2.39 ± 0.02</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>g CaCO(_3)/L</td>
<td>0.50 ± 0.00</td>
<td>12.38 ± 0.32</td>
</tr>
<tr>
<td>Total VFAs</td>
<td>g/L</td>
<td>0.07 ± 0.00</td>
<td>7.24 ± 0.02</td>
</tr>
</tbody>
</table>

\(^a\) Mean values (± standard deviation); \(^b\) In equivalent glucose

---

Table 8-2 : Chemical composition of fresh and ensiled sweet sorghum. Values correspond to mean ± standard deviation of measurement performed in duplicate.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>FS2</th>
<th>ES2</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.60 ± 0.04</td>
<td>4.62 ± 0.08</td>
</tr>
<tr>
<td>Moisture (% wet weight)</td>
<td>76.70 ± 0.11</td>
<td>77.69 ± 0.29</td>
</tr>
<tr>
<td>TS (% wet weight)</td>
<td>23.30 ± 0.09</td>
<td>22.31 ± 0.14</td>
</tr>
<tr>
<td>VS (%TS)</td>
<td>94.50 ± 0.17</td>
<td>94.36 ± 0.08</td>
</tr>
<tr>
<td>Ash (%TS)</td>
<td>5.50 ± 0.03</td>
<td>5.64 ± 0.04</td>
</tr>
<tr>
<td>TOC (%TS)</td>
<td>59.80 ± 0.04</td>
<td>51.00 ± 1.23</td>
</tr>
<tr>
<td>Total carbohydrates(^b) (%TS)</td>
<td>81.00 ± 0.33</td>
<td>28.91 ± 0.96</td>
</tr>
<tr>
<td>Soluble carbohydrates(^b) (%TS)</td>
<td>49.00 ± 0.28</td>
<td>2.90 ± 0.11</td>
</tr>
<tr>
<td>Cellulose (%TS)</td>
<td>23.00 ± 0.41</td>
<td>37.88 ± 2.11</td>
</tr>
<tr>
<td>Hemicellulose (%TS)</td>
<td>11.00 ± 0.72</td>
<td>17.50 ± 1.23</td>
</tr>
<tr>
<td>Lignin (%TS)</td>
<td>8.00 ± 0.44</td>
<td>16.88 ± 3.12</td>
</tr>
<tr>
<td>Total nitrogen, TKN (%TS)</td>
<td>0.10 ± 0.01</td>
<td>0.10 ± 0.00</td>
</tr>
<tr>
<td>Proteins (%TS)</td>
<td>0.70 ± 0.06</td>
<td>0.70 ± 0.00</td>
</tr>
<tr>
<td>Lactic acid (%TS)</td>
<td>N.D</td>
<td>1.15 ±0.03</td>
</tr>
<tr>
<td>Ethanol (%TS)</td>
<td>N.D</td>
<td>8.90 ±0.09</td>
</tr>
</tbody>
</table>

\(^a\) Mean values (± standard deviation); \(^b\) In equivalent glucose; N.D:No Detected
In particular, CW was characterized by high organic load mainly due to carbohydrates (lactose), whereas LCM was characterized by high concentration of volatile fatty acids and nitrogen. Sweet sorghum mainly consisted of soluble and insoluble polysaccharides (cellulose and hemicellulose) and high content of lignin. During the ensiling procedure soluble carbohydrates are utilized by fermentative bacteria for the production of volatile fatty acids, lactic acid and ethanol, depending on the metabolic pathway followed. Thus, the amount of soluble carbohydrates in the ensiled sorghum is low and lactic acid concentration was observed.

### 8.3.2 Bacterial growth model

The cumulative bio-hydrogen production profile from each batch experiment was fitted to a modified Gompertz bacterial growth model (Eq.8.1) using OriginPro version 8. This equation has been widely used to model gas production data (Mohd Yasin et al., 2011).

\[
H = P \exp \left\{ - \exp \left[ \frac{R_m}{P} (\lambda - t) + 1 \right] \right\}
\]

where \(H\) is the cumulative hydrogen production (mL); \(P\) is the maximum hydrogen production potential (mL); \(R_m\) is the maximum hydrogen production rate (mL/h); \(\lambda\) is the lag-phase duration (h); \(t\) is the time (h) and \(e\) is \(\exp(1) = 2.71828\).

The specific hydrogen production potential (SHPP) was obtained by dividing \(P\) with the substrate COD applied (Khanal et al., 2004). The maximum specific hydrogen production rate (SHPR\(_m\)) was determined by dividing \(R_m\) by the volatile suspended solids (VSS) added. The hydrogen conversion efficiency for different pH values was compared based on SHPP and SHPR\(_m\).
8.3.3 Effect of pH

Batch acidogenic experiments were performed to investigate the effect of controlled pH (5.0, 5.5, 6.0, 6.5) on the production of hydrogen and volatile fatty acids. The tested waste mixture consisted of 55% FS2 (8% dry matter sorghum stalks suspended in water), 40% CW and 5% LCM (v/v/v). This study is a sequel of a previous study in which equal organic loading was used consisting of 55% OMW, 40% CW and 5% LCM (Dareioti et al., 2014). The reason for using sweet sorghum was to replace other agricultural wastewaters such as OMW which will not be available during a full annual operation of a plant (OMW availability lies between October to February).

The consumption of total carbohydrates was high in all pH values tested (Fig. 8.1(a)). Maximum carbohydrates degradation (77%) was observed at pH 6.5. However, the soluble carbohydrates consumption was similar (~95-96%) at all pH values, suggesting that the microorganisms’ ability to consume sugars was not altered within this pH range. The dominant soluble end-products were volatile fatty acids (i.e. acetic, propionic, butyric), ethanol and lactic acid in all pH values tested (Fig. 8.1(b)). Both acetic and butyric acid are well known metabolites in the anaerobic hydrogen fermentation of carbohydrates, whereas propionic acid is considered an undesirable product of hydrogen fermentation (Kawagoshi et al., 2005). Acetic and butyric acid were the most abundant end-products at each pH. However, the concentrations of propionic acid and ethanol were relatively lower (<2300 ppm and 900 ppm, respectively) and limited amounts (<250 ppm) of other volatile fatty acids (i.e. i-butyric, valeric, i-valeric and caproic) were detected in all tests. Accumulation of lactic acid (as intermediate product) was observed at pH 5.0 (8.86 g lactic acid/L), whereas at higher pH it was metabolized during the process and thus not detected at the end of batch test (Fig. 8.1(b)).

![Figure 8-1](image-url)

**Figure 8-1:** Effect of pH on (a) carbohydrates consumption and (b) main soluble end-products.
As can be seen in Fig. 8.1(b), the distribution of main soluble end-products was similar at all pH values apart from pH 5.0. Hence, at these pH values biogas and hydrogen productivity was also approximately at the same level (Fig. 8.2(a)). Table 8.3 presents additional results at each pH tested, including hydrogen yield and as well as organic carbon and solids’ degradation. As shown, the highest degradation of total organic carbon was obtained at pH 5.5 (17.07%), as well as VS degradation (22.94%). Taken together, these data show that the pH value of 5.5 is the most suitable in terms of hydrolysis. Hydrogen yield (calculated as mol H₂/mol carbohydrate consumed) is a good indicator to the effectiveness of hydrogen production and represents the capability of microorganisms to convert carbohydrates into hydrogen gas. Fig. 8.2(b) presents the net hydrogen yield (moles of hydrogen produced per mole equivalent glucose of total consumed carbohydrates) at each pH tested. The maximum hydrogen yield of 0.52 mol H₂/mol equiv. glucose was observed at pH 5.5. These results indicate that pH had indeed an effect on hydrogen yield and thus pH adjustment of agro-waste mixture during anaerobic acidogenesis may determine the type of anaerobic fermentation pathway followed during the anaerobic bio-hydrogen process. In literature many studies have been reported about the effect of initial and controlled pH on hydrogen production using many different substrates. For instance, in the study of Van Ginkel et al. (2001) the highest rate (74.7 mL H₂/LR·h) of hydrogen production using sucrose and a heat-treated inoculum was observed at pH 5.5. Similarly, the maximum hydrogen content of 26.9% and production rate of 31.8 mL H₂/h were observed at pH 5.5 by Hernández and Rodríguez (2013) who studied pig manure fermentation in an Anaerobic Batch Reactor (ABR). Fang and Liu (2002) studied the conversion of glucose to hydrogen at different pH values (4.0 to 7.0) and suggested that pH 5.5 was the optimum with the highest hydrogen content (64.2%) and yield of 2.1 ± 0.1 mol H₂/mol glucose.

Figure 8-2: Effect of pH on (a) biogas and hydrogen production and (b) hydrogen yield.
Chittibabu et al. (2006) observed the maximum hydrogen production at initial pH 6.0. However, Davila-Vazquez et al. (2008) studied the effect of initial pH (3.88 – 8.12) on hydrogen production using three different substrates, namely lactose, cheese whey powder and glucose. pH 7.5 was found as the optimum for lactose and glucose, whereas pH 6.0 was suggested for the cheese whey powder. Wang and Wan (2009), who reported that batch reactors with non-regulated pH and treating sucrose are the systems most commonly studied, suggested that further investigations should focus rather on pH-controlled systems and on more complex organic wastes as substrates. The effect of controlled pH (3.5 to 6.0) was studied by Bengtsson et al. (2008) who suggested that optimum pH is around 5.25 – 5.5 for whey treatment and pH around 5.5 – 6.0 for paper mill effluent. Using food waste in thermophilic anaerobic fermentation, Mohd Yasin et al. (2011) found that the greatest hydrogen yield under controlled pH value of 5.5 was 1769.6 mL H₂/L-medium·d.

<table>
<thead>
<tr>
<th>pH</th>
<th>Yield (mol H₂/mol equiv.glucose)</th>
<th>TOC removal (%)</th>
<th>SOC removal (%)</th>
<th>TS removal (%)</th>
<th>VS removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>0.15</td>
<td>11.01</td>
<td>23.97</td>
<td>5.77</td>
<td>14.64</td>
</tr>
<tr>
<td>5.5</td>
<td>0.52</td>
<td>17.07</td>
<td>27.61</td>
<td>4.34</td>
<td>22.94</td>
</tr>
<tr>
<td>6.0</td>
<td>0.45</td>
<td>12.35</td>
<td>12.85</td>
<td>2.88</td>
<td>16.19</td>
</tr>
<tr>
<td>6.5</td>
<td>0.49</td>
<td>12.67</td>
<td>14.85</td>
<td>2.34</td>
<td>11.55</td>
</tr>
</tbody>
</table>

Fig. 8.3 displays the batch experimental results at the optimum pH 5.5, whereas the experimental results obtained in different pH values can be found in Appendix A (Fig. A.7–Fig. A.9). In particular, Fig. 8.3(a) presenting the concentration of total and soluble sugars over the fermentation experiment indicates that the achieved degradation was 61.95% and 96%, respectively. Carbohydrates fermentation contributed to the increase of volatile fatty acids, lactic acid and ethanol concentrations (Fig. 8.3(b)). The most abundant metabolic products were lactic and acetic acid during the first 10 h of batch experiment. Significant accumulation of butyric acid (~9.5 g/L) was observed after a period of 10 h, as a result of lactic acid bioconversion, which was also accompanied by simultaneous production of hydrogen (Fig. 8.3(c)). The produced biogas consisted exclusively of hydrogen and carbon dioxide. No methane was observed in any of the batch experiments that were conducted although LCM was used.
in the mixture. The complete absence of methane could be attributed to a number of reasons with the most valid ones being the use at a very low percentage (5% v/v) of a rather recalcitrant to biomethanization substrate (LCM), fermented under acidic conditions. Herein, it can be mentioned that pH can exert remarkable effects on end-products distribution. For instance, at the lowest pH tested in this study (5.0) accumulation of lactic acid, without further bioconversion, was observed due to kinetic limitations in the reactions converting lactic acid to butyric acid and subsequently to hydrogen production.

Figure 8-3: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and lactic acid and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of agro-waste mixture (in ratio of 55:40:5), at pH 5.5.
Anaerobic co-digestion of agro-industrial wastewaters and sweet sorghum in a two-stage system

Chapter 8

It is well known that lactic acid is produced from glucose via three metabolic pathways, i.e. the homofermentative (Eq. 8.2), the heterofermentative (Eq. 8.3) and the bifidum pathway (Eq. 8.4). In all three pathways the hydrogen balance is zero, i.e. no hydrogen is consumed nor produced.

\[ C_6H_{12}O_6 \rightarrow 2\text{CH}_3\text{CH(OH)}\text{COOH} \]  
(8.2)

\[ C_6H_{12}O_6 \rightarrow \text{CH}_3\text{CH(OH)}\text{COOH} + \text{CH}_3\text{CH}_2\text{OH} + \text{CO}_2 \]  
(8.3)

\[ 2C_6H_{12}O_6 \rightarrow 3\text{CH}_3\text{COOH} + 2\text{CH}_3\text{CH(OH)}\text{COOH} \]  
(8.4)

Lactic acid, as a major intermediate soluble product, may also decompose to acetic and propionic acid (Eq. 8.5) (Antonopoulou et al., 2008) or to butyric acid accompanied by simultaneous production of hydrogen via the following metabolic pathway (Eq. 8.6) (De Gioannis et al., 2012; Dareioti et al., 2014).

\[ 3\text{CH}_3\text{CH(OH)}\text{COOH} \rightarrow 2\text{CH}_3\text{CH}_2\text{COOH} + \text{CH}_3\text{COOH} + \text{CO}_2 + \text{H}_2\text{O} \]  
(8.5)

\[ 2\text{CH}_3\text{CH(OH)}\text{COOH} \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{CO}_2 + 2\text{H}_2 \]  
(8.6)

Moreover, with lactic acid depletion a simultaneous consumption of acetic acid and hydrogen was observed. According to Bhat and Barker (1947), in the C. kluyveri fermentation, butyric acid is probably formed by condensation of two moles of acetic acid with two moles of hydrogen (Eq. 8.7). Dareioti et al. (2014) reported briefly using mass balance calculations the bioconversion of acetic acid and hydrogen to butyric acid.

\[ 2\text{CH}_3\text{COOH} + 2\text{H}_2 \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \]  
(8.7)

Taking into account the aforementioned transformation pathways (Eqs. (8.2)-(8.7)), our experimental data obtained at the optimum pH (5.5) were used to calculate mass balances in order to estimate the pathways that result to hydrogen production under these conditions (Table 8.4). The calculations were carried out before and after the peak detection of lactic acid (8.5th h). First of all, the contribution of each pathway (Eqs. (8.2)-(8.4)) to the production of intermediates was studied (0-8.5 h). The ethanol formation was attributed to glucose degradation via heterofermentative pathway (Eq. 8.3) with simultaneous lactic acid production (Table 8.4, React. (2)). On the other hand, the produced acetic acid was attributed to bifidum pathway (Eq. 8.4), whereas the rest of glucose was decomposed to lactic acid via the homofermentative pathway (Eq. 8.2).
Table 8-4: Mass balances for hydrogen production at the optimum pH 5.5.

<table>
<thead>
<tr>
<th>Reactions for period (0-8.5 h)</th>
<th>Calculated values (mnoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
</tr>
<tr>
<td>1. C₆H₁₂O₆→2C₂H₅CH(OH)COOH</td>
<td>-6.76</td>
</tr>
<tr>
<td>2. C₆H₁₂O₆→CH₃CH(OH)COOH+CH₃CH₂OH+CO₂</td>
<td>-9.04</td>
</tr>
<tr>
<td>3. 2C₂H₅OH→3CH₃COOH+2CH₃CH(OH)COOH</td>
<td>-75.25</td>
</tr>
<tr>
<td>4. 3CH₃CH(OH)COOH→2CH₃CH₂COOH+CH₃COOH+CO₂+H₂O</td>
<td>-13.08</td>
</tr>
<tr>
<td>5. 2CH₃CH(OH)COOH→CH₃CH₂CH₂COOH+2CO₂+2H₂</td>
<td>-16.40</td>
</tr>
<tr>
<td>6. 2CH₃COOH+2H₂→CH₃CH₂CH₂COOH+2H₂O</td>
<td></td>
</tr>
</tbody>
</table>

Net calculated values  -91.05  +68.33  +9.04  +14.08  +105.63  +8.72  +4.80
Experimental values   -91.05  +64.17  +9.04  +14.08  +105.63  +8.72  +4.78
Error (%)            0.00     6.48     0.00     0.00     0.00     0.00     0.42

Reactions for period (8.5-44.5 h)

<table>
<thead>
<tr>
<th>Reactions for period (8.5-44.5 h)</th>
<th>Calculated values (mnoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7. C₆H₁₂O₆→2CH₃CH(OH)COOH</td>
<td>-10.67</td>
</tr>
<tr>
<td>8. C₆H₁₂O₆→CH₃CH(OH)COOH+CH₃CH₂OH+CO₂</td>
<td>-0.70</td>
</tr>
<tr>
<td>9. 2C₂H₅OH→3CH₃COOH+2CH₃CH(OH)COOH</td>
<td>-0.00</td>
</tr>
<tr>
<td>10. 3CH₃CH(CH)COOH→2CH₃CH₂COOH+CH₃COOH+CO₂+H₂O</td>
<td>-8.60</td>
</tr>
<tr>
<td>11. 2CH₃CH(CH)COOH→CH₃CH₂CH₂COOH+2CO₂+2H₂</td>
<td>-69.18</td>
</tr>
<tr>
<td>12. 2CH₃COOH+2H₂→CH₃CH₂CH₂COOH+2H₂O</td>
<td></td>
</tr>
</tbody>
</table>

Net calculated values  -113.7  -55.74  +0.70  +45.54  -19.03  +5.74  +47.28
Experimental values   -113.7  -55.74  +0.70  +45.54  -19.80  +5.74  +47.55
Error (%)            0.00     0.00     0.00     3.39     0.00     0.00     0.57

*: Production; -: Consumption.
Moreover, in the first period of experiment (0-8.5 h), a slight lactic acid depletion was observed. The propionic acid formation was attributed to lactic acid bioconversion with simultaneous acetic acid production, according to Eq. (8.5) (Table 8.4, React. (4)). The butyric acid production was correlated with lactic acid degradation (Table 8.4, React. (5)) with simultaneous hydrogen production and with acetic acid and hydrogen depletion (Table 8.4, React. (6)). Under these calculations, an error of 6.48% was calculated in the mass balances of lactic acid accumulation, which is attributed to the complexity of microbial populations present in the mixed culture used in our experiments.

Then, a calculation was carried out at the second phase of the experiment (8.5-44.5 h) during lactic acid bioconversion with simultaneous hydrogen production. The continued degradation of carbohydrates after the 8.5th h till the end of experiment was calculated with the same way as described in the first period, in order to estimate the further lactic and acetic acid production (Table 8.4, React. (8.5)–(8.7)). The Eq. (8.5) was used in order to correlate the propionic acid production via lactic acid consumption (Table 8.4, React. (10)), whereas the rest of lactic acid was decomposed to butyric acid as well as hydrogen, according to Eq. (8.6) (Table 8.4, React. (11)). Finally, an additional calculation was done, the acetic acid depletion with the corresponding hydrogen towards the rest butyric acid production (Table 8.4, React. (12)). According to Table 8.4, slight errors of 3.89% and 0.57% were estimated in the mass balances of acetic acid and hydrogen respectively, which however can be partially attributed to experimental errors and also to biomass production which was not encountered in all calculations.

Moreover, the cumulative hydrogen production data from the experiments, where fresh sorghum was used, were fitted using the modified Gompertz equation (Eq. 8.1) by using the “fit curve” function in OriginPro version 8. Fig. 8.4 depicts the hydrogen production based on experimental data and the simulations using the modified Gompertz model. The correlation coefficient ($r^2$) ranged between 0.966 – 0.997, indicating the perfect fit to the experimental data. Comparing each set of experimental data with the revelant model simulation, the parameters of hydrogen production potential ($P$), the maximum hydrogen production rate ($R_m$) and lag-phase time ($\lambda$) was determined (Table 8.5). The $P$ and $R_m$ values peaked at pH of 6.5 and 6.0, respectively. The optimum pH for hydrogen production was found to be pH 6.5. The lag time was almost similar to all pH values ranging from 7.61 to 11.11 h. The variation in SHPP and SHPR$_m$ against the operating pH is presented in Table 8.5. As shown, the SHPP increased when pH increased from 5.0 to 6.5. The SHPP peaked at 14.26 mL H$_2$/g COD applied at pH 6.5. In a previous work (Section 7.4.2), where a mixture of agro-industrial wastes was used, a peak of 15.11 mL H$_2$/g COD applied was observed at pH 6.0. However, the maximum specific hydrogen production rate, using sucrose and starch as substrates was obtained by Khanal et al. (2004) at initial pH range 5.5 – 5.7.
Taking into account the results obtained in this work and the concept of biorefinery as a whole, in which the best compromise between hydrogen and VFAs production has to be considered in an integrated way aiming to maximize energy production in the overall system comprising of an acidogenic and methanogenic reactor, the pH 5.5 was considered as optimum for further work. Moreover, the use of pH 5.5 implies the use of lower amount of alkaline solution for pH control and lower inhibition to the subsequent step of methanogenesis due to sodium cations compared to operation at higher pH values.

**Figure 8-4:** Cumulative hydrogen production (experimental data and modified Gompertz model simulation) at different pH values tested.

**Table 8-5 :** Kinetic parameters of hydrogen production estimated using the modified Gompertz equation.

<table>
<thead>
<tr>
<th>pH</th>
<th>P (mL)</th>
<th>Rₘ (mL/h)</th>
<th>λ (h)</th>
<th>r²</th>
<th>SHPP (mL H₂/g COD added)</th>
<th>SHPRₘ (mL H₂/g VSS·h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>420.75±7.50</td>
<td>6.71±0.11</td>
<td>7.65±0.38</td>
<td>0.997</td>
<td>3.243</td>
<td>0.090</td>
</tr>
<tr>
<td>5.5</td>
<td>1194.28±17.66</td>
<td>88.94±5.83</td>
<td>11.11±0.43</td>
<td>0.985</td>
<td>10.807</td>
<td>1.252</td>
</tr>
<tr>
<td>6.0</td>
<td>1056.75±18.16</td>
<td>143.15±10.71</td>
<td>7.61±0.30</td>
<td>0.981</td>
<td>9.983</td>
<td>1.988</td>
</tr>
<tr>
<td>6.5</td>
<td>1505.63±41.44</td>
<td>63.48±3.52</td>
<td>8.74±0.59</td>
<td>0.966</td>
<td>14.259</td>
<td>1.136</td>
</tr>
</tbody>
</table>
8.3.4 Heat-treated inoculum

In an attempt to estimate the effect of inoculum pretreatment on hydrogen productivity, a batch experiment was performed at the optimum pH 5.5, using heat-treated enriched anaerobic sludge (Fig. 8.5) which could potentially enhance the sludge’s clostridia characteristics (Lay, 2000). The total and soluble carbohydrates consumption during the course of this experiment is presented in Fig. 8.5(a), reaching up to 58.9% and 94.9%, respectively which are almost similar to the ones obtained with untreated inoculum. The main metabolic end-product observed was butyric acid (Fig. 8.5(b)), which was produced from the bio-conversion of lactic acid, while the concentration of acetic acid was substantially lower than in the previous batch experiments. The pretreatment of inoculum prior to fermentation led to different metabolic pathways via killing or inactivating one or more bacteria types present in the “typical” mixed culture. In particular, a significant accumulation of lactic acid was performed (13.7 g lactic acid/L) during the first 30 h of experiment, whereas acetic acid production of 1.60 g/L was also observed. This indicates a change in the main metabolic pathway followed towards homofermentation. Lactic acid concentration was almost two-fold the one measured in the previous experiments which resulted in higher production of butyric acid with simultaneous hydrogen production (Fig. 8.5(c)).

In Table 8.6 are reported the mass balance calculations obtained with the same way as described in the previous batch, using the experimental data of batch with heat-treated inoculum. The calculations were also carried out before and after the peak detection of lactic acid (30.5th h). As mentioned before, the main metabolic pathway of glucose degradation was the homofermentation (Table 8.6, React. (1)). For this reason, higher concentration of lactic acid was observed compared to previous batch experiment with untreated inoculum. Moreover, in the first period (0-30.5 h) no lactic acid bioconversion was observed, which occurred only in the second phase (30.5-139.5 h). Using mass balance calculations, a negligible error of 1.74% was obtained from the bioconversion of lactic acid. In the second period of experiment (30.5-139.5 h) calculations were also done, whereas higher hydrogen production was observed due to higher lactic acid amount, comparing with experiment with untreated sludge.

The negative impact of heat treatment of inoculum was the lower process rates (e.g. carbohydrates consumption, lactic acid production and bioconversion, hydrogen production etc) exhibited compared to the ones presented in all previous batch tests, where untreated inoculum was used. *Clostridium* species are considered to be the dominant organisms, responsible for butyrate fermentation (Dinopoulou et al., 1988). It has been reported that heat-treatment of sludge may eradicate the coexistence of bacteria in inoculums, which have an adverse effect on hydrogen fermentation, and accelerate an enrichment of hydrogen producing bacteria, such as spore forming *Clostridium* species, which are highly tolerant to extreme environments (Chen et al., 2002; Logan et al., 2002). Indeed, the overall hydrogen yield estimated in this test was...
increased (1.09 mol H₂/mol equivalent glucose) compared to the previous experiments where non-heat-treated inocula were used (0.52 mol H₂/mol equivalent glucose). This is in agreement with a previous study by Oh et al. (2003) in which fermentation of glucose with heat-treated culture at pH values of 6.2 and 7.5 gave better results compared to non-heat-treated one. However, Kawagoshi et al. (2005) observed no difference in the amount of hydrogen produced from heat-conditioned and unconditioned digested sludge.

Figure 8-5: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and lactic acid and (c) gaseous products (biogas and hydrogen) using heat-pretreated enriched anaerobic sludge, at pH 5.5.
Anaerobic co-digestion of agro-industrial wastewaters and sweet sorghum in a two-stage system

**Chapter 8**

### Table 8-6: Mass balances for hydrogen production using heat-treated inoculum at pH 5.5.

<table>
<thead>
<tr>
<th>Reactions for period (0-30.5 h)</th>
<th>Calculated values (mmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
</tr>
<tr>
<td>1. C₆H₁₂O₆→2CH₃CH(OH)COOH</td>
<td>-42.02</td>
</tr>
<tr>
<td>2. C₆H₁₂O₆→CH₃CH(OH)COOH+CH₃CH₂OH+CO₂</td>
<td>-0.96</td>
</tr>
<tr>
<td>3. 2C₆H₁₂O₆→3CH₃COOH+2CH₃CH(OH)COOH</td>
<td>-8.96</td>
</tr>
<tr>
<td>4. 3CH₃CH(OH)COOH→2CH₃CH₂COOH+CH₃COOH+CO₂+H₂O</td>
<td>-0.00</td>
</tr>
<tr>
<td>5. 2CH₃CH(OH)COOH→CH₃CH₃CH₂COOH+2CO₂+2H₂</td>
<td>-0.00</td>
</tr>
<tr>
<td>6. 2CH₃COOH+2H₂→CH₃CH₂CH₂COOH+2H₂O</td>
<td>+0.00</td>
</tr>
<tr>
<td><strong>Net calculated values</strong></td>
<td>-51.94</td>
</tr>
<tr>
<td><strong>Experimental values</strong></td>
<td>-51.94</td>
</tr>
<tr>
<td><strong>Error (%)</strong></td>
<td>0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reactions for period (30.5-139.5 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7. C₆H₁₂O₆→2CH₃CH(OH)COOH</td>
</tr>
<tr>
<td>8. C₆H₁₂O₆→CH₃CH(OH)COOH+CH₃CH₂OH+CO₂</td>
</tr>
<tr>
<td>9. 2C₆H₁₂O₆→3CH₃COOH+2CH₃CH(OH)COOH</td>
</tr>
<tr>
<td>10. 3CH₃CH(OH)COOH→2CH₃CH₂COOH+CH₃COOH+CO₂+H₂O</td>
</tr>
<tr>
<td>11. 2CH₃CH(OH)COOH→CH₃CH₃CH₂COOH+2CO₂+2H₂</td>
</tr>
<tr>
<td>12. 2CH₃COOH+2H₂→CH₃CH₂CH₂COOH+2H₂O</td>
</tr>
<tr>
<td><strong>Net calculated values</strong></td>
</tr>
<tr>
<td><strong>Experimental values</strong></td>
</tr>
<tr>
<td><strong>Error (%)</strong></td>
</tr>
</tbody>
</table>

+: production; -: consumption.
8.3.5 Substrate concentration

A batch experiment with the half initial substrate concentration (17.15 g total carbohydrates/L) was conducted at the optimum pH 5.5 in order to assess substrate inhibition phenomena to hydrogen and VFAs production (Fig. 8.6). Based on our results, no effect of initial substrate loading was identified, as the hydrogen yield (0.53 mol H$_2$/mol equiv. glucose) was comparable to the previously conducted batch experiment at pH 5.5 (0.52 mol H$_2$/mol equiv. glucose). In contrast to our results, Antonopoulou et al. (2011), conducting continuous experiments with different initial carbohydrates concentration in the range 9.89-20.99 g equiv. glucose/L, have reported that hydrogen productivity and yield depended significantly on the initial carbohydrate concentration used. Their maximum hydrogen yield ($0.74 \pm 0.02$ mol H$_2$/mol glucose consumed) was obtained at the concentration of 17.50 g carbohydrates/L, whereas the hydrogen yield decreased ($0.59 \pm 0.04$ mol H$_2$/mol glucose consumed) at the high concentration of 20.99 g carbohydrates/L. This behavior indicates that, under conditions, higher substrate concentrations may quickly become inhibitory through pH depletion, acid production or increased hydrogen partial pressure. Hence, removal of such inhibitory mechanisms is considered mandatory in the aim to achieve high hydrogen conversion efficiency and production rate at high substrate concentrations.

Few studies have suggested that higher hydrogen molar yield was found at low substrate concentration (Davila-Vazquez et al., 2008), whereas high initial substrate concentration caused high initial hydrogen production, increased hydrogen partial pressure and acid toxicity or pH inhibition. Salerno et al. (2006) found the highest hydrogen molar yield (1.17 mol H$_2$/mol glucose) using low glucose concentration (3.76 g/L) at pH 6.2, while Zheng and Yu (2005) attained hydrogen yield of 1.75 mol H$_2$/mol glucose at an initial glucose concentration of 10 g/L at pH 6.0. Based on the results of the current study, it is considered that the inhibitory/toxic thresholds are specific to each system and depend on the type of substrate and inoculum used.

![Figure 8-6](image_url)  

*Figure 8-6:* (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and lactic acid and (c) gaseous products (biogas and hydrogen) using half initial substrate concentration, at pH 5.5.
8.3.6 Effect of ensiled sorghum

The effect of using sweet sorghum obtained following an ensiling procedure, instead of fresh one, on hydrogen production was also assessed. The experiment was conducted at constant controlled pH of 5.5. Fig. 8.7(a) illustrates the total and soluble sugars bioconversion to soluble (Fig. 8.7(b)) and gaseous end-products (Fig. 8.7(c)) during the fermentation. 52.2% removal of total carbohydrates and 88.2% of soluble ones was observed during this experiment. The distribution of end-products was quite similar with the experiment with FS2. The most abundant metabolic end-products were the acetic and butyric acid, whereas subsequently the lactic acid was metabolized to butyric acid. However, the hydrogen production, i.e. 694.5 mL (Fig. 8.7(c)), was lower than the previous experiment and as a result the hydrogen yield calculated was thus lower (0.37 mol H₂/mol equiv. glucose) than the one obtained with fresh sorghum (0.52 mol H₂/mol equiv. glucose).

Figure 8-7: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and lactic acid and (c) gaseous products (biogas and hydrogen) using ensiled sorghum, at pH 5.5.
8.4 Anaerobic mesophilic co-digestion of ensiled sorghum, cheese whey and liquid cow manure in a two-stage CSTR system: Effect of hydraulic retention time

The experiments were carried out in two CSTR reactors, one used for acidogenesis and the other one for methanogenesis. The bioreactors’ setup is briefly described in Section 2.2.1. Both reactors were fed via a double-headed precise peristaltic pump (Watson Marlow Bredel 323). In this study, the acidogenic reactor was operated under controlled pH throughout the experimentation phase via automatic control (using a Hach PID-controller) by the addition of a solution mixture of NaOH/KOH (1.5 N/1.5 N) via a peristaltic pump, whereas the methanogenic reactor was operated at non-controlled pH conditions.

Experiments were conducted successively to determine the optimum HRT for maximum hydrogen and methane production. To this end, OLR was increased by decreasing the operating HRT. The acidogenic reactor was thus operated at six different HRTs of 5, 3, 2, 1, 0.75 and 0.5 d with a feeding mixture of pretreated ES3, CW and LCM in a ratio 55:40:5 (v/v/v), respectively. The pH in the acidogenic reactor was kept constant throughout the experimentation phase at pH 5.5, based on previous results (Section 8.3.3). Effluent from the acidogenic reactor was used for feeding the methanogenic one which was operated at three different HRTs (24, 16 and 12 days) equivalent to OLRs of 3.58, 5.36 and 7.15 kg COD/m³·d, respectively. The tested operating conditions in the two-stage system are summarized in Table 8.7.

| Table 8-7: Operating conditions in the acidogenic and methanogenic CSTR. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **HRT (d)**    | 5               | 3               | 2               | 1               | 0.75            | 0.5             |
| Flow rate (mL/d) | 100             | 167             | 250             | 500             | 667             | 1000            |
| OLR (kg VS/m³·d) | 11.56           | 19.27           | 28.91           | 57.81           | 77.08           | 115.62          |
| OLR (kg COD/m³·d) | 17.16           | 28.60           | 42.90           | 85.80           | 114.40          | 171.60          |
| **Methanogenic Reactor** |
| **HRT (d)**    | 24              | 16              | 12              |
| Flow rate (mL/d) | 167             | 250             | 333             |
| OLR (kg VS/m³·d) | 1.93            | 2.90            | 3.87            |
| OLR (kg COD/m³·d) | 3.58            | 5.36            | 7.15            |
8.4.1 Materials

The raw materials used in this study were cheese whey (CW), liquid cow manure (LCM) and ensiled sweet sorghum (ES3). The two agro-industrial wastewaters (CW and LCM) were collected from small local units in the area of Patras (Western Greece) according to Section 2.1.1. Table 8.8 represents the average values measured during the characterization of each wastewater. Significant differences in the composition of wastewater streams were detected. In particular, CW presented higher organic content ($93.21 \pm 2.99$ g COD/L), compared to LCM ($43.14 \pm 2.56$ g COD/L). CW was characterized by high organic load mainly due to carbohydrates (lactose) and low nitrogen content ($0.81$ g/L) in contrast with LCM ($2.78$ g/L). LCM contributes in the buffering capacity of the mixture as a consequence of its neutral pH ($7.24 \pm 0.18$) and alkalinity in high levels ($12.38 \pm 0.32$ g CaCO$_3$/L). It is important to take into consideration the fact that alkalinity should be high enough to avoid destabilization of the system caused by potential accumulation of volatile fatty acids.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>CW</th>
<th>LCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td>$6.12 \pm 0.04$</td>
<td>$7.24 \pm 0.18$</td>
</tr>
<tr>
<td>TS</td>
<td>g/L</td>
<td>$72.33 \pm 2.45$</td>
<td>$33.15 \pm 1.98$</td>
</tr>
<tr>
<td>VS</td>
<td>g/L</td>
<td>$62.40 \pm 1.62$</td>
<td>$22.50 \pm 0.98$</td>
</tr>
<tr>
<td>Total COD</td>
<td>g/L</td>
<td>$93.21 \pm 2.99$</td>
<td>$43.14 \pm 2.56$</td>
</tr>
<tr>
<td>Soluble COD</td>
<td>g/L</td>
<td>$38.96 \pm 3.05$</td>
<td>$15.05 \pm 0.32$</td>
</tr>
<tr>
<td>TOC</td>
<td>g/L</td>
<td>$39.49 \pm 0.33$</td>
<td>$16.72 \pm 0.24$</td>
</tr>
<tr>
<td>Total carbohydrates $^b$</td>
<td>g/L</td>
<td>$51.37 \pm 1.65$</td>
<td>$6.99 \pm 0.45$</td>
</tr>
<tr>
<td>Soluble carbohydrates $^b$</td>
<td>g/L</td>
<td>$40.63 \pm 1.99$</td>
<td>$0.45 \pm 0.05$</td>
</tr>
<tr>
<td>Total nitrogen, TKN</td>
<td>g/L</td>
<td>$0.81 \pm 0.03$</td>
<td>$2.78 \pm 0.00$</td>
</tr>
<tr>
<td>Ammonium nitrogen</td>
<td>g/L</td>
<td>$0.11 \pm 0.00$</td>
<td>$1.57 \pm 0.02$</td>
</tr>
<tr>
<td>Proteins</td>
<td>g/L</td>
<td>$5.06 \pm 0.19$</td>
<td>$17.38 \pm 0.00$</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>mg/L</td>
<td>$228.03 \pm 3.00$</td>
<td>$463.50 \pm 9.49$</td>
</tr>
<tr>
<td>Soluble phosphorus</td>
<td>mg/L</td>
<td>$57.50 \pm 0.91$</td>
<td>$21.68 \pm 0.04$</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>g CaCO$_3$/L</td>
<td>$0.50 \pm 0.00$</td>
<td>$12.38 \pm 0.32$</td>
</tr>
<tr>
<td>Total VFAs</td>
<td>mg/L</td>
<td>$0.00 \pm 0.00$</td>
<td>$4986.80 \pm 19.61$</td>
</tr>
</tbody>
</table>

$^a$ Mean values ($\pm$ standard deviation); $^b$ In equivalent glucose

Sweet sorghum (Sorghum Sudangrass hybrid-HoneyGraze BMR) was cultivated in Patras (Section 2.1.2-Table 2.1). It was harvested in November 2011 and finally the fresh chopped sorghum was ensiled. After ensiling, sorghum samples were dried at 55°C, ground into 1 mm particle size with a kitchen blender and sieved to powder of
< 315 μm diameter. The effect of mechanical pretreatment on methane production and hydrolysis kinetics has already been investigated by many authors. Sambusiti et al. (2013a), for example, found no significant differences in terms of methane yields and kinetic constants using ensiled sorghum forage milled into 2, 1, 0.5 and 0.25 mm particle sizes. The chemical composition of the ensiled sorghum used in this study, after drying and milling, is given in Table 8.9. The sorghum mainly consisted of polysaccharides (37.60% cellulose, 25.51% hemicellulose), whereas its total lignin content was 17.28%. These results are in accordance with the typical composition of sweet sorghum reported by Panagiotopoulos et al. (2010). The ash content of the total dry matter of ensiled sorghum stalks was rather low (5.93%). During the ensiling procedure soluble carbohydrates are utilized by fermentative bacteria for the production of volatile fatty acids, lactic acid and ethanol, depending on the followed metabolic pathway. Thus, the amount of soluble carbohydrates of ES3 is low in contrast to the increased content of lactic acid. Prior to its use as feeding material in mixture with CW and LCM, ES3 was subjected to alkaline pretreatment at 80°C for 2 h with the addition of alkaline solution 1.0% NaOH and 1.0% KOH (w/w), according to Section 4.5. Due to alkaline processing the pretreated ES3 had a final pH ranging between 12 and 13. It was thus neutralised to pH 7.0 with a concentrated HCl (37%) solution prior to its use in anaerobic digestion.

A mixture of pretreated ES3, CW and LCM (in a ratio of 55:40:5, v/v/v) was used, based on our previous study (Dareioti and Kornaros, 2014). In the present work, the ensiled sorghum was used to replace olive mill wastewater in the mixture because of its seasonal unavailability.

<table>
<thead>
<tr>
<th>Parameters a</th>
<th>ES3</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.10 ± 0.00</td>
</tr>
<tr>
<td>Moisture (% wet weight)</td>
<td>76.32 ± 0.10</td>
</tr>
<tr>
<td>TS (% wet weight)</td>
<td>23.73 ± 0.17</td>
</tr>
<tr>
<td>VS (%TS)</td>
<td>94.08 ± 3.15</td>
</tr>
<tr>
<td>Ash (%TS)</td>
<td>5.93 ± 3.15</td>
</tr>
<tr>
<td>TOC (%TS)</td>
<td>46.18 ± 0.00</td>
</tr>
<tr>
<td>Total carbohydrates b (%TS)</td>
<td>38.82 ± 1.29</td>
</tr>
<tr>
<td>Soluble carbohydrates b (%TS)</td>
<td>2.02 ± 0.06</td>
</tr>
<tr>
<td>Cellulose (%TS)</td>
<td>37.60 ± 5.37</td>
</tr>
<tr>
<td>Hemicellulose (%TS)</td>
<td>25.51 ± 3.66</td>
</tr>
<tr>
<td>Lignin (%TS)</td>
<td>17.28 ± 4.93</td>
</tr>
<tr>
<td>Total Nitrogen, TKN (%TS)</td>
<td>0.96 ± 0.45</td>
</tr>
<tr>
<td>Proteins (%TS)</td>
<td>6.00 ± 2.81</td>
</tr>
<tr>
<td>Lactic acid (%TS)</td>
<td>4.28 ± 0.00</td>
</tr>
</tbody>
</table>

a Mean values (± standard deviation); b In equivalent glucose
8.4.2 Effect of HRT in the acidogenic reactor

For the start-up of the system the acidogenic reactor was filled up with anaerobically digested sludge removed from the lab-scale acidogenic reactor fed with a waste mixture of olive mill wastewater (OMW), CW and LCM in a ratio 55:40:5 (v/v/v). The acidogenic reactor was initially operated batchwise for 48 h and then switched to continuous mode at HRT of 5 d and low organic loading rate (OLR), 17.16 kg COD/m³·d, which was subsequently increased during the course of the experiment.

As shown in Table 8.7, the acidogenic reactor was operated at different HRTs, i.e. 5, 3, 2, 1, 0.75 and 0.5 d. After reactor start-up, the initial HRT was set at 5 d for a period of 114 days. HRT was then decreased to 3 d, 2 d, 1 d, 0.75 d and 0.5 d for a period of 54, 55, 65, 36 and 19 days, respectively, reaching steady-state conditions in the reactor. The biogas produced from the acidogenic reactor consisted exclusively of hydrogen and carbon dioxide, whereas no methane was detected indicating the absence or complete inhibition of methanogens under the tested operating conditions. Fig. 8.8(a) illustrates the net biogas and hydrogen production rate as a function of experimental time, at standard temperature and pressure (STP) conditions. The fluctuation of produced biogas may be attributed to microbial population shifts taking place during the extended period of operation, whereas the complexity of the feeding medium and in particular the presence of various organic and inorganic materials may have also caused temporary inhibitory effects (Torkian et al., 2003).

Initially, the biogas production rate at HRT of 5 d was fluctuating with a mean value of 0.31 L/L_R·d containing 22.16% of hydrogen at the steady state (with the rest being mainly CO₂). The biogas and hydrogen production rate systematically increased when the HRT was decreased from 5 to 0.5 d, where the highest biogas and hydrogen production rates were obtained (5.69 and 2.14 L/L_R·d, respectively). A characteristic of the system under consideration is the strong fluctuations of biogas production and hence all relevant parameters not only during the transition from one phase to another, but also from one day to the next even within the same phase. Such fluctuations can be attributed to the instability of such a system operated as CSTR and were also observed in other studies (e.g. Mariakakis et al., 2011). It has been widely reported that the hydrogen productivity increases with decreasing HRT, which is however expected in continuously operating systems without kinetic limitations. For instance, Scoma et al. (2013) investigated the anaerobic acidogenic process of dephenolized olive mill waste using Packed Bed Biofilm Reactor and observed a significant increased production of a hydrogen-rich biogas when shorter HRTs (7, 5, 3 and 1 d) were applied. Moreover, Dareioti and Kornaros (2014) conducted experiments in the same system configuration in order to investigate the effect of HRT using the same mixture with olive mill waste instead of pretreated ensiled sorghum. Studying five different HRTs ranging from 5 to 0.75 d, an increase in hydrogen productivity with decreasing HRT was also observed. On the other hand, a different observation was reported during the digestion of sweet
Anaerobic co-digestion of agro-industrial wastewaters and sweet sorghum in a two-stage system

sorghum syrup, in which the hydrogen content and solvents production decreased with a reduction in HRT (Saraphirom and Reungsang, 2011).

Hydrogen yield (calculated as mol $H_2$/mol carbohydrate consumed) is a good indicator of the microbial populations’ effectiveness for hydrogen production and represents the capability of microorganisms to convert carbohydrates into hydrogen gas. The variation of average hydrogen production rate, hydrogen content and hydrogen yield observed at different HRTs is shown in Fig. 8.9. When operating at low HRTs, i.e. 0.75 and 0.5 d, a significant increase was observed in all these parameters. The hydrogen content increased with a decrease in HRT, up to 33.42% at HRT of 0.75 d. The maximum hydrogen yield 0.70 mol $H_2$/mol carbohydrate consumed was achieved at HRT 0.5 d, whereas the hydrogen yield was very low at higher HRT (0.25 mol $H_2$/mol carbohydrate consumed).

![Figure 8-8: Evolution of (a) biogas and hydrogen production rate and (b) main end-products during acidogenic reactor operation at different HRT.](image)

In summary, shortening the HRT to 0.5 d was sufficient to achieve the highest hydrogen productivity and reduce the diversity of microbial populations associated with elimination of product inhibitors. This low productivity at higher HRTs was likely due to bacteria limitation by the low substrate concentration supplied, which facilitates microbial population shift and growth of homoacetogens, mainly homoacetogenic clostridia, in the reactor (Chen et al., 2009). As regards soluble end-products concentration, significant production of volatile fatty acids (namely acetic, propionic, isobutyric, butyric and caproic acid) and lactic acid were noted as the most prominent ones at all tested HRTs (Fig. 8.8(b)). Valeric acid and ethanol were also measured in concentrations, though, less than 1000 ppm, whereas isovaleric acid was measured in trace concentrations. The complex and fluctuating end-product distribution of this
reactor is likely attributed to the complex nature of the wastewater mixture tested. As shown in Fig. 8.8(b) an appreciable amount of propionic acid was produced, especially at HRT of 3 d, which may be one of the reasons of low hydrogen productivity. The production of soluble end-products was a result of carbohydrates degradation. For all HRT studied, carbohydrate utilization efficiency was not affected by HRT changes and was over 34% and 86% for total and soluble, respectively (Fig. 8.10(a)). This is consistent with previous results that carbohydrates degradation of dairy wastewater was not affected by HRT changes (Fang and Yu, 2000), suggesting no inhibition or limitation of the acidogenic population to consume carbohydrates even at low retention time of microorganisms. In our study, the low degradation of total carbohydrates was due to the lignocellulosic structure of ensiled sorghum. However, maximization of soluble organic matter and VFAs concentration was crucial for the following methanogenic phase.

Figure 8-9: Effect of HRT on (a) hydrogen production rate, (b) hydrogen content and (c) hydrogen yield, during acidogenic reactor operation.
Fig. 8.10(b) presents the evolution of total (TCOD) and soluble COD (SCOD), in the effluent from the acidogenic reactor during the 343 days of its operation. No significant decrease was observed comparing their mean values in the influent and effluent stream. In our previous works operating acidogenic reactors at fixed HRT of 3 d without pH control, no COD removal was observed using a mixture of olive mill waste, cheese whey and liquid cow manure (Dareioti et al., 2009), or a slight decrease of TCOD (2.4%) was evident during co-digestion of 20% olive mill waste and 80% liquid cow manure (Dareioti et al., 2010), which is consistent with the low biogas production reported under these conditions. Fig. 8.10(c) depicts the TS and VS concentration in the acidogenic reactor effluent, which remained constant at 77.92 ± 7.59 g TS/L and 46.83 ± 5.97 g VS/L, respectively. VS removal of 18.99% was reached for all values of HRT tested, whereas negligible difference in TS concentration between influent and effluent was observed.

Figure 8-10: Evolution of (a) total and soluble carbohydrates concentration, (b) total and soluble COD concentration and (c) total and volatile solids concentration during acidogenic reactor operation at different HRT.
8.4.3 Effect of HRT in the methanogenic reactor

For the start-up of the methanogenic reactor was filled up with anaerobically digested sludge removed from the methanogenic reactor of a two-stage lab-scale system fed with a waste mixture of olive mill wastewater (OMW), CW and LCM in a ratio 55:40:5 (v/v/v). The continuous operation of the methanogenic reactor started at HRT of 24 d, while the reactor was being fed with acidified effluent from the acidogenic one.

A methane bioreactor was used for treating the acidified effluent of the first stage (acidogenic reactor) in order to assess the rate and extent of methanogenesis at three different HRTs (24, 16 and 12 d). Table 8.10 presents the experimental results, including methane yields and removal efficiencies obtained at steady-state conditions for the different HRTs. Firstly, at HRT of 24 d, biogas and methane production rates increased until the 17th day of operation up to 1.24 and 0.81 L/LR·d respectively, as shown in Fig. 8.11(a), whereas after that, the rates fluctuated and stabilized at mean values of 1.02 ± 0.18 and 0.63 ± 0.11 L/LR·d, respectively. Switching to the lower HRT of 16 d (and thus higher OLR of 5.36 kg COD/m³·d) at the 78th day, an increase in the produced volume of biogas was observed, which then stabilized at 1.52 ± 0.22 L biogas/LR·d and 0.90 ± 0.12 L CH₄/LR·d. Besides the increase in biogas volume a slight increase in methane content was also noticed, from 58.27 ± 1.03% to 58.58 ± 1.87% at the steady-state conditions of HRT of 24 and 16 d, respectively (Fig. 8.11(b)). Kavacik and Topaloglu (2010) obtained the highest biogas (1.51 L/LR·d) at HRT 5 d from the co-digestion of 50% cheese whey with 50% dairy manure (diluted 1:1) with methane content of 60% and suggested that co-digestion of these two wastes is more advantageous than processing each one separately. Finally, lowering the HRT from 16 to 12 d resulted to instability of reactor performance and also decrease of biogas and methane productivity. The reduction of methane production rate took place simultaneously with the increase of volatile fatty acids (VFA) concentration indicating inhibition of the methanogenic biomass by VFA accumulation (Fig. 8.11(c)). The high concentration of total VFA, during the operation at HRT of 12 d, was mainly due to the high acetic acid concentration (up to 10.17 g/L), while the concentration of propionic, butyric and caproic acid were also increased at a lower extent (up to 2.56 g/L, 3.72 g/L and 1.77 g/L, respectively), leading to methanogenesis inhibition with a consequent reduction of methane production. The effect of HRT on anaerobic digestion of poultry slaughterhouse wastes was examined by Salminen and Rintala (2002) who observed a gradual accumulation of total VFA with simultaneous decline in the methane yield in the range of HRT from 25 to 13 d. On the other hand, as shown in Fig. 8.11(c), total VFAs concentration remained lower than 0.5 g/L at HRT values of 24 and 16 d, indicating process stability. Some peaks were however periodically observed, especially during the initial start-up (day 1-48), which was overtaken after that. Koutrouli et al. (2009) studied the effect of HRT on two-stage digestion of two-phase olive mill waste (water-diluted 1:4) and observed that methane yield increased with decreasing HRT.
However, methane productivity reached the maximum value of $1.13 \pm 0.08$ L/LR·d at HRT 10 d, while the reactor failed at lower HRT tested (5 d).

![Graphs showing biogas and methane production rate, methane content, and volatile fatty acids concentration.](image)

**Figure 8-11:** Evolution of (a) biogas and methane production rate, (b) methane content and (c) main volatile fatty acids concentration during methanogenesis operated at different HRT.

The summarized average values of methane production rate, methane content and methane yields at steady-state conditions of each HRT tested in this study are presented in Table 8.10-Fig 8.12. The methane yield shown in Table 8.10 was determined from the experimental data at each HRT on the basis of volatile solids added (expressed thus as mL CH$_4$/g VS added) and the COD removed (expressed as mL CH$_4$/g COD removed). Methane yields of 326.42 and 310.34 mL CH$_4$/g VS added were thus calculated at HRT of 24 and 16 d respectively, whereas at the lower HRT (12 d) it was almost zero due to the negligible methane productivity. Hence, better performance and
higher methane yields were noticed in this study by co-digesting ensiled sorghum with CW and LCM compared to the results obtained in the study of anaerobic digestion of untreated and pretreated ensiled sorghum in two semi-continuous CSTR (Sambusiti et al., 2013b). In the latter study, 237 and 297 mL CH₄/g VS added was achieved for untreated and pretreated ensiled sorghum, respectively, suggesting that an alkaline pretreatment step (40°C for 24 h with the addition of 10% NaOH) prior to the anaerobic digestion could have a beneficial effect in enhancing methane production. Bayr et al. (2012) studied the co-digestion of rendering and slaughterhouse wastes and obtained a methane potential of 720 mL CH₄/g VS added in OLR of 1.0 and 1.5 kg VS/m³·d (HRT 50 d), in comparison to 262-572 mL CH₄/g VS added when using the two substrates separately.

### Table 8-10: Steady-state characteristics of the methanogenic CSTR for each HRT tested.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HRT (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24</td>
</tr>
<tr>
<td>pH</td>
<td>8.05 ± 0.06</td>
</tr>
<tr>
<td>Biogas (L/LR·d)</td>
<td>1.02 ± 0.18</td>
</tr>
<tr>
<td>CH₄ (L/LR·d)</td>
<td>0.63 ± 0.11</td>
</tr>
<tr>
<td>CH₄ (%)</td>
<td>58.27 ± 1.03</td>
</tr>
<tr>
<td>TVFAs (g/L)</td>
<td>0.30 ± 0.14</td>
</tr>
<tr>
<td>Yield CH₄ (mL CH₄/g VS added)</td>
<td>326.42 ± 56.9</td>
</tr>
<tr>
<td>Yield CH₄ (mL CH₄/g COD consumed)</td>
<td>223.09 ± 38.9</td>
</tr>
<tr>
<td>TCOD removed (%)</td>
<td>84.77 ± 8.71</td>
</tr>
<tr>
<td>SCOD removed (%)</td>
<td>84.05 ± 4.64</td>
</tr>
<tr>
<td>TS removed (%)</td>
<td>37.48 ± 2.29</td>
</tr>
<tr>
<td>VS removed (%)</td>
<td>65.97 ± 6.52</td>
</tr>
</tbody>
</table>

* The parameter values recorded in this operating condition do not correspond to steady state operation, since the bioreactor was in transition towards washout.

Comparing our present results with the ones obtained in a previous study (Dareioti and Kornaros, 2014), the presence of pretreated ensiled sorghum in the mixture instead of olive mill waste contributed to higher methane productivity, especially at lower operating HRTs. On the other hand, the methane yield, i.e. the conversion of organic matter to methane, was lower in this study (223.09 and 216.50 mL CH₄/g COD removed) compared with the same mixture with olive mill waste (294.37 and 316.08 mL CH₄/g COD removed), mainly due to the fact that sorghum is lignocellulosic biomass. Moreover, Rincón et al. (2008) found quite similar methane yield
Chapter 8 | Anaerobic co-digestion of agro-industrial wastewaters and sweet sorghum in a two-stage system

(244 mL CH₄/g COD removed) obtained in anaerobic digestion of two-phase olive mill solid residue at HRT of 17 d with OLR of 9.2 kg COD/m³·d.

As shown in Table 8.10, pH remained practically constant at high HRT values (24 and 16 d), with values 8.05 ± 0.06 and 8.00 ± 0.07 respectively, whereas at the lower HRT (12 d) pH decreased at 6.63 (Fig. 8.13(a)), as a result of VFA accumulation. The TVFA/alkalinity ratio can be used as a measure of process stability (Callaghan et al., 2002); namely when the ratio is less than 0.3-0.4 (equiv. acetic acid/equiv. CaCO₃) the process is considered to be stable without facing any acidification risk. At HRTs of 24 and 16 d the TVFA/alkalinity ratio was measured in acceptable values, i.e. ranging from 0.02 to 0.15 which were lower than the suggested failure limit values. However, at HRT

Figure 8-12: Effect of HRT on (a) methane production rate, (b) methane content and (c) methane yield.
12 d, a considerable increase in the TVFA/alkalinity ratio up to 2.32 was noticed, higher than the safety threshold-value, which was mainly due to VFAs accumulation with a simultaneous decrease in alkalinity to 7.73 g CaCO₃/L. Indeed, the process was destabilized and deteriorated due to inhibition of methanogens.

The evolution of total and soluble COD in the methanogenic reactor, as a function of experimental time, is plotted in Fig. 8.13(b). As can be seen, at the steady-state conditions of both HRTs of 24 and 16 d the COD concentration was almost stable, whereas at the shorter HRT of 12 d the concentration started to rise immediately after the transition, with a concomitant drop in methane production, following the evolution of TVFA. Although at HRT of 12 d most of soluble COD could be accounted for by TVFAs, this was not the case at the higher HRTs indicating the presence of other soluble products which were not detected. The total and soluble COD removal efficiency were both high and similar, namely 84.77% and 84.05% for 24 d HRT and 83.36% and 85.09% for 16 d HRT, respectively. At the lower HRT of 12 d the performance of the reactor deteriorated sharply performing 39.29% removal of total COD and 22.87% of soluble COD. In general, the percentages of organic matter and specifically of COD removal obtained in the present work were higher than those obtained in the anaerobic digestion of the same mixture with olive mill waste instead of ensiled sorghum (Dareioti and Kornaros, 2014). This is likely due to the presence of phenolic compounds in olive mill waste, which are microbiologically toxic and difficult to degrade anaerobically (Raposo et al., 2003). Colussi et al. (2013) observed a COD removal efficiency of over 80% in a two-stage anaerobic digestion using maize silage as substrate. On the other hand, Karim et al. (2005) reported methane production rates of 0.45 L CH₄/L·R·d in the digestion of dairy manure, whereas the removal percentage of TCOD was 50%.

The evolution of solids concentration (TS, VS) during the experimental operation at the three different HRTs is shown in Fig. 8.13(c). The highest removal efficiencies, in terms of TS and VS, were noticed at HRT of 16 d and were equal to 42.32% and 70.02%, respectively. The degradation of total carbohydrates in glucose equivalents was 90.22% during methanogenesis (data not shown) at HRT of 16 d whereas a slight decrease (86.19%) was observed at HRT of 12 d. The mean value of TKN concentration in the influent for all HRT was 763 mg/L (15% in the form of ammonium nitrogen with the rest being organic N). In the effluent, the mean TKN concentration was 730, 640 and 530 mg/L, whereas the mean ammonium nitrogen concentration was 24, 14 and 31 mg/L for the HRTs 24, 16 and 12 d, respectively. This change is due to ammonification process, ammonia nitrogen use in cellular synthesis processes and gaseous emissions of ammonia nitrogen due to increased pH.
Figure 8-13: Evolution of (a) pH, (b) total, soluble COD and TVFA (expressed in COD units) concentration and (b) total and volatile solids concentration during methanogenesis operated at different HRT.
8.5 Biochemical Methane Potential

Biochemical Methane Potential (BMP) of the mixture was also studied according to batch assay (Section 2.2.3). In Fig. 8.14 the cumulative methane production as a function of the digestion time, is presented. The calculated methane production of the mixture, after subtraction of the methane produced from the blank experiment, was 87.29 ± 4.39 mL of CH₄, whereas the methane potential was 445.72 ± 22.42 mL CH₄/g VS added. The obtained methane potential is higher than the respective value obtained from CSTR experiment (310.34 ± 41.3 mL CH₄/g VS added at HRT of 16 d), because of the fact that in BMP assay no inhibition was occurred.

Moreover, the cumulative biomethane production profile was fitted to a modified Gompertz bacterial growth model. The equation used (Eq. 8.8) is a modified form from Eq. (8.1).

\[
M(t) = P \exp \left\{ - \exp \left( \frac{R_m e}{P} (\lambda - t) + 1 \right) \right\} 
\]

(8.8)

where \( M(t) \) is the cumulative methane production (mL); \( P \) is the maximum methane production potential (mL); \( R_m \) is the maximum methane production rate (mL/d); \( \lambda \) is the lag-phase duration (d); \( t \) is the time (d) and \( e \) is \( \exp(1) = 2.71828 \).

Fig. 8.15 depicts the methane production based on experimental data and the simulation generated using the fitted modified Gompertz model. The correlation coefficient (\( R^2 \)) was 0.994, whereas the methane production potential (\( P \)) was 85.48 ± 1.09 mL, the maximum methane production rate (\( R_m \)) was 5.18 ± 0.40 mL/d and finally the lag-phase (\( \lambda \)) was 13.01 ± 0.60 d.

Figure 8-14: Cumulative methane production during the BMP assay from the mixture (55% ES3, 40% CW and 5% LCM) and blank sample. Errors bars represent the standard deviation for the replicates.
Chapter 8  Anaerobic co-digestion of agro-industrial wastewaters and sweet sorghum in a two-stage system

Figure 8-15: Cumulative methane production (experimental data and modified Gompertz model simulation) during BMP assay of ES3 / CW / LCM mixture (55 / 40 / 5, v/v/v).

8.6 Conclusions

Co-digestion of sorghum stalks, cheese whey and liquid cow manure (55:40:5, v/v/v) was efficiently demonstrated in a two-stage anaerobic system. First of all, batch experiments were carried out in order to investigate the effect of pH on hydrogen production and distribution of end-products. The maximum hydrogen yield was observed at pH 5.5, whereas the highest productivity was found at pH 6.5. No substrate inhibition was identified, at least within the tested organic loading limits. Heat treatment of inoculum resulted to higher hydrogen yield compared to the use of typical inoculum, most probably due to partial inactivation or death of hydrogen-consuming microbial populations. On the other hand, utilization of ensiled sorghum instead of fresh one led to lower hydrogen yield. Furthermore, the effect of hydraulic retention time (HRT) in a two-stage process was investigated using a mixture of pretreated ES3, CW and LCM at a ratio 55:40:5 (v/v/v). The highest hydrogen production rate (2.14 L/LR·d) and hydrogen yield (0.70 mol H₂/mol carbohydrates consumed) was achieved when the acidogenic reactor was operated at HRT 0.5 d. The highest methane productivity (0.90 L CH₄/LR·d) was achieved at HRT 16 d, whereas the methane yield was 223.09 and 216.50 mL CH₄/g COD consumed at HRTs of 24 and 16 d, respectively. Switching to a lower HRT of 12 d resulted to reactor instability and inhibition of methane production as a consequence of significant accumulation of VFAs.
8.7 References


Chapter 8

Anaerobic co-digestion of agro-industrial wastewaters and sweet sorghum in a two-stage system


Anaerobic co-digestion of agro-industrial wastewaters and sweet sorghum in a two-stage system

Chapter 8


Chapter 8

Anaerobic co-digestion of agro-industrial wastewaters and sweet sorghum in a two-stage system


Raposo, F., Borja, R., Sánchez, E., Martín, M.A., Martin, A., 2003. Inhibition kinetics of overall substrate and phenolics removals during the anaerobic digestion of two-


Chapter 9.

Implications of the OMW use in co-digestion mixtures in a two-stage system.

9.1 Abstract

The effect of co-digestion in the acidogenesis and the influence of substrate type and initial substrate concentration in the methanogenesis were studied in the aim to assess their effects of a selected mixture of agro-industrial wastes and sweet sorghum. This study was performed in batch reactors under mesophilic conditions (37°C) using two different types of substrate: mixture A (55% olive mill wastewater, 40% cheese whey and 5% liquid cow manure) and mixture B (55% water-sorghum, 40% cheese whey and 5% liquid cow manure). The acidogenic experiments were performed for each substrate at controlled pH throughout the course of each experiment. A comparison between the experimental and the predicted hydrogen yield which was calculated from the hydrogen yield of the individual fractions was performed. A higher hydrogen yield by 60.1% than the expected one was observed, using the mixture A, demonstrating a synergistic effect. Nevertheless, negligible difference on hydrogen yield was observed for the mixture B. The methanogenic experiments were carried out at two different initial organic substrate concentrations from each mixture, i.e. A1 with 73.15 g COD/L, A2 with 42.32 g COD/L, B1 with 78.15 g COD/L and B2 with 44.35 g COD/L. According to our results, the maximum methane yield of 346.65 mL CH₄/g VS added was measured at the B2 experiment, whereas at higher initial substrate concentration of mixture B (B1) the CH₄ yield decreased (284.12 mL CH₄/g VS added). On the other hand, higher inhibition and lower COD removal rates were measured in the case of mixture A, where olive mill wastewater was used instead of sweet sorghum. At high initial substrate concentration of mixture (A1) the CH₄ yield decreased along with a significant decrease in phenolics degradation (from 40% to 17.9%). The higher initial concentration of soluble phenolics in mixture A1 resulted in partial inhibition of the methanogenic process.
9.2 Introduction

Residual biomasses, such as plant biomass, energy crops, agricultural and industrial residues, animal manures, wastewater and other organic wastes represent spatially diffused sources of alternative substrates for anaerobic biotransformation to biogas, which is widely used as a source of renewable energy. Olive mills, cheese factories and cow farms are agro-industries that represent a considerable share of the Mediterranean countries economy. These industries generate millions of tons of wastewaters and large amounts of by-products, which are totally unexploited and in some cases dangerous for the environment. Multiple waste streams of organic substrates can be anaerobically co-digested to generate a homogeneous mixture increasing both process and equipment performance.

Co-digestion of various wastes, in order to enhance biogas production and overcome a number of problems such as nutrient imbalance, seasonal availability of wastes and presence of inhibiting compounds, among other factors, is an attractive approach for improving the efficiency of biotransformation (Dareioti et al., 2010; Ferrer et al., 2014). Many successful co-fermentation processes using different substrates have shown large increases in methane potential, compared with digestion of the monosubstrates (Labatut et al., 2011; Bertin et al., 2013). Up to now, the majority of studies report the influence of co-digestion on methane production and not on fermentative hydrogen production. For example, Pagés-Díaz et al. (2014) examined synergistic effects obtained from the combination of different mixtures using four substrates, and reported 31% increase of the expected yield which was calculated from the methane potential of the individual fractions.

The determination of the methanogenic potential of wastes can be determined by various kinetic factors, among which we have the substrate concentration (Sánchez et al., 2001; Fernández et al., 2010), microorganisms’ adaptation (Chamy and Ramos, 2011), alkalinity (Mockaitis et al., 2006), nutrient requirements (Takashima and Speece, 1989), inoculum/substrate ratio (Raposo et al., 2009), pH (Sánchez et al., 2000) and/or temperature (Sánchez et al., 2001). These trials will provide preliminary information about the process, analyzing various factors simultaneously in order to maximize the methane production and/or the biomethanization potential. Operating the reactor above the adequate range causes a destabilization in the process, which is reflected in a drop in the removal of organic matter and methane production. This would depend on the waste composition and biodegradability, which is linked to the production of volatile fatty acids (VFAs) and/or ammonia nitrogen, acclimatization and type of biomass and the reactor’s operating conditions (type and configuration of the digester(s), temperature and/or pH). Among the various factors that determine process performance, the initial organic matter concentration is especially important. Mackie and Bryant (1995) studied the anaerobic digestion of cattle wastes at mesophilic and thermophilic temperatures and found that methane yield was more affected in mesophilic digestion when the
organic load was increased and retention time reduced in a completely mixed digester on a laboratory scale.

This study aims to analyze various factors about the hydrogen and methane production of different agro-wastes mixtures in batch reactors under mesophilic conditions. Thereby the effect of co-digestion of agro-wastes on hydrogen production and also the substrate type and the initial substrate concentration on methane production were studied.

9.3 Materials and experimental set-up

The raw materials used in this study were olive mill wastewater (OMW), cheese whey (CW), liquid cow manure (LCM) and fresh sweet sorghum (FS2). All three agro-industrial wastewaters (OMW, CW, LCM) were collected from small local units in the area of Patras (Western Greece) according to Section 2.1.1. Sweet sorghum (*bicolor L. Moench var. Keller*) was cultivated at the University of Patras (Section 2.1.2-Table 2.1). Following their collection, all wastewaters and FS2 were immediately stored in the freezer at -18°C until subsequent use throughout the experiment period. Two different mixtures of agro-wastes were used for the experiments. Mixture A contained 55% OMW, 40% CW and 5% LCM, whereas mixture B contained 55% FS2 stalks, 40% CW and 5% LCM. The reason for using sweet sorghum in mixture B was to replace OMW which is not available throughout the year (seasonal production from November to February).

First of all, anaerobic batch experiments were performed to study the acidogenesis of the three agro-industrial wastes and sweet sorghum in order to evaluate the effect of co-digestion of the two mixtures (A and B). All experiments were conducted in a 1-L steel reactor under controlled temperature, pH and stirring rate conditions (Section 2.2.2- Fig. 2.4(a)). The working volume of the reactor, mono-digesting the substrates of the mixture A, was adjusted to 400 mL, whereas the amount of anaerobic sludge used as inoculum was 60 mL (15% v/v of working volume). The experimental set-up and also the acclimatized anaerobic inoculum used in mono-substrate experiments were the same with mixture’s experiment (Section 7.4.1; Dareioti et al., 2014). On the other hand, the reactor’s working volume, mono-digesting the substrates of the mixture B, was adjusted to 800 mL, whereas the amount of anaerobic sludge used as inoculum was 20% (v/v) of working volume. The acclimatized anaerobic inoculum used in these mono-substrate experiments was the same with the previous experiments and the same with mixture’s experiment (Section 8.3.3).

Finally, anaerobic batch experiments were performed to study the methanogenesis of the mixtures A and B. The mixtures were acidified effluents from acidogenic CSTR reactors operated at HRT of 1 day. The pH in the acidogenic reactors was kept constant at pH 6.0 and 5.5, which is the optimum pH for hydrogen production for each mixture (A: Section 7.4.1 and B: Section 8.3.3), throughout the experimentation phase for
mixture A and B, respectively. The amount of sweet sorghum used in the first stage (acidogenesis) was equivalent to the organic load of olive mill waste that was replaced, i.e. 80 g dry FS2/L. The initial concentration of total COD in both mixtures was about 100 g COD/L. All batch anaerobic co-digestion experiments were carried out in glass bottles shaken in an orbital shaking water bath (Grant OLS200) at 80 rpm at constant mesophilic temperature (37°C). The total volume capacity in each bottle was 500 mL, while the working volume was 450 mL. Each digester was loaded with 90 mL of inoculum (20% v/v) and 360 mL of the mixture. They were closed by rubber caps where two output ports were installed, one for sampling and the other one for biogas outlet which was collected in a glass syringe (Section 2.2.2 - Fig. 2.4(b)). For the experiments carried out in this study an acclimatized anaerobic inoculum was used, which was obtained from a laboratory anaerobic methanogenic CSTR reactor fed with mixture A. The total and volatile solids concentrations of the inoculum were 41.43 and 18.4 g/L, respectively. The study was aimed to evaluate the mesophilic methanogenic digestion at two different initial substrate concentrations (full-strength and half-strength) for each mixture (A and B). The initial substrate concentration (g COD/L) was changed by dilution of the mixture waste with distilled water.

9.4 Fermentative hydrogen production in batch experiments using agro-wastes: Effect of co-digestion under controlled pH.

In the present study, the effect of co-digestion on hydrogen production and acidogenesis of two different mixtures of agro-wastes was investigated. The mixtures tested were 55% OMW, 40% CW and 5% LCM (Mixture A), and 55% FS2 stalks, 40% CW and 5% LCM (Mixture B), respectively. Batch experiments were carried out using the mono-substrates of each mixture at controlled pH, throughout the course of each experiment, and then a comparison with the batch experiment of the mixture was occurred. The pH was chosen according to previous studies with the aim of finding the optimum value for each mixture (Section 7.4.1 and 8.3.3). In particular, for the mixture A, three batch experiments were performed at constant pH 6.0. The percent of OMW and CW in the experiments was the same with the mixture’s one in order to avoid any extra inhibition (S_{OMW}:55, S_{CW}:40), whereas LCM’s percent was selected 30% than 5% in order to measured satisfying productivity (S_{LCM}:30). On the other hand, the effect of co-digestion on the mixture B was investigated through batch experiments at controlled pH value of 5.5, whereas the substrates’ percent was S_{FS2}:55, S_{CW}:40, S_{LCM}:30 for the reasons mentioned previously. For batch experiments carried out in this investigation an acclimatized anaerobic inoculum was used, the same used in the batch experiments of each mixture (Section 7.4.1 and 8.3.3, respectively).

The quantity of produced biogas and its composition was monitored throughout of each experiment, whereas samples were collected for analysis, i.e. determination of carbohydrates, VFAs, alcohols etc.
Fig. 9.1 presents the acidogenic batch experiment of OMW (S_{OMW:55}) at pH 6.0. In particular, the consumption of total and soluble carbohydrates is associated with the rate of production of major products during the whole experimentation course. The consumption of carbohydrates, measured as equivalent glucose, (Fig. 9.1(a)) was 66.7% and 74.7% for total and soluble, respectively. The degradation of carbohydrates contributed to an increase of the concentration of acetic and propionic acid (Fig. 9.1(b)), whereas a slight increase of butyric acid was observed. The biogas was mainly composed of hydrogen and carbon dioxide (Fig. 9.1(c)). The production of biogas was 930.92 mL, whereas hydrogen production was 143.28 mL. The calculated hydrogen yield, from OMW treatment at pH 6.0, was 0.344 mol H_{2}/mol equivalent glucose consumed.

Figure 9-1: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of OMW (S_{OMW:55}), at pH 6.0.
Chapter 9 | Implications of the OMW use in co-digestion mixtures in a two-stage system

Fig. 9.2 illustrates the results obtained from the acidogenic batch experiment of CW (S_{CW}:40) at pH 6.0. In particular, the concentration of total and soluble carbohydrates decreased during the experimentation course, mainly the first 30 h. The consumption of carbohydrates, measured as equivalent glucose, (Fig. 9.2(a)) was high and equal to 78.2% and 97.6% for total and soluble, respectively. Ferchichi et al. (2005) observed equally high sugar consumption by 97% studying hydrogen production from cheese whey at different initial pH values (ranging from 5.0 to 9.0). The degradation of carbohydrates contributed to a significant increase of the concentration of acetic and propionic acid (Fig. 9.2(b)), whereas a slight increase of butyric acid was observed. The production of biogas was 732.82 mL, whereas hydrogen production was 212.62 mL (Fig. 9.2(c)). The calculated hydrogen yield was 0.477 mol H₂/mol equivalent glucose consumed, higher than yield obtained from OMW substrate.

![Figures](a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of CW (S_{CW}:40), at pH 6.0.

242
Fig. 9.3 shows the acidogenic batch experiment of LCM (S_{LCM:30}) at pH 6.0. In particular, the consumption of total and soluble carbohydrates was very low in this case because of the fact that LCM is characterized by low concentration of soluble sugars as lignocellulosic material. The consumption of carbohydrates, measured as equivalent glucose, (Fig. 9.3(a)) was 23.1% and 22.9% for total and soluble, respectively. The production of soluble end-products was negligible (Fig. 9.3(b)) with simultaneous low production of biogas and traces of hydrogen (Fig. 9.3(c)). The calculated hydrogen yield, from LCM treatment at pH 6.0, was 0.420 mol H\textsubscript{2}/mol equivalent glucose consumed.

Figure 9-3: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of LCM (S_{LCM:30}), at pH 6.0.
Moreover, the cumulative bio-hydrogen production profile from each batch experiment was fitted to a modified Gompertz bacterial growth model (Eq. (9.1)) using OriginPro version 8.

\[
H = P \exp \left\{ - \exp \left[ \frac{R_m}{P} (\lambda - t) + 1 \right] \right\} \tag{9.1}
\]

Fig. 9.4 depicts the bio-hydrogen production based on experimental data and the simulation generated using the fitted modified Gompertz model at three different substrates (OMW, CW and LCM). Comparing each set of experimental data with the relevant model simulation, the parameters of hydrogen production potential (P), the maximum hydrogen production rate (R_m) and lag-phase time (\lambda) were determined (Table 9.1). The modified Gompertz bacterial growth model was successful in interpreting the experimental production trend, as demonstrated by the high correlation coefficient (R²) values (0.994-0.999). As you can see in Fig. 9.4-Table 9.1, the maximum hydrogen potential (213.84 ± 1.10 mL) was observed at CW, which also presents the highest hydrogen production rate (19.57 ± 0.70 mL/h) as an easy-degradable substrate (Prazeres et al., 2012).

![Cumulative Hydrogen Production](image)

**Figure 9-4**: Cumulative hydrogen production (experimental data and modified Gompertz model simulation) during the batch acidogenesis of each substrate (OMW, CW and LCM) at controlled pH 6.0.

<table>
<thead>
<tr>
<th>Substrate (pH 6.0)</th>
<th>P (mL)</th>
<th>R_m (mL/h)</th>
<th>\lambda (h)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>OMW</td>
<td>143.62 ± 1.20</td>
<td>6.75 ± 0.22</td>
<td>5.97 ± 0.33</td>
<td>0.998</td>
</tr>
<tr>
<td>CW</td>
<td>213.84 ± 1.10</td>
<td>19.57 ± 0.70</td>
<td>3.02 ± 0.17</td>
<td>0.999</td>
</tr>
<tr>
<td>LCM</td>
<td>17.19 ± 0.34</td>
<td>0.45 ± 0.02</td>
<td>4.18 ± 0.87</td>
<td>0.994</td>
</tr>
</tbody>
</table>
A calculation was performed in order to estimate the effect of co-digestion on hydrogen production using agro-industrial wastewaters. The calculated theoretical hydrogen yield of the mixture A was obtained using the hydrogen yields of each mono-digestion and was equal to 0.401 mol H\textsubscript{2}/mol equivalent glucose consumed. The experimental hydrogen yield of the mixture (Section 7.4.1), at the same pH value of 6.0, was higher than theoretical calculated one and equal to 0.642 mol H\textsubscript{2}/mol equivalent glucose consumed (Fig. 9.5). As a result, the co-digestion efficiency of these agro-industrial wastes is greater than the expected one, clearly demonstrating a positive synergistic effect due to more balanced nutrient composition enhancing the anaerobic digestion process. Pagés Díaz et al. (2011) studied co-digestion of solid cattle slaughterhouse wastes with different agricultural residues, and reported up to 43% higher methane yields than the expected methane yields of different mixtures, which were attributed to synergistic effects because of better nutritional balance for the microorganisms.

![Figure 9-5: Comparative hydrogen yield between OMW, CW, LCM, theoretical calculated mixture yield and experimental mixture yield (The experimental mixture yield was obtained from Section 7.4.1).](image)

As mentioned previously, three batch experiments at controlled pH value of 5.5 were carried out with substrates' percent of S\textsubscript{FS2:55}, S\textsubscript{CW:40}, S\textsubscript{LCM:30} in order to investigate the effect of co-digestion of the mixture B. Fig. 9.6 presents the batch experiment of FS2 (S\textsubscript{FS2:55}) at controlled pH 5.5, throughout the course of experiment. In particular, the consumption of total and soluble carbohydrates is associated with the rate of production of major products during the whole experimentation course. The consumption of carbohydrates, measured as equivalent glucose, (Fig. 9.6(a)) was very low and equal to 16.7% and 87.16% for total and soluble, respectively. The reduction of total carbohydrates is due to soluble carbohydrates removal. The insoluble part remains the same because of the fact that fresh sorghum is characterized as lignocellulosic crop and consists of lignin, which acts
as a physical barrier preventing the degradation of cellulose and hemicellulose (Brodeur et al., 2011). The degradation of carbohydrates contributed to an increase of the concentration of butyric acid (Fig. 9.6(b)), whereas a slight increase of acetic and propionic acid was observed. Moreover, an increase of lactic acid (approximately 1.8 g/L) was observed the first 7.5 h of experiment and then it was consumed. The accumulation of butyric acid was observed as a result of lactic acid bioconversion, which was also accompanied by simultaneous production of hydrogen (Fig. 9.6(c)). The production of biogas was 765.97 mL, whereas hydrogen production was 153.70 mL. The calculated hydrogen yield, from FS2 treatment at pH 5.5, was 0.617 mol H₂/mol equivalent glucose consumed.

![Carbohydrates](image1)

![Main end-products](image2)

![Gaseous products](image3)

**Figure 9-6:** (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of FS2 stalks (SFS2:55), at pH 5.5.
Fig. 9.7 illustrates the results obtained from the acidogenic batch experiment of CW (SCW:40) at pH 5.5. In particular, the concentration of total and soluble carbohydrates decreased during the experimentation course, mainly the first 6 h (Fig. 9.7(a)), whereas the degradation was equal to 76.0% and 97.1% for total and soluble, respectively. The degradation was almost the same with the batch experiment of cheese whey at different controlled pH (6.0). Ferchichi et al. (2005) examined the hydrogen production from cheese whey at different initial pH values (ranging from 5.0 to 9.0), suggesting that the microorganisms’ ability to consume sugars did not alter within this initial pH range. A significant increase of the concentration of acetic and butyric and lactic acid (Fig. 9.7(b)) was observed. The production of biogas was 1818.67 mL, whereas hydrogen production was 602.99 mL (Fig. 9.7(c)). The calculated hydrogen yield was 0.395 mol H$_2$/mol equivalent glucose consumed, lower than yield obtained from CW experiment at different pH (6.0).

![Figure 9-7](image-url)

**Figure 9-7:** (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of CW (SCW:40), at pH 5.5.
Finally, Fig. 9.8 shows the acidogenic batch experiment of LCM ($S_{LCM:30}$) at pH 5.5. In particular, the consumption of total and soluble carbohydrates was insignificant in this case (Fig. 9.8(a)) because of the fact that LCM is characterized by low concentration of soluble sugars as lignocellulosic material. Manures often contain quantities of organic fibers, including straw bedding material, that are more difficult to degrade than the manure itself (Angelidaki and Ahring, 2000). The production of soluble end-products was also negligible (Fig. 9.8(b)) with simultaneous low production of biogas and traces of hydrogen (Fig. 9.8(c)). The calculated hydrogen yield obtained from LCM treatment at pH 5.5 was 0.189 mol H$_2$/mol equivalent glucose consumed, lower compared to the previous experiment of LCM at higher pH (6.0) of the experiment (0.420 mol H$_2$/mol equivalent glucose consumed).

![Graphs showing carbohydrate consumption, volatile fatty acids evolution, and biogas and hydrogen production](image)

**Figure 9-8:** (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of LCM ($S_{LCM:30}$), at pH 5.5.
Fig. 9.9 depicts the bio-hydrogen production based on experimental data and the simulation generated using the fitted modified Gompertz model at three different substrates (FS2, CW and LCM). Comparing each set of experimental data with the relevant model simulation, the parameters of hydrogen production potential (P), the maximum hydrogen production rate (Rm) and lag-phase time (λ) were determined (Table 9.2). The correlation coefficient (R²) ranged between 0.860 and 0.992. The different values between previous experiments and these ones (Table 9.1 and Table 9.2, respectively) is due to different working volumes used in the experiments. Moreover, the different duration of these two set of experiments is due to different acclimatized anaerobic inoculum used. Similarly, the maximum hydrogen potential was observed at CW, which also presents the highest hydrogen production rate as an easy-degradable substrate (Prazeres et al., 2012).

**Figure 9-9:** Cumulative hydrogen production (experimental data and modified Gompertz model simulation) during the batch acidogenesis of each substrate (FS2, CW and LCM) at controlled pH 5.5.

**Table 9-2:** Kinetic parameters of hydrogen production estimated using the modified Gompertz equation.

<table>
<thead>
<tr>
<th>Substrate (pH 5.5)</th>
<th>P (mL)</th>
<th>Rm (mL/h)</th>
<th>λ (h)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS2</td>
<td>162.25 ± 3.43</td>
<td>14.99 ± 0.85</td>
<td>2.97 ± 0.28</td>
<td>0.992</td>
</tr>
<tr>
<td>CW</td>
<td>658.24 ± 40.80</td>
<td>62.29 ± 5.02</td>
<td>0.95 ± 0.40</td>
<td>0.980</td>
</tr>
<tr>
<td>LCM</td>
<td>2.70 ± 1.64</td>
<td>0.33 ± 0.09</td>
<td>0.58 ± 0.85</td>
<td>0.860</td>
</tr>
</tbody>
</table>
A calculation was performed in order to estimate the effect of co-digestion on hydrogen production using a mixture of agro-industrial wastewaters and sweet fresh sorghum. The calculated theoretical hydrogen yield of the mixture B was obtained using the hydrogen yields of each mono-digestion and was equal to 0.507 mol H$_2$/mol equivalent glucose consumed. The experimental hydrogen yield of the mixture (Section 8.3.3), at the same pH value of 5.5, was almost the same with theoretical calculated one and equal to 0.52 mol H$_2$/mol equivalent glucose consumed (Fig. 9.10). As a result, the efficiency of these agro-industrial wastes co-digested with sweet fresh sorghum is similar than the expected one, so no synergism obtained in the digestion medium in this mixture treatment.

**Figure 9-10:** Comparative hydrogen yield between FS2, CW, LCM, theoretical calculated mixture yield and experimental mixture yield (The experimental mixture yield was obtained from Section 8.3.3).
9.5 A comparative study on methanogenesis of agro-wastes mixtures: Effect of substrate type and initial concentration.

The results obtained from the characterization of each substrate at initial batch conditions (after mixing with sludge inoculum) are shown in Table 9.3. All mixtures were rich in total volatile fatty acids (TVFAs) as anticipated due to the pretreatment of each mixture in the anaerobic acidogenic phase. The VFAs were mostly acetic, propionic and butyric acid, plus smaller quantities of ibutyric, valeric, i-valeric and caproic acid. In all experiments pH was 7.41 ± 0.04. The total COD was slightly higher in mixture B, compared to mixture A, as a result of its higher content in polysaccharides (cellulose and hemicellulose) due to the presence of sweet sorghum.

Fig. 9.11 shows the evolution of biogas and methane produced in both mixtures. The initial substrate concentration affected the total production and the temporal evolution in mixture A, since biogas and methane production rates decreased at the higher substrate concentration batch (A1), which implied that microbial activity was inhibited, presumably because of the higher phenolics concentration. However, in experiments B1 and B2 the biogas and methane production rates were quite similar.

Table 9-3: Initial characteristics of acidified mixtures used in the experiments.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>A1*</th>
<th>A2**</th>
<th>B1*</th>
<th>B2**</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS</td>
<td>g/L</td>
<td>65.20</td>
<td>39.84</td>
<td>60.77</td>
<td>36.10</td>
</tr>
<tr>
<td>VS</td>
<td>g/L</td>
<td>35.96</td>
<td>20.58</td>
<td>43.01</td>
<td>22.53</td>
</tr>
<tr>
<td>Total COD</td>
<td>g/L</td>
<td>73.15</td>
<td>42.32</td>
<td>78.15</td>
<td>44.35</td>
</tr>
<tr>
<td>Total organic carbon</td>
<td>g/L</td>
<td>29.00</td>
<td>16.69</td>
<td>29.50</td>
<td>17.06</td>
</tr>
<tr>
<td>Soluble organic carbon</td>
<td>g/L</td>
<td>14.10</td>
<td>7.50</td>
<td>10.45</td>
<td>6.45</td>
</tr>
<tr>
<td>Total carbohydrates</td>
<td>g equiv.gluc/L</td>
<td>2.84</td>
<td>1.36</td>
<td>9.81</td>
<td>4.52</td>
</tr>
<tr>
<td>Soluble carbohydrates</td>
<td>g equiv.gluc/L</td>
<td>0.88</td>
<td>0.45</td>
<td>0.89</td>
<td>0.48</td>
</tr>
<tr>
<td>TVFAs</td>
<td>g/L</td>
<td>16.11</td>
<td>8.09</td>
<td>15.62</td>
<td>8.44</td>
</tr>
</tbody>
</table>

* ‘1’: full-strength mixture; ** ‘2’: half-strength mixture

Moreover, the cumulative biomethane production profile from each experiment was fitted to a modified Gompertz bacterial growth model (Eq. 9.2).

\[ M(t) = P \exp \left\{ - \exp \left[ \frac{R_m}{P} \left( \lambda - t \right) + 1 \right] \right\} \]  (9.2)
Chapter 9  
Implications of the OMW use in co-digestion mixtures in a two-stage system

Fig. 9.11: Biogas and CH₄ production in each mixture (□: biogas (full-strength), ■: biogas (half-strength), △: CH₄ (full-strength), ▲: CH₄ (half-strength)).

Fig. 9.12 depicts the methane production based on experimental data and the simulation generated using the fitted modified Gompertz model at two different initial substrate concentrations (full-strength and half-strength) for each mixture (A and B). Comparing each set of experimental data with the relevant model simulation, the parameters of methane production potential (P), the maximum methane production rate (Rₘ) and lag-phase time (λ) were determined (Table 9.4). The modified Gompertz bacterial growth model was successful in interpreting the experimental production trend, as demonstrated by the high correlation coefficient (R²) values (0.990-0.995). As mentioned previously, the methanogenesis of full-strength mixture 55% OMW, 40% CW and 5% LCM (A1) led to lower methane production rate (0.14 L/d) and higher lag-phase (5.31 days) compared to half-strength one (A2), mainly due to higher phenols concentration in the A1 mixture. On the other hand, the methanogenesis of mixture 55% FS2 stalks, 40% CW and 5% LCM led to negligible effect of initial substrate concentration to methane production rate. As you can see, the operational substrate concentration varies according to the nature of the feedstock materials.

Fig. 9.13 shows the behavior of VFAs obtained for each mixture and for different initial substrate concentration as a function of the digestion time. As shown in many studies the conversion rates of VFAs to methane vary in the order of acetic >butyric >propionic acid (Ren et al., 2003). Before being degraded to methane, all VFAs are firstly degraded to acetic acid. Comparing all the experiments carried out, it was not observed any accumulation of VFAs at the end of batches (except temporary ones for propionic acid), whereas all experiments showed optimal microbial activity, reaching stable values after about 30 days from inoculation.
Implications of the OMW use in co-digestion mixtures in a two-stage system

Chapter 9

(a) 

(b) 

Figure 9-12: Cumulative methane production (experimental data and modified Gompertz model simulation) during the batch methanogenesis of a) mixture A and b) mixture B.

Table 9-4: Kinetic parameters of methane production of two different mixtures (A and B) estimated using the modified Gompertz equation.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>P (L)</th>
<th>R_m (L/d)</th>
<th>λ (d)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1*</td>
<td>3.82 ± 0.03</td>
<td>0.14 ± 0.00</td>
<td>5.31 ± 0.23</td>
<td>0.995</td>
</tr>
<tr>
<td>A2**</td>
<td>2.83 ± 0.02</td>
<td>0.21 ± 0.00</td>
<td>1.86 ± 0.22</td>
<td>0.990</td>
</tr>
<tr>
<td>B1*</td>
<td>5.35 ± 0.04</td>
<td>0.28 ± 0.00</td>
<td>4.60 ± 0.21</td>
<td>0.994</td>
</tr>
<tr>
<td>B2**</td>
<td>3.35 ± 0.02</td>
<td>0.32 ± 0.01</td>
<td>5.10 ± 0.16</td>
<td>0.994</td>
</tr>
</tbody>
</table>

* '1': full-strength mixture; ** '2': half-strength mixture

Table 9.5 summarizes the effects of the type of substrate and the different initial substrate concentration, especially on the methane yield (per g of COD removed or g VS added). Treating the mixture A, the maximum methane yield of 323.62 mL CH₄/g VS added was measured in experiment A2, whereas at higher initial substrate concentration the methane yield decreased to 230.53 mL CH₄/g VS added. This yield is higher compared to previous one (203.11 mL CH₄/g VS added) obtained using the same mixture in a two-stage CSTR system at HRT 25 d (Section 7.5.2). On the other hand, the calculated methane yield obtained from BMP assay of the same mixture (Section 7.6) was higher and equal to 472.41 mL CH₄/g VS added, may be due to the limitation of the inhibitions. In mixture A the methane yield and the methane content decreased as the concentration of substrate increased. Comparing the two results, it can
be seen that there was a significant difference on phenolics degradation between A1 and A2 (19% and 40%, respectively). As described previously, the increase of the initial substrate concentration was achieved by the addition of more concentrated mixture. Thus, more concentrated OMW resulted in higher amounts of phenolics compounds that could inhibit the anaerobic process. It was thus verified that the higher initial concentration of total soluble phenolics may cause inhibition on methane production rates and reduction to methane yields. Raposo et al. (2003) studied the mesophilic anaerobic digestion of two-phase olive mill effluents and observed decrease of phenols removal from 90 to 50% when organic loading rate increased from 0.86 to 5.38 g total COD/L.

Moreover, increasing the initial substrate concentration of mixture B (from B2 to B1), a decrease of methane yield was observed from 346.65 to 284.12 mL CH₄/g VS added, respectively. Although the increase of initial substrate concentration caused a reduction of the COD removal efficiency, no significant differences were found between B1 and B2 in terms of methane yield per g COD removed (306.03 and 287.77 mL CH₄/g COD removed).

As also shown in Table 9.5, an increase in the initial concentration of organic matter caused a reduction of COD removal in both mixtures (Sánchez et al., 2001). Furthermore, the organic matter removal efficiency was higher using sweet sorghum (mixture B) instead of olive mill (mixture A).

### Table 9-5: Removal efficiencies and methane yields during methanogenesis of mixtures A and B.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>A1*</th>
<th>A2**</th>
<th>B1*</th>
<th>B2**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biogas (L/Lₚ)</td>
<td>13.62</td>
<td>9.50</td>
<td>20.42</td>
<td>12.91</td>
</tr>
<tr>
<td>CH₄ (L/Lₚ)</td>
<td>8.29</td>
<td>6.66</td>
<td>12.22</td>
<td>7.81</td>
</tr>
<tr>
<td>CH₄ composition (%)</td>
<td>54.0</td>
<td>67.0</td>
<td>59.8</td>
<td>60.4</td>
</tr>
<tr>
<td>CH₄ yield (mL CH₄/g COD removed)</td>
<td>246.37</td>
<td>313.56</td>
<td>306.03</td>
<td>287.77</td>
</tr>
<tr>
<td>CH₄ yield (mL CH₄/g VS added)</td>
<td>230.53</td>
<td>323.62</td>
<td>284.12</td>
<td>346.65</td>
</tr>
<tr>
<td>Total COD removal (%)</td>
<td>46.0</td>
<td>50.2</td>
<td>51.1</td>
<td>61.2</td>
</tr>
<tr>
<td>Total organic carbon removal (%)</td>
<td>32.0</td>
<td>36.6</td>
<td>45.1</td>
<td>45.8</td>
</tr>
<tr>
<td>Phenolics degradation (%)</td>
<td>17.9</td>
<td>40.0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*1*: full-strength mixture; *2*: half-strength mixture
9.6 Conclusions

In this study, the effect of co-digestion on batch fermentative hydrogen production was investigated. Co-digestion improves hydrogen yield due to the positive synergism established in the digestion medium and the supply of missing nutrients by the co-digestion. Nevertheless, the effect of co-digestion in acidogenesis varies according to the feedstock. Moreover, the effect of substrate type and substrate concentration on methane production was studied. The process was carried out at two different mixtures (mixture A: 55% olive mill wastewater, 40% cheese whey and 5% liquid cow manure) and mixture B: 55% water-sorghum, 40% cheese whey and 5% liquid cow manure) and at two different initial organic substrate concentrations from each mixture. Increase of the initial substrate concentration led to the decrease of the COD removal and the methane yield. Especially, the efficiency and the methane production rate of the mixture A were lower compared to the mixture B, due to long-chain fatty acids (LCFA), tannins or most probably phenolic compounds, which are responsible for the toxicity of methanogenic bacteria.

Figure 9-13: Evolution of main volatile fatty acids during methanogenesis of each mixture.
Chapter 9 | Implications of the OMW use in co-digestion mixtures in a two-stage system

9.7 References


Implications of the OMW use in co-digestion mixtures in a two-stage system

Chapter 9


Chapter 10.

Anaerobic co-digestion of cow manure and sweet sorghum in a two-stage system.

10.1 Abstract

The aim of the present study was to determine the optimal pH and hydraulic retention time (HRT) values for treating mixture of sorghum biomass with liquid cow manure (in a ratio 95:5, v/v) in a two-stage system. Batch experiments were performed to investigate the effect of controlled pH (4.5, 5.0, 5.5, 6.0) on the production of bio-hydrogen and VFAs. The maximum hydrogen yield of 0.92 mol H₂/mol carbohydrates consumed was obtained at pH 5.0, whereas the greatest degradation of carbohydrates and VFAs productivity was observed at pH 6.0. Further investigation of the effect of HRT on hydrogen and methane production was carried out. A continuously stirred tank reactor (CSTR) was used under mesophilic conditions (37°C) in order to enhance acidogenesis and methanogenesis. Maximum H₂ yield of 1.68 mol H₂/mol carbohydrates consumed was observed at HRT of 5 d with H₂ productivity of 0.13 L/L·R·d. On the other hand, highest CH₄ production rate of 0.44 L/L·R·d was achieved at the HRT of 25 d with methane yield of 295.3 mL/g VS added, whereas at lower HRT of 20 d the process appeared to be inhibited and/or overloaded, as indicated by the accumulation of VFAs and the decline in the CH₄ productivity.
10.2 Introduction

It is clear that renewable resources have received great interest from the international community during the last decades and play a crucial role in the current CO₂-mitigation policy. In this regard, energy from biomass and waste is seen as one of the most dominant future renewable energy sources, especially since that a continuous power generation from these sources can be guaranteed, unlike other types such as solar energy and wind energy. Thus, organic waste materials like energy crops and manure are of specific importance since these sources do not compete with food crops in agricultural land usage. The various technologies that are available for power generation from biomass and waste can be subdivided into thermochemical, biochemical and physicochemical conversion processes. Anaerobic digestion, classified within the biochemical conversion processes, is a robust process and is widely applied. Various types of biomass and waste, can be anaerobically co-digested to generate a homogeneous mixture increasing both process and equipment performance. This technology is an attractive option to improve the yields of the anaerobic digestion of substrates due to the positive synergisms established in the digestion medium; a fact that increases the economic viability of the biogas plants (Mata-Alvarez et al., 2000). Furthermore, two-stage anaerobic treatment process has several advantages over the conventional one, since it permits the selection and the enrichment of different bacteria in each anaerobic digester and increases the stability of the whole process by controlling the acidification phase in the first digester and hence preventing the overloading and/or the inhibition of the methanogenic population in the second digester (Schievano et al., 2012). At the first stage of acidogenesis generation of biological hydrogen occurs whereas, at the second stage of methanogenesis, methane evolves. Optimum environmental and operational conditions for each microbial community may be achieved in such a separated two-reactor system resulting in the production of biogas. The valorization of the produced biogas (consisting of CO₂, CH₄ and/or H₂ and trace gases such as H₂S and N₂) is energy efficient and environmentally friendly because of the low emission of hazardous pollutants.

A series of operational parameters including pH (Dareioti et al., 2014), temperature (Lin et al., 2011), reactor configuration (Nasir et al., 2012), organic loading rate (Mariakakis et al., 2011) and hydraulic retention time (Rincón et al., 2008; Dareioti and Kornaros, 2014) have been investigated, as contributory factors for biogas production. Among these factors, pH has been found to be crucial to the distribution of acidogenic products (Ren et al., 1997; Dareioti et al., 2014). Although a substantial number of studies have been conducted on the optimal pH range for fermentative hydrogen production, the results are often inconsistent due to differences in substrate and seed type and other operating conditions adopted (Wu et al., 2010). It is acknowledged that low pH values result in inhibition of the hydrogenase activity, which is regarded to as a key factor explaining the influence of pH on fermentative hydrogen production (Mohd Yasin et al., 2011). The metabolic pathways involving acetate and butyrate production
appear to be favored at pH ranging from 4.5 to 6.0, while lower or higher pH are believed to promote ethanol and propionate production, respectively (Guo et al., 2010).

In addition, hydraulic retention time (HRT) is reported as one of the most important parameters significantly affecting microbial ecology and characteristics in CSTR operational systems and must be controlled for treating wastewaters. Continuous anaerobic digestion experiments are time-consuming and complex, so methane productivity testing is generally based on the results of batch tests (Antonopoulou and Lyberatos, 2013). However, batch tests may fail to truly predict full-scale anaerobic reactors performance, due to their dependency on inoculums type, on the substrate to inoculum ratio, and on the batch nature of the test. Therefore, in order to monitor possible inhibition effects due to the addition of chemicals and in order to evaluate the anaerobic digestion performances in terms of biogas production, tests with continuous reactors are needed in order to confirm and quantify the effect on anaerobic digestion of a specific substrate and/or specific operational conditions (Carrère et al., 2010). Furthermore, anaerobic digestion of lignocellulosic substrates (Colussi et al., 2013), or raw sorghum as substrate (Sambusiti et al., 2013) has been poorly studied in continuous anaerobic reactors and especially in a two-stage system for hydrogen and methane production, respectively.

Recently, some authors reported experiences of anaerobic continuous laboratory scale reactors treating the hydrolysate fraction (syrup) of sweet sorghum (Antonopoulou et al., 2008; Saraphirom and Reungsang, 2011), wheat straw (Kaparaju et al., 2009) and oat straw (Gomez-Tovar et al., 2012). The last few years, the interest is increased to evaluate the anaerobic co-digestion of energy crops with other wastes. Giuliano et al. (2013) found that the co-digestion of manures, energy crops and agro-wastes, using pilot scale CSTRs, was viable at all operating conditions tested. Appels et al. (2011) suggested that yields can be improved by co-digestion with other wastes since mixing with other residues provides the necessary nutrients to improve the digester efficiency. Synergism of the dairy manure co-digestion has been identified as a positive increase in the specific methane yield as compared to the individual substrates (Atandi and Rahman, 2012).

Hence, this study aimed to investigate the effect of pH values and the effect of various HRT values in a two-stage continuous anaerobic process co-digesting sorghum with liquid cow manure.
10.3 Materials

The substrates used in the present study corresponded to two varieties of sorghum (FS1 and ES3) and liquid cow manure (LCM). A mixture of sorghum (FS1 or ES3) and LCM in a ratio of 95:5 (v/v) was used. This mixture composition used is in continuation of previous experiment (Section 8.4; Dareioti and Kornaros, 2015). In this experiment the sorghum was used to replace other substrates (such as cheese whey) because of their seasonal availability.

Among energy crops, sorghum is a C4, heat- and drought - tolerant and highly productive crop, with a high photosynthetic efficiency. It requires less water than corn, so it is likely to be grown as a replacement to corn and it produces better yields than corn in hotter and drier areas. In the present work two varieties of sorghum were used (FS1 and ES3). Their characteristics are presented in Section 2.1.2 (Table 2.1). The chemical composition of both sorghums, after drying and milling, is given in Table 10.1. The whole characterization, apart from moisture and total solids (TS), was obtained after drying and milling of sorghum. The sorghum mainly consisted of high percentage of polysaccharides and lignin (Panagiotopoulos et al., 2010). The ash content of the total dry matter of fresh sorghum stalks was rather low. Fresh sorghum was characterized by higher percentage of carbohydrates compared with ensiled one. During the ensiling procedure soluble carbohydrates are utilized by fermentative bacteria for the production of volatile fatty acids, lactic acid and ethanol, depending on the metabolic pathway. Thus, the amount of soluble carbohydrates of ensiled sorghum is low and lactic acid concentration was observed.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>FS1</th>
<th>ES3</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.4 ± 0.01</td>
<td>4.10 ± 0.00</td>
</tr>
<tr>
<td>Moisture (% wet weight)</td>
<td>74.0 ± 0.12</td>
<td>76.32 ± 0.10</td>
</tr>
<tr>
<td>TS (% wet weight)</td>
<td>26.0 ± 0.03</td>
<td>23.73 ± 0.17</td>
</tr>
<tr>
<td>VS (%TS)</td>
<td>96.2 ± 0.51</td>
<td>94.08 ± 3.15</td>
</tr>
<tr>
<td>Ash (%TS)</td>
<td>3.8 ± 0.09</td>
<td>5.93 ± 3.15</td>
</tr>
<tr>
<td>TOC (%TS)</td>
<td>57.0 ± 0.05</td>
<td>46.18 ± 0.00</td>
</tr>
<tr>
<td>Total carbohydrates (%TS)</td>
<td>58.0 ± 0.91</td>
<td>38.82 ± 1.29</td>
</tr>
<tr>
<td>Soluble carbohydrates (%TS)</td>
<td>28.0 ± 0.30</td>
<td>2.02 ± 0.06</td>
</tr>
<tr>
<td>Cellulose (%TS)</td>
<td>22.0 ± 1.31</td>
<td>37.60 ± 5.37</td>
</tr>
<tr>
<td>Hemicellulose (%TS)</td>
<td>12.0 ± 1.55</td>
<td>25.51 ± 3.66</td>
</tr>
<tr>
<td>Lignin (%TS)</td>
<td>9.0 ± 2.01</td>
<td>24.38 ± 1.93</td>
</tr>
<tr>
<td>Total nitrogen, TKN (%TS)</td>
<td>0.2 ± 0.01</td>
<td>0.96 ± 0.45</td>
</tr>
<tr>
<td>Proteins (%TS)</td>
<td>1.2 ± 0.06</td>
<td>6.00 ± 2.81</td>
</tr>
<tr>
<td>Lactic acid (%TS)</td>
<td>N.D</td>
<td>4.28 ± 0.00</td>
</tr>
<tr>
<td>Ethanol (%TS)</td>
<td>N.D</td>
<td>N.D</td>
</tr>
</tbody>
</table>

* Mean values (± standard deviation); ‡ In equivalent glucose; N.D: No Detected
On the other hand, LCM is one of the most polluting agro-industrial wastewaters. Manure is a frequently used feedstock for anaerobic digesters because it is readily available and very suitable for the development of anaerobic microorganisms because of its high nitrogen content. However, the ammonia concentration in some types of manure exceeds the inhibition threshold concentration. Therefore, manure is frequently applied in co-digestion with other wastes that are characterized by low nitrogen concentrations (Ward et al., 2008). Table 10.2 represents the average values of LCM characterization. High nitrogen content is an important attribute of manure wastes and also contribute in buffering capacity of the mixture as a consequence of neutral pH (7.24 ± 0.18) and alkalinity in high levels (12.38 ± 0.32 g CaCO₃/L). It is important to take into consideration the fact that the alkalinity should be high enough to avoid the destabilization of the system originated by the possible accumulation of volatile fatty acids.

Table 10-2: Chemical composition of liquid cow manure (LCM).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>LCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-</td>
<td>7.24 ± 0.18</td>
</tr>
<tr>
<td>TS</td>
<td>g/L</td>
<td>33.15 ± 1.98</td>
</tr>
<tr>
<td>VS</td>
<td>g/L</td>
<td>22.50 ± 0.98</td>
</tr>
<tr>
<td>Total COD</td>
<td>g/L</td>
<td>43.14 ± 2.56</td>
</tr>
<tr>
<td>Soluble COD</td>
<td>g/L</td>
<td>15.05 ± 0.32</td>
</tr>
<tr>
<td>TOC</td>
<td>g/L</td>
<td>16.72 ± 0.24</td>
</tr>
<tr>
<td>Total carbohydrates</td>
<td>g/L</td>
<td>6.99 ± 0.45</td>
</tr>
<tr>
<td>Soluble carbohydrates</td>
<td>g/L</td>
<td>0.45 ± 0.05</td>
</tr>
<tr>
<td>Total nitrogen, TKN</td>
<td>g/L</td>
<td>2.78 ± 0.00</td>
</tr>
<tr>
<td>Ammonium nitrogen</td>
<td>g/L</td>
<td>1.57 ± 0.02</td>
</tr>
<tr>
<td>Proteins</td>
<td>g/L</td>
<td>17.38 ± 0.00</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>mg/L</td>
<td>463.50 ± 9.49</td>
</tr>
<tr>
<td>Soluble phosphorus</td>
<td>mg/L</td>
<td>21.68 ± 0.04</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>g CaCO₃/L</td>
<td>12.38 ± 0.32</td>
</tr>
<tr>
<td>Total VFAs</td>
<td>mg/L</td>
<td>4986.80 ± 19.61</td>
</tr>
</tbody>
</table>

a Mean values (± standard deviation); b In equivalent glucose
10.4 Effect of pH

Anaerobic acidogenesis batch experiments were studied using mixture of FS1 and LCM (in a ratio of 95:5, v/v) and were performed at different constant pH values, namely 4.5, 5.0, 5.5 and 6.0. The experiments were conducted using steel reactor (Section 2.2.2) at constant temperature (37°C). The operating volume was 900 mL, whereas the acclimatized anaerobic culture seed sludge (20% of the working volume) was an effluent of a lab-scale anaerobic acidogenic reactor (CSTR) fed with a mixture of 55% OMW, 40% CW and 5% LCM (Section 7.5.1). Prior to batch experiments centrifugation (4500 rpm) of the inoculum was performed to remove the soluble part of culture medium. The pH of the mixed liquor was kept constant throughout the experimental course via automatic control (using a Hach PID-controller) by adding drops of NaOH or HCl (6 N) solutions.

Fig. 10.1(a) displays the net biogas and hydrogen production in the batch reactor at different pH values tested. As can be seen, the hydrogen productivity at low pH value of 4.5 was almost minimum and equal to 40.75 mL. The maximum production of 1706.69 mL was obtained at pH 5.0, whereas increasing to pH values of 5.5 and 6.0 the hydrogen productivity was lead to 1045.53 and 1358.83 mL, respectively. Over the course of the anaerobic digestion experiments, no methane production was detected indicating that only acidogenesis was active. The pH of the growth medium is an important parameter to many fermentation processes. The search for the best pH is usually important in designing a fermentation process, especially for fermentations involving acid products. The consumption of total carbohydrates (equivalent glucose) was increased switching to higher pH values with the maximum degradation (56.9%) observed at pH 6.0 (Fig. 10.1(b)).

Figure 10-1: Effect of pH on (a) biogas and hydrogen production and (b) carbohydrates consumption.
This low degradation can be explained due to the fact that sorghum is a lignocellulosic material. However, soluble carbohydrates consumption was independent at different pH values, with the highest percentage of 91.96% at pH 5.5, despite their simultaneous production due to hydrolysis of total carbohydrates. Mohd Yasin et al. (2011) found that in anaerobic fermentation of food waste at thermophilic conditions (55°C), the controlled pH value of 6.0 caused increase in carbohydrates consumption (78%), compared with lower pHs of 5.0 and 5.5. Degradation of carbohydrates during anaerobic conditions is accompanied by the production of hydrogen and various metabolic soluble end-products, mainly volatile fatty acids (i.e. acetic, propionic, and butyric acids), lactic acid, and alcohols (ethanol), depending on the microbial species present and the prevailing conditions. The analysis of the metabolic products provides useful information on the evolution of the process and can be used to clarify the observed hydrogen generation yields. In the present study, the course of soluble metabolites’ concentration was monitored during the process. Fig. 10.2(a) shows the most abundant end-products, namely acetic, propionic, butyric, lactic acid and ethanol, at four different pH conditions. However, lower amount of i-butyric acid (<900 mg/L) and limited amounts (<300 mg/L) of other volatile fatty acids (i.e. valeric, i-valeric and caproic) were detected in all pH cases. Also, as seen in Fig. 10.2(a), the variation of pH value exerted remarkable effect on metabolic products distribution. Ethanol concentration was higher (1200 mg/L) at the lowest value of pH 4.5 which is in agreement with previous study (Ren et al., 1997) that suggested a pH value of about 4.5 as optimum for maximizing the production of ethanol. Lactic acid, as an intermediate fermentation product, firstly was produced and subsequently was metabolized. This metabolic change was influenced by pH conditions and it was observed accumulation of lactic acid apart from pH value of 5.0. The conversion of lactic acid may be occurred into propionic and acetic acid with no hydrogen production or into butyric acid with simultaneous hydrogen production (Dareioti et al., 2014).

![Figure 10-2: Effect of pH on (a) main soluble end-products and (b) hydrogen yield.](image-url)
Hence, at pH 5.0 was obtained the highest amount of butyric acid with simultaneous hydrogen production, as a result of negligible kinetic limitation in the metabolic reactions. This clearly suggests that the intense hydrogen production phase was mainly associated with the production of butyric acid. The total VFAs concentration was highest at pH 6.0 (16.79 g/L) due to the fact that acetic and propionic acid were increased with increasing pH, indicating that the greatest VFAs production was occurred at pH 6.0, which was similar to Jiang et al. (2013). The acetic acid concentration at pH value of 6.0 was 9.54-fold higher than pH 5.0, whereas propionic acid concentration was 3.1-fold higher, respectively. Zhang et al. (2013) reported that acetic acid was main product at pH 5.0 whereas butyric acid was dominant at pH 6.0, during anaerobic acidogenesis of kitchen wastes. Fig. 10.2(b) depicts the hydrogen yield (moles of hydrogen produced per mole equivalent glucose of total consumed carbohydrates) at each pH value tested. The maximum hydrogen yield was observed at pH 5.0 and was equal to 0.92 mol H$_2$/mol equivalent glucose consumed. In current study, high hydrogen yield is associated with butyric acid production, whereas low yields with the production of end-products such as propionic acid, ethanol and lactic acid which are accompanied by a negative or zero hydrogen. The estimated hydrogen yield was higher than other yields, which were obtained by previous experiment (Section 7.4.1). For example, an optimum pH value of 6.0 was found with hydrogen yield 0.64 mol H$_2$/mol equivalent glucose consumed, using mixture of olive mill waste, cheese whey and liquid cow manure (in a ratio 55:40:5).

In Fig. 10.3 is shown the acidogenenic experiment of the mixture at pH value of 5.0, whereas the experimental results obtained in different pH values (4.5, 5.5, 6.0) can be found in Appendix A (Fig. A.10–Fig. A.12). In particular, the consumption of total and soluble sugars is presented associated with the rate of production of major products during the whole experiment course. Degradation of carbohydrates (Fig. 10.3(a)) was contributed to an increase of the concentration of volatile fatty acids, lactic acid and ethanol (Fig. 10.3(b)). The most abundant metabolic end-product was the butyric acid (approximately 8.4 g butyric acid/L), mainly after the decreasing concentration of acetic acid and lactic acid. The increasing concentration curve for butyric acid was synchronized with the increasing curve for hydrogen production (Fig. 10.3(c)).

The modified Gompertz bacterial growth model (Eq. 10.1) used to describe the product formation in the hydrogen production process (using OriginPro version 8). H is the cumulative hydrogen production (mL); P is the maximum hydrogen production potential (mL); $R_m$ is the maximum hydrogen production rate (mL/h); $\lambda$ is the lag-phase duration (h); $t$ is the time (h) and $e$ is exp(1) = 2.71828.

$$H = P \exp \left\{ - \exp \left[ \frac{R_m e}{P} (\lambda - t) + 1 \right] \right\}$$  (10.1)
Fig. 10.4 depicts the hydrogen production for experimental data and modified Gompertz model simulation at four different controlled pH conditions. The correlation coefficient ($r^2$) range was 0.983–0.996. Comparing the experimental data with model simulation, the parameters of hydrogen production potential ($P$), the maximum hydrogen production rate ($R_m$), and lag phase time ($\lambda$) was determined. The parameters of the fitted equation for the hydrogen were summarized in Table 10.3. The maximum $P$ and $R_m$ peaks were obtained at pH of 5.0 and were equal to 1759.29 mL and 92.53 mL H$_2$/h, respectively. When the acidogenic reactor was operated at pH 5.5 had the longest lag time of 15.89 h, whereas at pH value of 4.5 the lag phase was no measurable due to the limited hydrogen production.

Figure 10-3: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of mixture (95% FS1 and 5%LCM), at pH 5.0.
Chapter 10 | Anaerobic co-digestion of cow manure and sweet sorghum in a two-stage system

Figure 10-4: Cumulative hydrogen production (experimental data and modified Gompertz model simulation) at different pH values tested.

Table 10-3: Kinetic parameters of hydrogen production estimated using the modified Gompertz equation.

<table>
<thead>
<tr>
<th>pH</th>
<th>P (mL)</th>
<th>$R_m$ (mL/h)</th>
<th>$\lambda$ (h)</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>39.38 ± 0.62</td>
<td>1.71 ± 0.08</td>
<td>-</td>
<td>0.983</td>
</tr>
<tr>
<td>5.0</td>
<td>1759.29 ± 13.77</td>
<td>92.53 ± 2.24</td>
<td>7.49 ± 0.20</td>
<td>0.996</td>
</tr>
<tr>
<td>5.5</td>
<td>1701.10 ± 115.44</td>
<td>22.19 ± 0.46</td>
<td>15.89 ± 0.69</td>
<td>0.986</td>
</tr>
<tr>
<td>6.0</td>
<td>1365.80 ± 11.47</td>
<td>91.38 ± 2.34</td>
<td>0.48 ± 0.19</td>
<td>0.993</td>
</tr>
</tbody>
</table>
Furthermore, batch experiment with the pretreated ES3 in the mixture was conducted in order to assess the impact of ensiled sorghum, instead of fresh one, on hydrogen and VFAs production. Prior to its use, ES3 was subjected to alkaline pretreatment at 80°C for 2 h with the addition of alkaline solution 0.5% NaOH and 0.5% KOH (w/w) as described in Section 4.5. The efficiency of the process, using pretreated ES3 than FS1, was lower as it can be seen in Fig. 10.5. The carbohydrates degradation was lower (16.06%), which may be explained because of ensiled sorghum was characterized by low soluble sugar concentration compared with fresh one (Fig. 10.5(a)). As a result, the distribution of main metabolites was different, an accumulation of lactic acid was observed with simultaneous lower butyric acid production (Fig. 10.5(b)) and lower hydrogen productivity (586.30 mL), respectively (Fig. 10.5(c)).

Figure 10-5: (a) Consumption of carbohydrates, b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of mixture (95% ES3 and 5%LCM), at pH 5.0.
10.5 Effect of HRT

Separate operation of a two-stage process was carried out, using a mixture of pretreated ES3 and LCM (in a ratio of 95:5, v/v) in order to assess the HRT impact on hydrogen and methane productivity. In this case, we considered that it was more practical to use ensiled sorghum instead of fresh one because of seasonal availability after ensiling procedure. Experiments were carried out in a CSTR reactor, which was made with a double wall, having an operating volume of 500 mL (Section 2.2.1). Firstly, it was conducted the acidogenesis step and subsequently the methanogenesis step. In this study, pH in the acidogenic reactor was kept constant at 5.0 (according to Section 10.4) throughout the experimentation phase via automatic control (using a Hach PID-controller) by the addition of a solution mixture of NaOH (1.5 N) and KOH (1.5 N) via a peristaltic pump. For the start-up, an acclimated anaerobic sludge was used from a two-stage system (Section 8.4.3) fed with the waste mixture of pretreated ensiled sorghum (ES3), cheese whey and liquid cow manure in a ratio 55:40:5 (v/v/v). Prior to its use as feeding material in mixture with LCM, ES3 was subjected to alkaline pretreatment at 80°C for 2 h with the addition of alkaline solution 0.5% NaOH and 0.5% KOH (w/w) as described in Section 4.5.

Experiments were conducted successively to determine the optimum HRT for maximum hydrogen and methane production. The acidogenic reactor was operated at three different HRTs of 3, 5 and 8 d with a feed mixture of pretreated ES3 and LCM in a ratio 95:5 (v/v), respectively. The effluent from acidogenic reactor was homogenized and was preserved at −18ºC until subsequent use in methanogenesis which was operated at two different HRTs (20 and 25 d) equivalent to OLRs of 2.23 and 1.78 kg COD/m³·day, respectively. The experimental design conditions in the two-stage system were tested at various HRTs as shown in Table 10.4.

<table>
<thead>
<tr>
<th>Operating conditions</th>
<th>Acidogenic Reactor</th>
<th>Methanogenic Reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HRT (d)</strong></td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Flow rate (mL/d)</td>
<td>167</td>
<td>100</td>
</tr>
<tr>
<td>OLR (kg VS/m³·d)</td>
<td>12.83</td>
<td>7.70</td>
</tr>
<tr>
<td>OLR (kg COD/m³·d)</td>
<td>23.03</td>
<td>13.82</td>
</tr>
<tr>
<td><strong>HRT (d)</strong></td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Flow rate (mL/d)</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>OLR (kg VS/m³·d)</td>
<td>1.86</td>
<td>1.49</td>
</tr>
<tr>
<td>OLR (kg COD/m³·d)</td>
<td>2.23</td>
<td>1.78</td>
</tr>
</tbody>
</table>
10.5.1 Effect of HRT in the acidogenic reactor

Continuous hydrogen production was operated in CSTR with different HRTs i.e. 3, 5, and 8 d, at constant pH of 5.0, using mixture of pretreated ES3 and LCM (in a ratio of 95:5, v/v). After reactor start-up, the initial HRT was set at 3 d for a period of 51 days. HRT was then increased to 5 d and 8 d for a period of 41 and 34 days, respectively. The biogas produced from the acidogenic reactor consisted exclusively of hydrogen and carbon dioxide, whereas no traces of methane were detected in the whole period of our investigation, confirming that this method completely suppresses methanogens growth.

Fig. 10.6(a) illustrates the net biogas and hydrogen production rate as a function of experimental time (at normal temperature and pressure conditions). As shown in Fig. 10.6(a), the reactor performance was characterized by fluctuations of biogas and hydrogen production rates, maybe due to the complexity of the feeding medium. Initially biogas production rate at HRT of 3 d was fluctuating with a mean value of 0.36 L/LR·d containing 21.98% of hydrogen (Fig. 10.6(b)) at the steady state (with the rest being mainly CO₂). The maximum hydrogen production rate of 0.13 L/LR·d, corresponding to a yield of 1.68 mol H₂/mol carbohydrate consumed (209 mL H₂/g carbohydrate) was obtained at HRT of 5 d. It was observed a considerably higher hydrogen yield and lower hydrogen production rate compare to those results in our previous experiment (Section 8.4.2; Dareioti and Kornaros, 2015) fed with 55% pretreated ES3, 40% CW and 5% LCM. As regards soluble end-products concentration, significant production of volatile fatty acids (namely acetic, butyric and caproic acid) and lactic acid were noted as the most prominent ones at all tested HRTs (Fig. 10.6(c)). Propionic, isobutyric, valeric acid and ethanol were also measured in concentrations, though, less than 400 ppm, whereas isovaleric acid was measured in trace concentrations. It is noticeable that the highest amount of TVFAs, under steady-state conditions, was observed at HRT of 5 d (approximately 7.88 g/L) with minimum lactic acid concentration. A problem of the system (at 60th day of operation) led to a temporary accumulation of lactic and acetic acid with no production of butyric acid but the system was returned in stability after few days. However, lactic remained at low and zero concentration after increasing the HRT from 5 d to 8 d, whereas at lower HRT of 3 d the lactic acid was not fully degraded, confirming that lactic acid can be degraded by the enriched microorganisms which they demand retention time higher than 3 d. The production of soluble end-products was a result of carbohydrates degradation. For all HRTs studied, carbohydrate utilization efficiency was not affected significantly by HRT changes and was over 11.26 ± 1.3% and 40.30 ± 6.9% for total and soluble, respectively. The solids concentration remained constant, whereas negligible difference of organic matter was observed between influent and effluent of the acidogenic reactor. Table 10.5 presents the performance of acidogenic reactor considering the main parameters at each HRT.
Figure 10-6: Evolution of (a) biogas and hydrogen production rates, (b) hydrogen content and (c) main soluble end-products during acidogenesis for each HRT tested.

Table 10-5: Fermentation performance under steady-state conditions for each HRT.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HRT (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Biogas (L/LR·d)</td>
<td>0.36</td>
</tr>
<tr>
<td>H₂ (L/LR·d)</td>
<td>0.09</td>
</tr>
<tr>
<td>H₂ (%)</td>
<td>21.98</td>
</tr>
<tr>
<td>Yield (mol H₂/mol carbohydrates consumed)</td>
<td>0.63</td>
</tr>
<tr>
<td>Yield (mL H₂/g carbohydrates consumed)</td>
<td>78.63</td>
</tr>
</tbody>
</table>
10.5.2 Effect of HRT in the methanogenic reactor

The homogenized acidogenic effluent was fed into the methanogenic reactor for methane production at two different different hydraulic retention times (HRTs of 20 and 25 d). The influent of the methanogenic digester was rich in VFAs, as anticipated, having a contribution of 73.5% in terms of COD, compared to the soluble COD concentration. Methane production and pH variation at two different HRTs are shown in Fig. 10.7(a). No hydrogen was detected in the second stage. At the initial HRT of 20 d, a methane production rate of 0.43 L/LR·d and a yield of 230.9 mL CH₄/g VS added was achieved until the 40th day of operation, whereas biogas contained with 54.7% of methane. However, a further operation resulted in a significant reduction of methane production with simultaneous accumulation of VFAs (up to 6.76 g/L), indicating organic overloading (Fig. 10.7(b)). Increasing the HRT at 25 d, methane production was increased and subsequently reached optimal conditions with 56.9% of methane. An average methane production rate of 0.44 L/LR·d, corresponding to an average yield of 295.3 mL CH₄/g VS added was obtained. According to Sambusiti et al. (2013), similar methane yields of 237 and 297 mL CH₄/g VS added were noticed in anaerobic digestion of untreated and pretreated ensiled sorghum respectively, in two semi-continuous CSTR. Moreover, the co-digestion of liquid cow manure with different share of maize was investigated using continuous reactors, where the highest specific methane yield (259 mL CH₄/g VS added) was obtained when the share of maize in the feedstock was 40% (Seppälä, et al., 2013).

The pH fluctuated within a stable range of 7.5-8.14, apart from the period of reactor instability in which the pH decreased and led to the inhibition of the methanogenic biomass. The effluent pH was significantly higher than the influent pH as a direct consequence of the degradation of VFAs (Kongjan et al., 2013). The concentration of VFAs was significantly lower in methanogenic effluent than influent and especially at HRT of 25 d a complete degradation was observed (Fig. 10.7(b)). Therefore, an accumulation of them, after the 40th day of operation at HRT of 20 d, implies that the methanogens were not functioning as expected, with reduction of methane production rate. The effect of HRT on the anaerobic digestion of poultry slaughterhouse wastes was examined by Salminen and Rintala (2002) who observed a gradual accumulation of total VFA and concomitant decline in the methane yield at shorter HRT, in the range from 25 to 13 d.

The TVFA/alkalinity ratio can be used as a measure of process stability (Callaghan et al., 2002): when this ratio is less than 0.3-0.4 (equiv. acetic acid/equiv. CaCO₃) the process is considered to be operating favourably without acidification risk. When an accumulation of VFAs was observed at HRT of 20 d, the TVFA/alkalinity ratio was estimated 1.17, higher than the safety threshold value. These values indicate a destabilization and deterioration of the process, as a result of microorganisms’ inhibition at HRT of 20 d. Increasing the HRT at 25 d, the ratio decreased at 0.88 after 15 days and then at zero after a long period of operation.
The evolution of total and soluble COD as a function of experimental time of methanogenic reactor is plotted in Fig. 10.7(c). The removal of COD in conjunction with gas production in the anaerobic digester provided evidence of effective microbial activity from methanogenic bacteria. The total and soluble COD removal efficiencies were 49.40% and 68.80% for 25 d HRT, respectively. Karim et al. (2005) reported similar methane production rate of 0.45 L CH$_4$/LR·d in the digestion of dairy manure, whereas the removal percentage of total COD was 50%. Fig. 10.7(c) illustrates also the evolution of TVFAs (expressed in units of COD). Total VFAs concentration followed the same general trend as the values of soluble COD accounting for most of the soluble COD. Moreover, it were estimated the removal efficiencies, in terms of TS and VS and total carbohydrates at HRT of 25 d. The TS and VS removals were equal to 27.95% and
52.72% respectively, whereas the degradation of total carbohydrates in glucose equivalents was 87.5% in methanogenesis. It is well known that alkaline pretreatment removes lignin and a part of the hemicellulose, and thus efficiently increases the accessibility of microorganisms to the cellulose (Antonopoulou and Lyberatos, 2013).

### 10.6 Biochemical Methane Potential

Biochemical Methane Potential (BMP) of the mixture was also studied according to batch assay (Section 2.2.3). Fig. 10.8 illustrates the cumulative methane production as a function of the digestion time. The calculated methane production of the mixture, after subtraction of the methane produced from the blank experiment, was 75.79 ± 4.08 mL of CH$_4$, whereas the methane potential obtained was 464.91 ± 25.02 mL CH$_4$/g VS added. The calculated methane potential is higher than the respective value obtained from CSTR experiment (295.3 mL CH$_4$/g VS added at HRT of 25 d), because of the fact that in BMP assay no inhibition was occurred.

Moreover, the cumulative biomethane production profile was fitted to a modified Gompertz bacterial growth model. The equation used (Eq. 10.2) is a modified form from Eq. (10.1).

$$M(t) = P \exp \left\{ - \exp \left[ \frac{R_m e}{P} (\lambda - t) + 1 \right] \right\}$$

(10.2)

where $M(t)$ is the cumulative methane production (mL); $P$ is the maximum methane production potential (mL); $R_m$ is the maximum methane production rate (mL/d); $\lambda$ is the lag-phase duration (d); $t$ is the time (d) and $e$ is $\exp(1) = 2.71828$.

![Figure 10-8: Cumulative methane production during the BMP assay from the mixture (95% ES3 and 5% LCM) and blank sample. Errors bars represent the standard deviation for the replicates.](image-url)
Fig. 10.9 depicts the methane production based on experimental data and the simulation generated using the fitted modified Gompertz model. The correlation coefficient ($R^2$) was 0.994, whereas the methane production potential ($P$) was $74.08 \pm 0.90$ mL, the maximum methane production rate ($R_m$) was $4.67 \pm 0.39$ mL/d and finally the lag-phase ($\lambda$) was $12.61 \pm 0.62$ d. As it can be seen, the maximum methane production rate ($R_m$) was decreased as the percent of sorghum in the mixture increased. In particular, the $R_m$ of this mixture (4.67 mL/d) was lower than 5.18 mL/d (Section 8.5; mixture of 55%ES3, 40%CW and 5%LCM) and 5.85 mL/d (Section 7.6; mixture of 55%OMW, 40%CW and 5%LCM). This reduction was attributed to the fact that sorghum is a lignocellulosic material.

![Cumulative methane production (experimental data and modified Gompertz model simulation) during BMP assay of ES3 / LCM mixture (95 / 5, v/v).](image)

**Figure 10-9:** Cumulative methane production (experimental data and modified Gompertz model simulation) during BMP assay of ES3 / LCM mixture (95 / 5, v/v).

### 10.7 Conclusions

Based on the results of this study, it has been demonstrated that co-digestion of sorghum biomass and cow manure (95:5, v/v) in a two-stage process is a sustainable and environmentally-attractive method to treat these wastes. In acidogenesis, optimal pH value of 5.0 was obtained with highest hydrogen yield of 0.92 mol $H_2$/mol carbohydrates consumed, whereas HRT of 5 d led to the highest hydrogen production rate of 0.13 $L/L_{R}\cdot d$ and hydrogen yield of 1.68 mol $H_2$/mol carbohydrates consumed. However, in methanogenesis the highest yield of 295.3 mL CH$_4$/g VS added, was achieved at HRT 25 d, whereas an instability of reactor performance was observed at lower HRT of 20 d.
10.8 References


Chapter 10 | Anaerobic co-digestion of cow manure and sweet sorghum in a two-stage system


Chapter 11.

ADM1-based modeling of methane production in a two-stage system

11.1 Abstract

The anaerobic digestion model No. 1 (ADM1), conceived by the international water association (IWA) task group for mathematical modeling of anaerobic digestion processes is a structured generic model which includes multiples steps describing biochemical and physicochemical processes encountered in the anaerobic degradation of complex organic substrates and a common platform for further model enhancement and validation of dynamic simulations for a variety of anaerobic processes. In this study the ADM1 model was modified and applied to simulate the mesophilic anaerobic (co)-digestion of different substrates (mostly acidified effluents). Since the ADM1 does not account for metabolic products such as lactic acid, ethanol and caproic acid that are crucial during the fermentative hydrogen production process, the structure of the model was modified to include lactate, ethanol and caproate among the metabolites and to improve the predictions. The model was applied to lab-scale CSTR reactors fed with different substrates such as raw liquid cow manure and four different acidified mixtures. The simulations results indicated that the modified ADM1 was able to predict reasonably well the steady-state results of gas flows, methane, pH and total volatile fatty acids (TVFAs). Also the reactor failure observed at HRT of 12 days, treating the acidified mixture of 55% ensiled sorghum, 40% cheese whey and 5% liquid cow manure, was predicted and well justified by the modified ADM1.
11.2 Introduction

Anaerobic Digestion (AD) is a chain of interconnected biological reactions, where the organic matter (in the form of carbohydrates, proteins, lipids or more complex compounds), is transformed into methane, carbon dioxide and anaerobic biomass, in an oxygen-free environment. This biological process is used to simultaneously treat waste and wastewater and to produce biogas. In order to describe the performance and kinetics of the anaerobic digestion process for methane generation, several anaerobic digestion models were developed during the last 30 years (Gavala et al., 2003). Initially, model development considered organic matter as a whole and did not account for the composition of the feedstock. More recent model approaches consider complex feed compositions (carbohydrate, protein, volatile fatty acids (VFA) and other organics) yielding more accurate results (Lyberatos and Skiadas, 1999). The latest developed model is the Anaerobic Digestion Model No.1 (ADM1), which was developed by the International Water Association’s (IWA) Task Group (Batstone et al., 2002). The strength of this model is in its consideration of separate biomass fractions and their decay, apart from incorporating four main stages of anaerobic degradation, and dividing them into 31 processes and 33 groups of fractions. Moreover, the model includes a composite fraction \( X_C \), which represents a complex substrate. The composite fraction \( X_C \) is degraded into carbohydrates \( X_{Ch} \), proteins \( X_{pr} \), lipids \( X_{li} \) and inerts \( X_I \) fractions during the disintegration step (Batstone et al., 2002). Additionally, due to its capability to describe the biogas production rate and composition, the ADM1 was commonly used as an anaerobic degradation model for different substances, like grass silage (Koch et al., 2009, 2010), sweet sorghum (Antonopoulou et al., 2012), agro-waste (Gali et al., 2009), olive pulp waste (Kalfas et al., 2006), cattle manure (Myint et al., 2007; Wichern et al., 2008), cattle manure and co-substrates (Lübken et al., 2007). All these studies show the increasing trend of applications of ADM1 model as well as the necessity of appropriate modifications in its structure in order to simulate different anaerobic digestion concepts.

ADM1 was originally developed to describe anaerobic digestion of sludge from waste water treatment plants. The model includes the three overall biochemical (cellular) steps namely as acidogenesis, acetogenesis (anaerobic oxidation of organic acids) and methanogenesis as well as extracellular (partly non-biological) disintegration step and an extracellular hydrolysis step (Fig. 11.1). Three of the processes (hydrolysis, acidogenesis and acetogenesis) have a number of parallel reactions. The disintegration phase represents degradation of composite fraction \( X_C \) into carbohydrates \( X_{Ch} \), proteins \( X_{pr} \), lipids \( X_{li} \) and inerts \( X_I \) fractions. Further enzymatic degradation of the non-inert fractions into monosaccharides \( S_{su} \), amino acids \( S_{aa} \) and long chain fatty acids \( S_{fa} \) represents the hydrolysis stage. Decay processes are described producing inert and degradable particulate organic matter which again undergoes the disintegration step. The physicochemical process of stripping the gaseous compounds, hydrogen, methane and carbon dioxide, is included to represent the production of biogas.
ADM1-based modeling of methane production in a two-stage system

Chapter 11

283

Figure 11-1: Schematic description of ADM1 model including biochemical processes (1) acidogenesis from sugars, (2) acidogenesis from amino acids, (3) acetogenesis from LCFA, (4) acetogenesis from propionate, (5) acetogenesis from butyrate and valerate, (6) acetoclastic methanogenesis and (7) hydrogenotrophic methanogenesis.

11.3 Kinetic model

The ADM1 was developed by IWA task group for mathematical modeling of anaerobic digestion processes (Batstone et al., 2002). In the present study, the model was implemented in Aquasim 2.0 (Reichert, 1998), as a constant volume mixed-liquid compartment and a gas compartment linked by a diffusive gas link.

Biochemical and acid–base equilibrium processes were implemented in the form of differential equations (DE) and implicit algebraic equations (AE). The process rate and stoichiometry matrix for biochemical reactions are given in Tables 11.1 (soluble components) and 11.2 (particulate components). Disintegration and hydrolysis are described in ADM1 using first order kinetics. The disintegration kinetic constant for composite degradation is described as $k_{dis}$, the hydrolysis constant for the hydrolysis of carbohydrates, lipids and proteins are $k_{hyd,ch}$, $k_{hyd,li}$ and $k_{hyd,pr}$, respectively (Batstone et al., 2002). The key rate equation is substrate uptake, which is based on substrate Monod-type kinetics, where $k_m$ is the Monod maximum specific uptake rate (kg COS_S/kg COD_X·d), $k_s$ is the half saturation constant (kg COD_S/m³) and X is the concentration of degrading microorganisms (kg COD/m³). Biomass growth is implicit in substrate uptake.
<table>
<thead>
<tr>
<th>Component $j$</th>
<th>Process $i$</th>
<th>$S_1$</th>
<th>$S_{12}$</th>
<th>$S_{14}$</th>
<th>$S_5$</th>
<th>$S_6$</th>
<th>$S_9$</th>
<th>$S_{10}$</th>
<th>$S_{11}$</th>
<th>$S_{12}$</th>
<th>$S_{13}$</th>
<th>$S_{14}$</th>
<th>$S_{15}$</th>
<th>Rate ($\rho_j$, kg COD.m$^{-3}$.d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Disintegration</td>
<td>$k_{d1}X_c$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Hydrolysis of Carbohydrates</td>
<td>$k_{d2,1}X_c$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Hydrolysis of Proteins</td>
<td>$k_{d3,2}X_p$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Hydrolysis of Lipids</td>
<td>$k_{d4,3}X_l$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Uptake of Sugars</td>
<td>$-1$</td>
<td>$(1-Y_{m1})f_{m1}$</td>
<td>$(1-Y_{m2})f_{m2}$</td>
<td>$(1-Y_{m3})f_{m3}$</td>
<td>$(1-Y_{m4})f_{m4}$</td>
<td>$-\sum_{i=12,14,28}(1-Y_{c4})N_{biom}$</td>
<td>$k_{u5,1}S_{m1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Uptake of Amino Acids</td>
<td>$-1$</td>
<td>$(1-Y_{m1})f_{m1}$</td>
<td>$(1-Y_{m2})f_{m2}$</td>
<td>$(1-Y_{m3})f_{m3}$</td>
<td>$(1-Y_{m4})f_{m4}$</td>
<td>$-\sum_{i=12,14,28}(1-Y_{c4})N_{biom}$</td>
<td>$k_{u6,1}S_{m1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Uptake of LCFA</td>
<td>$-1$</td>
<td>$(1-Y_{m1})0.7$</td>
<td>$(1-Y_{m2})0.3$</td>
<td>$(1-Y_{m3})0.3$</td>
<td>$(1-Y_{m4})0.3$</td>
<td>$-\sum_{i=12,14,28}(1-Y_{c4})N_{biom}$</td>
<td>$k_{u7,1}S_{m1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 Uptake of Lactate</td>
<td>$-1$</td>
<td>$(1-Y_{m1})f_{m1}$</td>
<td>$(1-Y_{m2})f_{m2}$</td>
<td>$(1-Y_{m3})f_{m3}$</td>
<td>$(1-Y_{m4})f_{m4}$</td>
<td>$-\sum_{i=12,14,28}(1-Y_{c4})N_{biom}$</td>
<td>$k_{u8,1}S_{m1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Uptake of Caproate</td>
<td>$-1$</td>
<td>$(1-Y_{c4})0.625$</td>
<td>$(1-Y_{c4})0.25$</td>
<td>$(1-Y_{c4})0.125$</td>
<td>$(1-Y_{c4})0.0625$</td>
<td>$-\sum_{i=12,14,28}(1-Y_{c4})N_{biom}$</td>
<td>$k_{u9,1}S_{c4}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Uptake of Valerate</td>
<td>$-1$</td>
<td>$(1-Y_{c4})0.54$</td>
<td>$(1-Y_{c4})0.31$</td>
<td>$(1-Y_{c4})0.15$</td>
<td>$(1-Y_{c4})0.075$</td>
<td>$-\sum_{i=12,14,28}(1-Y_{c4})N_{biom}$</td>
<td>$k_{u10,1}S_{c4}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 Uptake of Butyrate</td>
<td>$-1$</td>
<td>$(1-Y_{c4})0.8$</td>
<td>$(1-Y_{c4})0.2$</td>
<td>$(1-Y_{c4})0.1$</td>
<td>$(1-Y_{c4})0.05$</td>
<td>$-\sum_{i=12,14,28}(1-Y_{c4})N_{biom}$</td>
<td>$k_{u11,1}S_{c4}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 Uptake of Propionate</td>
<td>$-1$</td>
<td>$(1-Y_{c4})0.57$</td>
<td>$(1-Y_{c4})0.33$</td>
<td>$(1-Y_{c4})0.1$</td>
<td>$(1-Y_{c4})0.05$</td>
<td>$-\sum_{i=12,14,28}(1-Y_{c4})N_{biom}$</td>
<td>$k_{u12,1}S_{c4}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 Uptake of Acetate</td>
<td>$-1$</td>
<td>$(1-Y_{c4})0.9$</td>
<td>$(1-Y_{c4})0.3$</td>
<td>$(1-Y_{c4})0.1$</td>
<td>$(1-Y_{c4})0.03$</td>
<td>$-\sum_{i=12,14,28}(1-Y_{c4})N_{biom}$</td>
<td>$k_{u13,1}S_{c4}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 Uptake of Hydrogen</td>
<td>$-1$</td>
<td>$(1-Y_{c4})0.9$</td>
<td>$(1-Y_{c4})0.3$</td>
<td>$(1-Y_{c4})0.1$</td>
<td>$(1-Y_{c4})0.03$</td>
<td>$-\sum_{i=12,14,28}(1-Y_{c4})N_{biom}$</td>
<td>$k_{u14,1}S_{c4}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 Decay of $X_m$</td>
<td>$k_{d15}X_m$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 Decay of $X_m$</td>
<td>$k_{d16}X_m$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 Decay of $X_m$</td>
<td>$k_{d17}X_m$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 Decay of $X_{12}$</td>
<td>$k_{d18}X_{12}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 Decay of $X_a$</td>
<td>$k_{d19}X_a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 Decay of $X_{14}$</td>
<td>$k_{d20}X_{14}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 Decay of $X_{12}$</td>
<td>$k_{d21}X_{12}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 11-1: Biochemical rate coefficients ($v_{ij}$) and kinetic rate equations ($\rho_i$) for particulate components ($i=16-28; j=1-22$)

Chapter 11 ADM1-based modeling of methane production in a two-stage system
### Table 11-1: Biochemical rate coefficients ($v_{ij}$) and kinetic rate equations ($\rho_j$) for particulate components ($i=16-28$; $j=1-22$).

<table>
<thead>
<tr>
<th>Process</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
<th>26</th>
<th>27</th>
<th>28</th>
<th>Rate ($\rho_j$, kg COD m$^{-3}$d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disintegration</td>
<td>$X_i$</td>
<td>$X_{16}$</td>
<td>$X_{17}$</td>
<td>$X_{18}$</td>
<td>$X_{19}$</td>
<td>$X_{20}$</td>
<td>$X_{21}$</td>
<td>$X_{22}$</td>
<td>$X_{23}$</td>
<td>$X_{24}$</td>
<td>$X_{25}$</td>
<td>$X_{26}$</td>
<td>$X_{27}$</td>
<td>$X_{28}$</td>
</tr>
<tr>
<td>Hydrolysis of Carbohydrates</td>
<td>-1</td>
<td>$f_{13,3C}$</td>
<td>$f_{14,3C}$</td>
<td>$f_{15,3C}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrolysis of Proteins</td>
<td>-1</td>
<td>$k_{3,14}X_i^C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrolysis of Lipids</td>
<td>-1</td>
<td>$k_{3,15}X_i^C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uptake of Sugars</td>
<td>Ym</td>
<td>$k_{3,16}X_i^C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uptake of Amino Acids</td>
<td>Ym</td>
<td>$k_{3,17}X_i^C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uptake of LCFA</td>
<td>Ym</td>
<td>$k_{3,18}X_i^C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uptake of Lactate</td>
<td>Ym</td>
<td>$k_{3,19}X_i^C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uptake of Caproate</td>
<td>Ym</td>
<td>$k_{3,20}X_i^C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uptake of Valerate</td>
<td>Ym</td>
<td>$k_{3,21}X_i^C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uptake of Butyrate</td>
<td>Ym</td>
<td>$k_{3,22}X_i^C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uptake of Acetate</td>
<td>Ym</td>
<td>$k_{3,23}X_i^C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uptake of Hydrogen</td>
<td>Ym</td>
<td>$k_{3,24}X_i^C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decay of $X_{24}$</td>
<td>1</td>
<td>-1</td>
<td>$k_{3,25}X_i^C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decay of $X_{25}$</td>
<td>1</td>
<td>-1</td>
<td>$k_{3,26}X_i^C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decay of $X_{26}$</td>
<td>1</td>
<td>-1</td>
<td>$k_{3,27}X_i^C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decay of $X_{27}$</td>
<td>1</td>
<td>-1</td>
<td>$k_{3,28}X_i^C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Composites (kg COD m$^{-3}$)**
- Carbohydrates
- Proteins
- Lipids
- Sugar degraders
- Amino acid degraders
- LCFA degraders
- Propionate degraders
- Aceto degraders
- H2 degraders
- Particulate inerts (kg mole C m$^{-3}$)

**Inhibition factors**
- $I_1 = I_{1,15}I_{1,16}$
- $I_2 = I_{1,17}I_{1,18}$
- $I_3 = I_{1,21}I_{1,22}$

**Notes:**
- The table includes the rate coefficients and kinetic rate equations for various biochemical processes.
- The processes include disintegration, hydrolysis of carbohydrates, proteins, and lipids, uptake of sugars, amino acids, LCFA, lactate, caproate, valerate, butyrate, acetate, and hydrogen.
- Decay rates for specific components are also included.

**References:**
- ADM1-based modeling of methane production in a two-stage system | Chapter 11

<table>
<thead>
<tr>
<th>Component</th>
<th>Composites</th>
<th>Carbohydrates</th>
<th>Proteins</th>
<th>Lipids</th>
<th>Sugar degraders</th>
<th>Amino acid degraders</th>
<th>LCFA degraders</th>
<th>Propionate degraders</th>
<th>Aceto degraders</th>
<th>H2 degraders</th>
<th>Particulate inerts</th>
<th>Inhibition factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_i$</td>
<td>(kg COD m$^{-3}$)</td>
<td>(kg COD m$^{-3}$)</td>
<td>(kg COD m$^{-3}$)</td>
<td>(kg COD m$^{-3}$)</td>
<td>(kg COD m$^{-3}$)</td>
<td>(kg COD m$^{-3}$)</td>
<td>(kg COD m$^{-3}$)</td>
<td>(kg COD m$^{-3}$)</td>
<td>(kg COD m$^{-3}$)</td>
<td>(kg COD m$^{-3}$)</td>
<td>(kg mole C m$^{-3}$)</td>
<td>$I_{1,15}I_{1,16}$, $I_{1,17}I_{1,18}$, $I_{1,21}I_{1,22}$</td>
</tr>
</tbody>
</table>
Inhibition kinetics were comprehensively reviewed by Batstone et al. (2002). The general expressions for inhibition terms can be found in Tables 11.1 and 11.2 but the individual inhibitions are shown in Table 11.3. For the pH inhibition, $pH_{UL}$ is the point at which the organisms are not inhibited and $pH_{LL}$ is the point at which inhibition is complete. pH inhibition is used for all intracellular processes in the ADM1 ($I_{pH}$), with different parameters for acetogens and acidogens ($I_{pH,ac}$), hydrogen-utilising methanogens ($I_{pH,h2}$) and aceticlastic methanogens ($I_{pH,meth}$). Batstone et al. (2002) proposed the recommended values for these parameters and they also reported the suggested values of inhibition constants ($K_S$ and $K_I$) (see Table 6.2).

<table>
<thead>
<tr>
<th>Term</th>
<th>Expression</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{pH}$</td>
<td>$I = \exp\left(-3 \left(\frac{pH - pH_{UL}}{pH_{UL} - pH_{LL}}\right)^2\right)$</td>
<td>pH inhibition</td>
</tr>
<tr>
<td></td>
<td>$else I = 1 if \ pH &gt; pH_{UL}$</td>
<td>(when low pH inhibition occurs)</td>
</tr>
<tr>
<td>$I_{IN,lim}$</td>
<td>$1/\left(1 + K_{S,N,IN} / S_{IN}\right)$</td>
<td>Lack of inorganic nitrogen</td>
</tr>
<tr>
<td>$I_{NH3,Xac}$</td>
<td>$1/\left(1 + S_{NH3} / K_{I,NH3}\right)$</td>
<td>Inhibition of NH$_3$ in acetate degradation</td>
</tr>
<tr>
<td>$I_{h2,fa}$</td>
<td>$1/\left(1 + S_{h2} / K_{I,h2,fa}\right)$</td>
<td>Inhibition of H$_2$ in fetties degradation</td>
</tr>
<tr>
<td>$I_{h2,c4}$</td>
<td>$1/\left(1 + S_{h2} / K_{I,h2,c4}\right)$</td>
<td>Inhibition of H$_2$ in c4 degradation</td>
</tr>
<tr>
<td>$I_{h2,pro}$</td>
<td>$1/\left(1 + S_{h2} / K_{I,h2,pro}\right)$</td>
<td>Inhibition of H$_2$ in propionate degradation</td>
</tr>
</tbody>
</table>

The structure of the model was modified in order to make the model more reliable for describing the methane production process from agro-industrial wastes and sweet sorghum stalks. Lactate and ethanol are excluded from the ADM1 model mainly due to their low impact to the overall anaerobic digestion process and low to medium loaded systems. However, these metabolites were detected as by-products of biohydrogen production process in our experiments tested. Especially lactate was detected in high concentrations as a significant intermediate product in many biohydrogen experiments. Moreover, caproate was detected in high concentration in the effluent of the acidogenic stage. Thus, their inclusion in the model was considered necessary.

The inclusion of lactate, ethanol and caproate in the model was achieved by the addition of four extra state variables accounting for soluble lactate ($S_{lac}$), ethanol ($S_{eth}$), caproate ($S_{cap}$) and lactate degrading organisms ($X_{lac}$), respectively. Caproate was assumed to be consumed from the same group of acetogenic microorganisms ($X_{c4}$) that
degrade also valerate and butyrate with maximum specific uptake rate $k_{m,c4}$. On the other hand, ethanol concentration in our experiments was negligible thus it was assumed only as a product of sugars’ fermentation. Furthermore, three equilibrium equations were included in the ADM1 to model acid-base equilibrium due to lactate, ethanol and caproate contributions (caproate’s pKa = 4.88, lactate is a strong acid, pKa = 3.86 while ethanol is a weak base, pKa = 15.9). In addition, a dynamic equation for the decay of lactate degrading microorganisms (first-order rate equation) was added.

According to the experimental results obtained previously (see Section 7.4.2, Section 8.3.3), products from the fermentation of sugars contained in mixtures of agro-industrial wastes and sweet sorghum included lactate, acetate and ethanol. The stoichiometric reactions for the production of lactate and ethanol are described by the reactions i.e. the homofermentative (Eq. 11.1), the heterofermentative (Eq. 11.2) and the bifidum pathway (Eq. 11.3).

\[
\begin{align*}
C_6H_{12}O_6 & \rightarrow 2CH_3CH(OH)COOH \quad (11.1) \\
C_6H_{12}O_6 & \rightarrow CH_3CH(OH)COOH + CH_3CH_2OH + CO_2 \quad (11.2) \\
2C_6H_{12}O_6 & \rightarrow 3CH_3COOH + 2CH_3CH(OH)COOH \quad (11.3)
\end{align*}
\]

The fraction of monosaccharide which is degraded via the first, second and third reaction of the above equations can be expressed as $n_{1,su}$, $n_{2,su}$ and $n_{3,su}$ respectively. Based on ADM1, the sum of aforementioned coefficients could be equal to one (Eq. 11.4). The stoichiometric coefficients of lactate, acetate and ethanol were calculated as a function of these fractions and presented in Table 11.4. Thus, the model was modified also in terms of sugars uptake ($j=5$; Tables 11.1, 11.2) based on new stoichiometric coefficients.

\[
n_{3,su} = 1 - n_{2,su} - n_{1,su} \quad (11.4)
\]

<table>
<thead>
<tr>
<th>Products of glucose degradation</th>
<th>Stoichiometric coefficients, kg COD/kg COD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>$f_{\text{lac,su}} = 0.5 \left(1 + n_{1,su}\right)$</td>
</tr>
<tr>
<td>Acetate</td>
<td>$f_{\text{ac,su}} = 0.5 \left(1 - n_{1,su} + n_{2,su}\right)$</td>
</tr>
<tr>
<td>Ethanol</td>
<td>$f_{\text{eth,su}} = 0.5 \ n_{2,su}$</td>
</tr>
</tbody>
</table>
Experimental data obtained in previous work (Section 7.4, Section 8.3.3) showed that lactate concentration decreased during the fermentation, implying that lactate was an intermediate product. Lactate can be degraded mainly to butyrate, acetate, propionate and hydrogen according to the following reactions:

$$3\text{CH}_3\text{CH(OH)COOH} \rightarrow 2\text{CH}_3\text{CH}_2\text{COOH} + \text{CH}_3\text{COOH} + \text{CO}_2 + \text{H}_2\text{O} \quad (11.5)$$

$$2\text{CH}_3\text{CH(OH)COOH} \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{CO}_2 + 2\text{H}_2 \quad (11.6)$$

The fraction of lactate which is degraded via the first and second reaction of the above equations can be expressed as $n_{1,lac}$ and $n_{2,lac}$ respectively. The sum of these coefficients could also be equal to one. Table 11.5 presents the stoichiometric coefficient of each product of lactate degradation. On the other hand, the stoichiometric coefficients of amino acids and composites degradation used in the model were suggested by Batstone et al. (2002), according to Table 6.1.

### Table 11-4 : Stoichiometric coefficients from lactate uptake.

<table>
<thead>
<tr>
<th>Products of lactate degradation</th>
<th>Stoichiometric coefficients, kg COD/kg COD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrate</td>
<td>$f_{bu,lac} = 0.83 \ (1 - n_{1,lac})$</td>
</tr>
<tr>
<td>Acetate</td>
<td>$f_{ac,lac} = 0.22 \ n_{1,lac}$</td>
</tr>
<tr>
<td>Propionate</td>
<td>$f_{pro,lac} = 0.78 \ n_{1,lac}$</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>$f_{h2,lac} = 0.17 \ (1 - n_{1,lac})$</td>
</tr>
</tbody>
</table>

Lactate uptake rate (Eq. 11.7) was modeled according to Monod-type kinetics ($j=8$; Tables 11.1, 11.2), including inhibition factors to account for pH inhibition and limitation of microbial growth due to the lack of inorganic nitrogen:

$$\text{Lactate uptake rate} : k_{m,lac} \frac{S_{lac}}{k_{s,lac} + S_{lac}} \cdot X_{lac} \cdot I_{\text{pH,ac}} \cdot I_{\text{IN,lim}} \quad (11.7)$$

where $k_{m,lac}$ is the Monod maximum specific rate (kg COS$_S$/kg COD$_x$·d), $k_{s,lac}$ is the half saturation value (kg COD$_S$/m$^3$), $S_{lac}$ is the concentration of lactate (kg COD/m$^3$), $X_{lac}$ is the concentration of lactate degrading microorganisms (kg COD/m$^3$), $I_{\text{pH,ac}}$ is the
inhibition factor for pH for acidogens/acetogens microorganisms and $I_{IN,lim}$ is the factor concerning the limitation of growth due to the lack of inorganic nitrogen. The parameter values of lactate uptake were chosen to be the same with sugar’s values according to Table 6.2 by Batstone et al. (2002).

As mentioned previously caproate was detected in high concentration in the effluent of the acidogenic stage and thus in the influent of the methanogenic stage. For this reason, an extra process describing the degradation of caproate was included into the model (j=9; Tables 11.1, 11.2). Caproate uptake rate (Eq. 11.8) was modeled according to Monod-type kinetics, including inhibition factors to account for pH inhibition, limitation of microbial growth due to the lack of inorganic nitrogen and hydrogen inhibition and also including a competitive uptake which refers to c4 microorganisms. With the addition of caproate in the model, modifications of valerate and butyrate uptake rates were occurred in terms of competitive uptake (j=10 and 11; Tables 11.1, 11.2).

\[
\text{Caproate uptake rate} : \frac{S_{cap}}{k_{s,cap} + S_{cap}} X_{c4} \frac{1}{1 + \frac{S_{bu} + S_{va}}{S_{cap}}} I_{pH,ac} I_{IN,lim} I_{h2,c4} (11.8)
\]

\[
\text{Valerate uptake rate} : \frac{S_{va}}{k_{s,va} + S_{va}} X_{c4} \frac{1}{1 + \frac{S_{bu} + S_{cap}}{S_{va}}} I_{pH,ac} I_{IN,lim} I_{h2,c4} (11.9)
\]

\[
\text{Butyrate uptake rate} : \frac{S_{bu}}{k_{s,bu} + S_{bu}} X_{c4} \frac{1}{1 + \frac{S_{va} + S_{cap}}{S_{bu}}} I_{pH,ac} I_{IN,lim} I_{h2,c4} (11.10)
\]
11.4 Modeling of methane production of raw LCM

The experiment was carried out in a single-stage CSTR system as described in Section 6.4.1. The influent of the methanogenic reactor was liquid cow manure (LCM). The reactor was operated for the first 65 days at HRT 16 d, whereas then the HRT was increased at 20 d. Although the LCM was consisted of low carbohydrates content, it is characterized by high proteins content and also high concentration of VFAs. The feed characteristics varied and the respective variations were taken into account.

Thus, the modified ADM1 was used to predict the performance of the methanogenic bioreactor using as input the raw LCM. The suggested biochemical parameter values given in Table 6.2 of the Scientific and Technical Report of ADM1 (Batstone et al., 2002) were set as initial values of the model used. Parameter estimation was achieved only in the maximum specific uptake rate of propionate (km,pro) in order to simulate the evolution of propionate in the reactor. The model estimated value was km,pro = 1.97 kg COD/kg COD·d and the respective proposed by Batstone et al. (2002) was km,pro = 13 kg COD/kg COD·d. The other maximum specific uptake rates of acetate and butyrate were kept as suggested by Batstone et al. (2002) and equal to km,ac = 8 kg COD/kg COD·d and km,c4 = 20 kg COD/kg COD·d, respectively. The low km,pro, obtained by parameter estimation, was due to microbial population’s inability to consume the propionic acid which was existed in the raw material.

The ADM1 simulation of the biogas and methane production rates, pH, main volatile fatty acids and COD against the respective experimental data of methanogenic reactor is shown in Fig. 11.2 and 11.3. A good agreement between simulation results and measurements was achieved. A good prediction of the measured biogas production is an indicator for a realistic influent characterization. However, the predicted pH value was a bit underestimated from the model.

![Figure 11-2: Experimental and model prediction of (a) biogas and methane production and (b) pH value during the methanogenesis of LCM.](image-url)
Modeling of methane production from different acidified effluents

11.5.1 Acidified mixture of 55%OMW, 40%CW and 5%LCM

The experiment was carried out in a two-stage CSTR system as described in Section 5.4. The substrate treated in this scenario was a mixture of olive mill wastewater (OMW), cheese whey (CW) and liquid cow manure (LCM) at a ratio of 55:40:5 (v/v/v). The methanogenic reactor (2\textsuperscript{nd} stage) was operated at HRT 16\textsuperscript{d} and was fed with acidified effluent of the 1\textsuperscript{st} stage of acidogenesis, which was consisted of soluble components like VFAs, mainly acetate. The concentration of soluble sugars, and acetate contained in the feed was determined in terms of COD. The concentration of VFAs in the feed varied and the respective variations were taken into account.

Thus, the modified ADM1 was used to predict the performance of the methanogenic bioreactor using as input the effluent characteristics of acidogenic reactor. The kinetic parameters were considered fixed since they are generally known to have limited variability in anaerobic systems (Batstone et al., 2002). Nevertheless, in order to achieve the best agreement between measured and simulated values, some kinetic parameters had to be adjusted. The parameters was chosen to adjust were the maximum specific uptake rates of acetate ($k_{m, ac}$) and propionate ($k_{m, pro}$) and also the coefficients of sugar and lactate degradation ($n_{1, su}$, $n_{2, su}$, $n_{3, su}$, $n_{1, lac}$ and $n_{2, lac}$) for this experiment. Table 11.6 presents the new estimated coefficients obtained from parameter estimation.

The values of $k_m$ for acetate and propionate were estimated from the respective experiment using non linear parameter estimation while the other model parameters
were kept as suggested in the scientific and technical report of ADM1 (Batstone et al., 2002). Thus the maximum specific uptake rate of acetate ($k_{m,ac}$) was determined, using the following standard parameter values: the yield of biomass on acetate, $Y_{ac} = 0.05$ kg COD/kg COD, half saturation value, $k_{s,ac} = 0.15$ kg COD/m$^3$ and the first order decay rate for the acetate degrading microorganisms, $k_{dec,ac} = 0.02$ d$^{-1}$. The model estimated value was $k_{m,ac} = 3.34$ kg COD/kg COD·d and the respective proposed by Batstone et al. (2002) was $k_{m,ac} = 8$ kg COD/kg COD·d.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Estimated value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n_1, su$</td>
<td>0.7409</td>
</tr>
<tr>
<td>$n_2, su$</td>
<td>0</td>
</tr>
<tr>
<td>$n_3, su$</td>
<td>0.2591</td>
</tr>
<tr>
<td>$n_1, lac$</td>
<td>0.7086</td>
</tr>
<tr>
<td>$n_2, lac$</td>
<td>0.2914</td>
</tr>
</tbody>
</table>

The maximum specific uptake rate of propionate ($k_{m,pro}$) was determined, using the following standard parameter values reported by Batstone et al. (2002) ($Y_{pro} = 0.04$ kg COD/kg COD, half saturation value, $k_{s,pro} = 0.1$ kg COD/m$^3$ and the first order decay rate for the propionate degrading microorganisms, $k_{dec, pro} = 0.02$ d$^{-1}$). As you can see in Fig. 11.3(a) the propionate was degraded more slowly than the acetate. This fact confirmed the experimental results of Wiegant et al. (1986) according which propionate is the most slowly stabilized intermediate in anaerobic systems. The model parameter estimation led to a $k_{m,pro} = 2.57$ kg COD/kg COD·d, whereas the respective proposed by Batstone et al. (2002) was $k_{m,pro} = 13$ kg COD/kg COD·d.

The values estimated for acetate and propionate were similar with those reported in Dereli et al. (2010) ($k_{m,ac} = 4$ kg COD/kg COD·d) and Koutrouli et al. (2009) ($k_{m,pro} = 2$ kg COD/kg COD·d).

The ADM1 simulation of the biogas and methane production rates, pH, main volatile fatty acids and COD against the respective experimental data of methanogenic reactor is shown in Fig. 11.4 and 11.5. The model was able to satisfactorily predict the experimental data, especially the evolution of acetate and propionate over the course of experiment. The biogas and methane production rates were slightly underestimated from the model.
Figure 11-4: Experimental and model prediction of (a) biogas and methane production and (b) pH value during the methanogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v).

Figure 11-5: Experimental and model predicted (a) main volatile fatty acids concentrations and (b) soluble and particulate COD during the methanogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v).

11.5.2 Acidified mixture of 90%CW and 10%LCM

The experiment was also carried out in a two-stage CSTR system as described in Section 5.5. The substrate treated in this scenario was a mixture of cheese whey (CW) and liquid cow manure (LCM) at a ratio of 90:10 (v/v). The methanogenic reactor, which was fed with acidified effluent of the 1st stage of acidogenesis, was operated at HRT of 16 days. The acidified effluent was characterized by high content of soluble
components like VFAs, mainly acetate. The concentration of soluble sugars, and acetate contained in the feed was determined in terms of COD. The concentration of VFAs in the feed varied and the respective variations were taken into account.

Thus, the modified ADM1 was used to predict the performance of the methanogenic bioreactor using as input the effluent characteristics of acidogenic reactor. The suggested biochemical parameter values given in Table 6.2 of the Scientific and Technical Report of ADM1 (Batstone et al., 2002) were set as initial values of the model used. To achieve the best agreement between measured and simulated values, kinetic parameters had to be adjusted. The parameters was chosen to adjust were the maximum specific uptake rates of acetate ($k_{m,ac}$) and propionate ($k_{m,pro}$) and also the coefficients of sugar and lactate degradation ($n_{1,su}$, $n_{2,su}$, $n_{3,su}$, $n_{1,lac}$ and $n_{2,lac}$) for this experiment. Table 11.7 presents the new estimated coefficients obtained from parameter estimation. In particular, the fractions of lactate degradation was very different compared to previous experiment, may be due to different microbial populations in the system.

### Table 11-6 : Estimated coefficients for sugars and lactate degradation treating the acidified mixture of 90%CW and 10%LCM

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Estimated value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n_{1,su}$</td>
<td>0</td>
</tr>
<tr>
<td>$n_{2,su}$</td>
<td>0</td>
</tr>
<tr>
<td>$n_{3,su}$</td>
<td>1</td>
</tr>
<tr>
<td>$n_{1,lac}$</td>
<td>0.1371</td>
</tr>
<tr>
<td>$n_{2,lac}$</td>
<td>0.8629</td>
</tr>
</tbody>
</table>

The values of $k_m$ for acetate and propionate were estimated from the respective experiment using non linear parameter estimation while the other model parameters were kept as suggested in the scientific and technical report of ADM1 (Batstone et al., 2002). Thus the maximum specific uptake rate of acetate ($k_{m,ac}$) was determined, using the following standard parameter values: the yield of biomass on acetate, $Y_{ac} = 0.05$ kg COD/kg COD, half saturation value, $k_{s,ac} = 0.15$ kg COD/m$^3$ and the first order decay rate for the acetate degrading microorganisms, $k_{dec,ac} = 0.02$ d$^{-1}$. The model estimated value was $k_{m,ac} = 7.54$ kg COD/kg COD·d, similar to proposed one (8 kg COD/kg COD·d) by Batstone et al. (2002). The value estimated for acetate was similar with those reported in Koutrouli et al. (2009) who reported $k_{m,ac}$ equal to 8.34 kg COD/kg COD·d, treating acidified olive pulp from a two-stage system.

The maximum specific uptake rate of propionate ($k_{m,pro}$) was also determined, using the following standard parameter values reported by Batstone et al. (2002) ($Y_{pro} = 0.04$ kg COD/kg COD, half saturation value, $k_{s,pro} = 0.1$ kg COD/m$^3$ and the
first order decay rate for the propionate degrading microorganisms, $k_{\text{dec, pro}} = 0.02 \text{ d}^{-1}$). The model parameter estimation led to a $k_{\text{m, pro}} = 3.31 \text{ kg COD/kg COD·d}$, whereas the respective proposed by Batstone et al. (2002) was $k_{\text{m, pro}} = 13 \text{ kg COD/kg COD·d}$.

Fig. 11.6(a) shows the comparison between simulation results and measurements for biogas and methane production, whereas in Fig. 11.6(b) is presented the model prediction and the experimental data of pH. The methane curve revealed discrepancies between measurements and simulation results. Moreover, the acetate and propionate concentrations were underestimated from the model (Fig. 11.7(a))

![Figure 11-6](image1.png)

![Figure 11-7](image2.png)

**Figure 11-6:** Experimental and model prediction of (a) biogas and methane production and (b) pH value during the methanogenesis of CW/LCM mixture (90/10, v/v).

**Figure 11-7:** Experimental and model predicted (a) main volatile fatty acids concentrations and (b) soluble and particulate COD during the methanogenesis of CW/LCM mixture (90/10, v/v).
11.5.3 Acidified mixture of 20%OMW and 80%LCM

The experiment was carried out in a two-stage CSTR system (as described in Section 5.7) treating a mixture of 20% OMW and 80% LCM. The methanogenic, operated at HRT of 16 d, was fed with the acidified effluent of the acidogenic reactor. The acidified effluent was rich in soluble components such as VFAs (mainly acetate), soluble sugars etc. After determining the concentration of soluble components in terms of COD, these values were inserted to the model in order to predict the performance of the methanogenic reactor.

Firstly, as initial values of the modified model, the suggested biochemical parameter values given in Table 6.2 of the Scientific and Technical Report of ADM1 (Batstone et al., 2002) were used. In order to achieve better agreements between measured and simulated values, parameter estimation (non linear) was occurred. The parameters was chosen to adjust were the maximum specific uptake rates of acetate \( k_{m,ac} \) and propionate \( k_{m,pro} \) and also the coefficients of sugar and lactate degradation \( n_1, su, n_2, su, n_3, su, n_1, lac, \) and \( n_2, lac \) for this experiment. As you can see in Table 11.8 the new estimated coefficients obtained from parameter estimation were presented.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Estimated value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n_{1, su} )</td>
<td>0.004</td>
</tr>
<tr>
<td>( n_{2, su} )</td>
<td>0.533</td>
</tr>
<tr>
<td>( n_{3, su} )</td>
<td>0.463</td>
</tr>
<tr>
<td>( n_{1, lac} )</td>
<td>0.588</td>
</tr>
<tr>
<td>( n_{2, lac} )</td>
<td>0.412</td>
</tr>
</tbody>
</table>

The maximum specific uptake rate of acetate \( k_{m,ac} \) was determined, using the following standard parameter values: the yield of biomass on acetate, \( Y_{ac} = 0.05 \) kg COD/kg COD, half saturation value, \( k_{s,ac} = 0.15 \) kg COD/m\(^3\) and the first order decay rate for the acetate degrading microorganisms, \( k_{dec,ac} = 0.02 \) d\(^{-1}\). The model estimated value was \( k_{m,ac} = 11.01 \) kg COD/kg COD·d, higher than proposed one (8 kg COD/kg COD·d) by Batstone et al. (2002). On the other hand, the maximum specific uptake rate of propionate \( k_{m,pro} \) was also determined, equal to \( k_{m,pro} = 3.55 \) kg COD/kg COD·d, whereas the respective proposed by Batstone et al. (2002) was \( k_{m,pro} = 13 \) kg COD/kg COD·d.

Simulated curves for biogas and methane production rate are presented in Fig. 11.8(a). A satisfying prediction was obtained between simulation results and measurements for biogas and methane production, especially the first days of operation.
Then, slight discrepancies can be observed between simulated and experimental results, whereas the biogas and methane peaks at 40\textsuperscript{th} day of operation could not be reproduced by the model. Fig. 11.8(b) illustrates the model prediction and the experimental data of pH value, which is overestimated by the model. Moreover, VFAs were fluctuated in low concentrations during the course of experiment (Fig. 11.9(a)), whilst a good simulation was achieved in soluble and particulate COD (Fig. 11.9(b)).

![Figure 11-8: Experimental and model prediction of (a) biogas and methane production and (b) pH value during the methanogenesis of OMW/LCM mixture (20/80, v/v).](image)

![Figure 11-9: Experimental and model predicted (a) main volatile fatty acids concentrations and (b) soluble and particulate COD during the methanogenesis of OMW/LCM mixture (20/80, v/v).](image)
11.5.4 Acidified mixture of 55%ES3 stalks, 40%CW and 5%LCM

Modeling was used to predict the effect of HRT on methane production in a mesophilic CSTR fed with 55% ES3, 40% CW and 5% LCM (Section 8.4.3). The methanogenic, operated at three different HRTs of 24, 16 and 12 d, was fed with the acidified effluent of the acidogenic reactor. The acidified effluent was rich in soluble components such as VFAs, lactic acid etc. The concentration of soluble products contained in the feed was determined in terms of COD. The concentration of VFAs and lactic acid in the feed varied and the respective variations were taken into account.

Many parameters, principally those with low sensitivity on model outputs, have been applied without any optimization. Hydrolysis constants for carbohydrates, proteins and lipids are also among those parameters that have not been optimized. Thus, the suggested biochemical parameter values given in Table 6.2 of the Scientific and Technical Report of ADM1 (Batstone et al., 2002) were used. In order to achieve better agreements between measured and simulated values, non linear parameter estimation was occurred. In particular, the parameters was chosen to adjust were the maximum specific uptake rates of acetate (km,ac), propionate (km,pro) and butyrate (km,c4). The coefficients for sugar and lactate degradation were selected using mass balance calculations, obtained by acidogenic batch experiment (Section 8.4.3) and considering that the microbial population cannot change dramatically. Table 11.9 presents the selected coefficients obtained from mass balance calculations.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Selected value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n1,su</td>
<td>0.074</td>
</tr>
<tr>
<td>n2,su</td>
<td>0.099</td>
</tr>
<tr>
<td>n3,su</td>
<td>0.827</td>
</tr>
<tr>
<td>n1,lac</td>
<td>0.444</td>
</tr>
<tr>
<td>n2,lac</td>
<td>0.556</td>
</tr>
</tbody>
</table>

The maximum specific uptake rates of acetate, propionate and butyrate were determined via the parameter estimation and using the standard parameter values proposed by Batstone et al. (2002). The model estimated values were presented in Table 11.10, which were lower than ones suggested by the Scientific and Technical Report of ADM1.
Table 11-9: Estimated maximum specific uptake rates treating the acidified mixture of 55%ES3, 40%CW and 5%LCM

<table>
<thead>
<tr>
<th>Maximum Specific Uptake Rate</th>
<th>Estimated value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{m,ac}$</td>
<td>2.50</td>
</tr>
<tr>
<td>$k_{m,pro}$</td>
<td>2.55</td>
</tr>
<tr>
<td>$k_{m,c4}$</td>
<td>2.50</td>
</tr>
</tbody>
</table>

As can be seen in Figs. 11.10 and 11.11, the correlation between experimental and simulation data, using the acidified mixture of 55%ES3, 40%CW and 5%LCM, is very good. In particular, the simulation data predicted more methane production than the experimental data (Figs. 11.10(a)). This could be due to some kind of inhibition occurring inside the methanogenic reactor that the model cannot totally predict, like slowly degradation of lignin. Leaving this fact aside, the correlation between model and experimental values is satisfactory considering the slopes of the profiles. Even if there is a discrepancy between the curves, the shapes of the curves do not vary very much. These variations are mainly attributed to variations in the proportion of biodegradable COD, which can be mainly attributed to temporal variations in the mixture biodegradability. Moreover, the model predicts the decline of the biogas production as a response of the load increase. From Fig. 11.10(b) it can be seen that the pH underestimated, especially after the second HRT tested, mainly due to higher estimated acetate concentration (Fig. 11.11(a)).

The comparison of model outputs and experimental data for the VFAs production is exemplary shown in Fig. 11.11(a). Particularly, the VFAs production in the first process stage is well predicted by the model. Then an accumulation of acetate was predicted at HRT of 16 d which indicates the difficulty to accurately predict the behavior under high loading conditions. Finally, Fig. 11.11(b) illustrates the soluble COD correlation between simulation and experimental data. The overestimation at HRT 16 d was a result of the estimated concentration of VFAs (mainly acetate). Girault et al. (2012) studied the model prediction of methane production in a mesophilic CSTR fed with waste activated sludge and pig slurry and found a perfect simulation.
Figure 11-10: Experimental and model prediction of (a) biogas and methane production and (b) pH value during the methanogenesis of ES3/CW/LCM mixture (55/40/5, v/v/v).

Figure 11-11: Experimental and model predicted (a) main volatile fatty acids concentrations and (b) soluble COD during the methanogenesis of ES3/CW/LCM mixture (55/40/5, v/v/v).
11.6 Conclusions

The Anaerobic Digestion Model No. 1 (ADM1) was used to simulate the biochemical conversion of the process and to calculate biogas production and composition. In order to facilitate model application, an approach for a detailed inflow characterization of the substrate used and modifications of ADM1 were suggested. The present study focused on the anaerobic methane production from raw liquid cow manure or acidified mixtures of agro-industrial wastes and sweet sorghum that were the effluents of hydrogen producing bioreactors. The structure of the model was modified to include lactate, caproate and ethanol among the metabolites produced in the acidogenic stage.

The obtained results indicate that the ADM1 is capable of simulating biogas production from such substrates, after precise characterization of the substrates and adjustment of the kinetic constants. In fact, gas flow and methane were predicted quite well at steady-state periods, whereas the correlation between model and experimental values was satisfactory considering the slopes of the profiles.

Therefore, the ADM1 could be a valuable tool for process design even in the case of a two-stage anaerobic process, whereas further modifications could further improve the predictions for the methane production. In addition, research will focus on broadening the database and testing the transferability to industrial-sized biogas plants.

11.7 References


Chapter 11  ADM1-based modeling of methane production in a two-stage system


12.1 Abstract

The main objective of this study was to evaluate the exploitation of digestate (effluent) from an anaerobic methanogenic reactor treating mixtures of agro-wastes. Coagulation/flocculation process is considered as a nonexpensive and effective method to reduce organic and inorganic content of agro-industrial wastewaters. A COD removal of 52.53% was observed using poly-(diallyldimethylammonium chloride) with concentration 1.5 g/L. The remaining organic compounds, in supernatant phase, were further treated with a combined ultrafiltration/nanofiltration system and further COD reduction was obtained. The final permeate could be used for diluting the waste prior to anaerobic digestion or for irrigation purposes. On the other hand, the concentrates were enriched in total organic and solid content and can be mixed with the solid fractions from the previous step of coagulation/flocculation for further conversion through composting. Moreover, study was conducted in order to evaluate the sludge transformation using earthworms (vermicomposting) directly to compost, in relation to sludge quality of agroindustrial wastes (cow manure, anaerobic sludge mixtures) and to investigate the effects of different sludge composition on compost physico-chemical properties. In particular, the purpose of this study was to investigate the biostabilization of a) mixed anaerobic sludge (AS1), originating from the effluents of a laboratory anaerobic co-digestion system treating agricultural and agro-industrial waste mixture (olive mill wastewater, cheese whey and liquid cow manure in ratio 55: 40: 5) mixed with solid cow dung (CD) and b) anaerobic sludge (AS2) produced in excess from the Wastewater Treatment Plant (WWTP) of Patras, using the earthworm *Eisenia fetida*. The mixtures with AS2 gave better results in terms of increased N-P-K concentration values compared with mixtures using AS1. Finally, the growth rate of earthworms was higher for the mixture of 85% CD-15% AS2 and 80% CD-20% AS2 with longer duration of vermicomposting.
12.2 Introduction

Anaerobic digestion of organic wastes is clearly encouraged by current regulations in Europe. In complement to the energy supply it represents, this biological treatment process also allows the recycling of organic matter and nutrients contained in biodegradable wastes. Indeed the digestion effluent can be further promoted as soil improver or fertilizer. There are many strategies to treat effluents from anaerobic digesters such as membrane filtration of liquid fraction and composting of solid one. Membrane filtration includes technology such as microfiltration, ultrafiltration, nanofiltration and reverse osmosis, for the fractionation of compounds from liquid solutions (Paraskeva et al., 2007a). On the other hand, composting is the digestion of waste combined with a solid substrate: this substrate can be straw, sesame bark, olive leaves, vineyard leaves, wood chips, animal manure, etc (Kaushik and Garg, 2004).

In particular, membrane filtration has been successfully implemented in the industry, enabling the reuse of large quantities of water, minimizing the expenses of wastewater disposal. Ultrafiltration (UF), nanofiltration (NF) and/or reverse osmosis (RO) are pressure-driven, membrane filtration processes that are used to separate and concentrate macro-molecules and colloids from wastewater. A fluid is placed under pressure on one side of a perforated membrane of a measured pore size. All materials smaller than the measured pore size pass through the membrane, leaving large contaminants concentrated on the feed side of the membrane. Moreover, membrane filtration also leads to the removal of microorganisms from the wastewaters. A possible pre-treatment method, before the membrane filtration, is coagulation/flocculation with the use of polyelectrolytes, which is widely used for the treatment of industrial wastewaters (Zagklis et al., 2012). By administering the appropriate dosage of coagulants and polyelectrolytes the contained chemical oxygen demand (COD) and total solids (TS) can be reduced significantly. Zagklis et al. (2012) have achieved a COD reduction of 93% with the use of poly-(diallyldimethylammonium chloride). After coagulation/flocculation has removed most of the contained COD and TS, any remaining organic content can be removed with the use of membrane filtration.

On the other hand, the anaerobic effluents are rich in nutrients that can valuable to plant growth and soil fertility. Prior to their application in agricultural fields bio-composting of such wastes ensures stabilization of compost avoiding potential risk of pathogens (Ahmad et al., 2007). An innovative discipline of vermiculture biotechnology, the breeding and propagation of earthworms and the use of their castings has become an important tool of waste recycling all over the world. Epigeic worms, e.g. Eisenia foetida, have been used in converting organic wastes (agro waste and domestic refuse) into vermicompost. Many research studies investigate the ability of Eisenia foetida to transform sludges i.e. sewage, paper sludge amended with cow dung into added-value product (Elvira et al., 1998; Kaushik and Garg, 2004; Gupta and Garg, 2008). However limited vermicompost studies examine degradation of anaerobic sludges.
12.3 **A combined coagulation/flocculation and membrane filtration process for the treatment of anaerobic digestion effluent.**

In the present work, the final effluent, obtained from the anaerobic digestion treating a mixture of agro-wastes (Section 8.4.3), was treated further because of the fact that was not fit for recycling or disposal to the environment. The membrane system was used for further purification of the anaerobic effluent. For this, the effluent was collected throughout the operation of the methanogenic digester in a 25-L plastic bin. The first treatment step was the implementation of polyelectrolyte for the coagulation of the anaerobic effluent, whereas the second step of the treatment was the use of membrane filtration, which diminished the organic content of the waste. In collaboration with the laboratory of Transport Phenomena and Physicochemical Hydrodynamics (Associate Professor Paraskeva, C.A.), the selection of polyelectrolyte and the coagulation/flocculation process was obtained.

12.3.1 **Materials**

12.3.1.1 **Waste composition**

The waste used was an anaerobic effluent, which was obtained from an anaerobic methanogenic CSTR digester from a two-stage system operated at steady-state conditions at hydraulic retention time (HRT) of 16 days under mesophilic conditions (37°C). The methanogenic effluent was produced from co-digested mixture of 55% pretreated ensiled sorghum, 40% cheese whey and 5% liquid cow manure (Section 8.4.3). The main characteristics of the effluent are presented in Table 12.1.

| **Table 12-1 : Characteristics of methanogenic effluent.** |
|---|---|---|
| **Parameters** | **Units** | **Methanogenic effluent** |
| pH | - | 7.82 ± 0.01 |
| TSS | g/L | 7.5 ± 0.1 |
| VSS | g/L | 5.8 ± 0.0 |
| TS | g/L | 40.75 ± 0.18 |
| VS | g/L | 10.65 ± 0.03 |
| COD | g/L | 15.44 ± 0.04 |
| ζ potential (mean values) | mV | -18.2 |
| particle size (mean values) | nm | 99.60 (93.9%) |
| | | 679.3 (6.1%) |
12.3.1.2 Polyelectrolyte

The polyelectrolyte used in this study was poly-(diallyldimethylammonium chloride) (P-DADMAC) average $M_w < 100,000$, 35 wt % in H$_2$O, CAS 26062-79-3, supplied by Sigma-Aldrich. The polyelectrolyte was chosen according to Zagklis et al. (2012), who studied series of polyelectrolytes in order to increase the removal percentages of COD before the use of ultrafiltration/reverse osmosis system.

12.3.2 Experimental design

12.3.2.1 Coagulation/Flocculation.

The coagulation/flocculation experiments were carried out in a jar test apparatus, with six beakers of 600 mL in volume (Picture 12.1). Different concentrations of polyelectrolyte P-DADMAC were tested (0.5-4.0 g/L). Initially, 300 mL of waste was placed in each beaker and the addition of coagulant was occurred. The next step was rapid mixing for 3 min (at 250 rpm) for the homogenization of the waste and the neutralization of the suspended particles and then slow mixing for 10 min (at 150 rpm) for agglomeration of the formed flocs. The waste was finally left for its sedimentation and after two hours, samples were collected from the supernatant phase for further analysis.

Picture 12-1: Coagulation/flocculation with the use of poly-(diallyldimethylammonium chloride).
12.3.2.2 Membrane filtration

In the membrane filtration experiment, the supernatant phase of the waste (after coagulation/flocculation using the P-DADMAC polyelectrolyte) was initially treated with an ultrafiltration unit (UF). The ultrafiltration filtrate was then fed to a nanofiltration unit (NF). The UF and NF modules used were both Sartorius Vivaflow 50 (Picture 12.2(a)). Cross-flow filtration was implemented, whereas a pressure indicator was also used. Both units contain an inbuilt polyethersulfone (PES) membrane with 100,000 and 3,000 MWCO for UF and NF, respectively, and an active area of 50 cm². Thin channel flip-flow recirculation path provides high cross flow velocities with minimum pump requirements. Picture 12.2(b) shows the experimental set-up which consists of the membrane filtration module, a peristaltic pump and a pressure indicator. A known amount of initial sample was used, the concentrate returned to initial beaker, whereas the permeate was collected in separate bottles.

![Picture 12-2: (a) Membrane filtration module (1: input, 2: concentrate, 3: permeate and 4: pressure indicator) and (b) lab-scale experimental set-up.](image)

12.3.3 Results and Discussion

First of all, the coagulation/flocculation experiments were performed. Table 12.2 presents the main characteristics of supernatant phase, obtained after the sedimentation, for each concentration of polyelectrolyte used (0.5-4.0 g/L). Fig. 12.1 shows the variation of ζ potential, COD, and TSS with poly-(diallyldimethylammonium chloride) (P-DADMAC) concentration. The best results occurred in experiment with concentration of 1.5 g/L of polyelectrolyte, where the reduction of COD achieved reaches 52.53% and TSS reduction 84.66%. For this reason, we chose the concentration of 1.5 g/L as optimum, for the coagulation/flocculation of the methanogenic effluent.
Table 12-2: Main characteristics of methanogenic effluent after coagulation/flocculation at different polyelectrolyte concentrations.

<table>
<thead>
<tr>
<th>Concentration P-DADMAC (g/L)</th>
<th>COD (g/L)</th>
<th>TSS (g/L)</th>
<th>( \zeta ) potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>12.18 ± 0.20</td>
<td>5.90 ± 0.12</td>
<td>-13.4</td>
</tr>
<tr>
<td>1.0</td>
<td>10.18 ± 0.13</td>
<td>2.50 ± 0.02</td>
<td>-10.4</td>
</tr>
<tr>
<td>1.5</td>
<td>7.33 ± 0.04</td>
<td>1.15 ± 0.07</td>
<td>-9.07</td>
</tr>
<tr>
<td>2.0</td>
<td>8.73 ± 0.09</td>
<td>2.40 ± 0.21</td>
<td>-8.05</td>
</tr>
<tr>
<td>3.0</td>
<td>10.27 ± 0.25</td>
<td>2.35 ± 0.11</td>
<td>-5.68</td>
</tr>
<tr>
<td>5.0</td>
<td>11.22 ± 0.17</td>
<td>2.15 ± 0.04</td>
<td>-2.88</td>
</tr>
</tbody>
</table>

![Zeta Potential, COD, and TSS reduction of supernatant phase in coagulation/flocculation experiments with different concentration of poly-(diallyldimethylammonium chloride).](image)

Figure 12-1: \( \zeta \) potential, COD, and TSS reduction of supernatant phase in coagulation/flocculation experiments with different concentration of poly-(diallyldimethylammonium chloride).

Then an adequate amount of the waste (anaerobic effluent) was treated using the polyelectrolyte (P-DADMAC) at concentration 1.5 g/L for further treatment in membrane filtration system. Picture 12.3(a) presents the six beakers with the waste at sedimentation step, whereas at Picture 12.3(b) the agglomeration of the formed flocs was observed. For extra removal efficiencies, a centrifugation was performed (two times at 4000 rpm for 15 min). As a result, a further decrease of TSS by 34.78% (from 1.15 to 0.75 g/L) was obtained, whereas the COD decreased from 7.33 to 6.02 g/L (17.87%). Turano et al. (2002) proposed an integrated centrifugation–ultrafiltration (UF) system, where in the centrifugation step, most of the suspended solids are removed in order to decrease membrane fouling and to increase the efficiency in the UF. Moreover, Paraskeva et al. (2007b) suggested the utilization of a pretreatment step (filter press) to remove solids and fats prior to the treatment of the filtrate in a membrane system, i.e. ultrafiltration and reverse osmosis.
Although the proposed method reduces significantly the organic content of the waste, the final product is not suited as irrigation water or for disposal to receptors aqueous as the COD is still higher than the approved concentrations (6.02 g/L). For its further treatment membrane filtration was examined. Initially, the waste obtained after the centrifugation, was treated with an ultrafiltration unit (UF). The transmembrane pressure (TMP) was kept at 1 bar. One thing that is of primary importance is that UF provides a “clean” solution appropriate to feed next treatment process (NF). Water and low-molecular weight solutes (MW < 100,000) passed through the UF membrane (permeate), while high-molecular weight solutes and suspended material were retained (rejected) in the concentrate stream of UF (concentrate). The permeate stream of the UF unit was used as feed stream for the NF unit. Physicochemical analysis of the collected compounds in UF and NF, is shown in Table 12.3. UF led to the removal of 36.38% of the organic matter and 7.83% reduction of TS.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Feed UF</th>
<th>Concentrate</th>
<th>Permeate (Feed NF)</th>
<th>Concentrate</th>
<th>Permeate</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD (g/L)</td>
<td>6.02 ± 0.08</td>
<td>8.79 ± 0.44</td>
<td>3.83 ± 0.05</td>
<td>4.53 ± 0.12</td>
<td>2.46 ± 0.03</td>
</tr>
<tr>
<td>TS (g/L)</td>
<td>31.93 ± 0.10</td>
<td>34.53 ± 0.11</td>
<td>29.43 ± 0.11</td>
<td>45.10 ± 0.21</td>
<td>27.84 ± 0.09</td>
</tr>
</tbody>
</table>

An unpleasant result was that the flux that was acquired was low for an ultrafiltration process (Fig. 12.2), which might have been caused by clogging of membrane pores by the suspended polymers. Permeate flux profiles show, typically, an initial sharp drop from the value obtained with water, then a smoother but continuous decay until a steady...
state is reached. That kind of time-dependent profile is caused by both concentration polarization and fouling (Turano et al., 2002). Although a thorough cleaning of membrane with 250 mL solution of 0.5 mM NaOCl in 0.5 M NaOH was occurred at the end of experiment, the flux rate wasn’t recovered.

![Flux results throughout the ultrafiltration (UF) membrane.](image)

Figure 12-2: Flux results throughout the ultrafiltration (UF) membrane.

Moreover, the NF unit succeeded in reducing the organic matter by 35.77% and the TS by 5.40%. Stamatelatou et al. (2009) examined further purification (filtering press, UF and RO) of the anaerobic effluent from a periodic anaerobic baffled reactor (PABR) and suggested that the filtering press and the UF unit could be omitted without any significant loss in the final permeate stream, whereas RO brought about extremely low organic matter.

The mean flux values of the different stages of treatment are presented in Table 12.4. As expected, fouling phenomena occurred. It must be stated that the feed of membrane near the end of the operational period contains large quantities of suspended solids as a result of concentrate. This had a major effect in the process and caused extended irreversible fouling in the UF membrane, which did not reverse after cleaning with NaOH solution. Fouling reduces the permeate fluxes and determines both efficiency decrease and variation of membrane selectivity. It also makes the process highly expensive owing to repeated plant shut-down for cleaning and washing the membranes. The fouling is an irreversible phenomenon comprising the effect of surface fouling, adsorption, gel layer formation, pore blocking or reduction of pore diameters, cake formation and adhesion of particles on the membrane (Turano et al., 2002). Membrane fouling depends on several factors, such as membrane characteristics, feed solution properties, such as molecular size of solutes and their interaction with the membrane, operating conditions (trans-membrane pressure, flow rate, temperature). Finally, Picture 12.4 presents the samples obtained from UF and NF respectively. The final permeate could be used for diluting the waste prior to anaerobic digestion, and for
irrigation purposes, or for other water requirements in agro-wastes establishments. The concentrates, on the other hand, were enriched in total organic and solid content. Both UF and NF concentrates can be mixed with the solid fractions from the previous step of coagulation/flocculation and be further converted to compost.

**Table 12-4**: Flux and fouling results of the membrane filtration of anaerobic effluent.

<table>
<thead>
<tr>
<th>TMP (bar)</th>
<th>Flux, L/(h(\cdot)m(^2))</th>
<th>Water After Cleaning</th>
<th>% Flux Drop</th>
<th>% Reversible Flux Drop</th>
<th>% Irreversible Flux Drop</th>
</tr>
</thead>
<tbody>
<tr>
<td>UF</td>
<td>1</td>
<td>303.26</td>
<td>8.73</td>
<td>93</td>
<td>97</td>
</tr>
<tr>
<td>NF</td>
<td>1</td>
<td>12</td>
<td>14.72</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

**Picture 12-4**: From left to right: the feed (UF), the concentrate (UF), the permeate (UF) or feed (NF), the concentrate (NF) and the permeate (NF)

**12.3.4 Conclusions**

Further purification of the anaerobic effluent through coagulation/flocculation and treatment in membrane filtration system (UF and NF) was achieved with encouraging results. In particular, high removal efficiency (COD reduction by 52.53%) was obtained using the polyelectrolyte poly-(diallyldimethylammonium chloride). The supernatant phase was initially treated with an UF unit and was then fed to a NF unit. Uf led to the removal of 36.38% of the organic matter and 7.83% reduction of TS. With the use of NF, the organic matter was reduced further by 35.77%. Overall, the combined treatment with the anaerobic digester and the membrane units was shown to be more effective than anaerobic digestion alone or the direct membrane separation of raw wastes.
12.4 Vermi-conversion of anaerobic sludges by *Eisenia foetida* earthworms

The main objective of this study was to evaluate the process performance of sludge transformation, using the earthworms *Eisenia foetida*, in relation to sludge quality used (mixtures of cow manure with anaerobic sludges) and to investigate the effects of different sludge composition on physicochemical properties of final product material, regarding its potential use as composting material.

12.4.1 Materials

Fresh cow dung (CD) was collected from a local dairy farm in Patras breeding 230 cows in total. Additionally, fresh anaerobic sludges were obtained from methanogenic anaerobic sludge (AS1), originating from the effluents of a laboratory anaerobic co-digestion system treating agro-industrial waste mixture (olive mill wastewater, cheese whey and liquid cow manure in ratio 55:40:5), and from the municipal Wastewater Treatment Plant (WWTP) of Patras (AS2). Table 12.5 presents the physicochemical analysis of the cow dung and anaerobic sludges after drying.

### Table 12.5: Main characteristics of cow dung (CD) and anaerobic sludges (AS1 and AS2).

|     | Moisture (%) | VS (%) | pH  | EC  (dS/cm) | TOC (%) | TKN
|-----|--------------|--------|-----|-------------|---------|-----
| CD  | 71.8         | 94.0   | 9.0 | 0.94        | 52.57   | 4.94 |
| AS1 | 79.2         | 88.8   | 8.9 | 3.78        | 53.82   | 36.00|
| AS2 | 79.5         | 51.5   | 8.6 | 1.05        | 35.15   | 32.00|

* total Kjeldahl nitrogen (TKN); † total phosphorus; ‡ total potassium

12.4.2 Experimental design

Prior to experimental testing all sludges (30% dry matter) were pretreated by manually mixing and aeration once a day for 15 days. Thus, the characteristic smell of putrescible substances was reduced and biotoxic compounds formed under anaerobiosis were decreased (Masciandaro et al., 2000). 200 g (on dry weight basis) of sludge mixture was then placed into 5-L plastic conical pots (Picture 12.5(a)) perforated at their bottom (for excess water to drain out) and was enriched with clitellated specimens of *Eisenia foetida* earthworms (Picture 12.5(b)) spread on the sludge surface in each pot. All substrate quantities are referred to dry weight basis at 105°C.
The growth, maturation and mortality of earthworms were monitored in a range of different feed mixtures in the laboratory under controlled environmental conditions (constant temperature at 20 ± 2°C) (Table 12.6). The addition of 10 or 20 earthworms was occurred in already composted samples. The moisture of treated material was maintained at about 70% by water spraying the sludge surface every 2 days securing appropriate moisture levels for worm growth. Vermistabilization of CD and sludge mixtures was estimated evaluating the vermicompost as fertilizer quality. The parameters that were monitored during vermicomposting were EC, pH, volatile solids and important plant nutrients such as N, P, K. Additionally the growth and reproduction of *Eisenia foetida* earthworms were also determined in vermi-reactors of different waste mixtures. The time of initial mixing of cow dung with anaerobic sludges before the inoculation of earthworms was set to t = 0 day after the aeration of sludge mixtures.

### 12.4.3 Results and Discussion

Considering the difference in characteristics of anaerobic sludges of municipal sewage sludge and co-digested methanogenic agroindustrial mixture (i.e. 55% olive mill, 40% cheese whey and 5% liquid cow manure) sludge, all the feed mixtures were analyzed for different physicochemical quality parameters. Table 12.7 presents the physicochemical analysis of the initial feed mixtures (after mixing different compositions of cow dung and anaerobic sludges), whereas Table 12.8 illustrates the vermicompost obtained at the termination of experiments.

The pH values of vermicompost as compared to initial values did not significantly changed. In some experiments, pH value decreased ~20% after the course of composting. The pH shift is considered to be directly related to the reduction in volatile solids and to the growth of earthworm biomass (Gupta and Garg, 2008). A decrease of volatile solids (~9.5%) was observed in some experiments.
Table 12-6: Compositions of the different feed mixtures tested of cow dung (CD) and anaerobic sludges of co-digested methanogenic agro-industrial mixture sludge (AS1) and municipal sewage sludge (AS2).

<table>
<thead>
<tr>
<th>A/A</th>
<th>CD (%)</th>
<th>SA1 (%)</th>
<th>SA2 (%)</th>
<th>No. of earthworms</th>
<th>duration (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>63</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>54</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>36</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>10</td>
<td>-</td>
<td>5</td>
<td>48</td>
</tr>
<tr>
<td>6</td>
<td>90</td>
<td>-</td>
<td>10</td>
<td>5</td>
<td>55</td>
</tr>
<tr>
<td>7</td>
<td>90</td>
<td>10</td>
<td>-</td>
<td>10</td>
<td>73</td>
</tr>
<tr>
<td>8</td>
<td>90</td>
<td>-</td>
<td>10</td>
<td>10</td>
<td>73</td>
</tr>
<tr>
<td>9</td>
<td>85</td>
<td>15</td>
<td>-</td>
<td>20</td>
<td>63</td>
</tr>
<tr>
<td>10</td>
<td>85</td>
<td>-</td>
<td>15</td>
<td>20</td>
<td>32</td>
</tr>
<tr>
<td>11</td>
<td>80</td>
<td>20</td>
<td>-</td>
<td>20</td>
<td>70</td>
</tr>
<tr>
<td>12</td>
<td>80</td>
<td>-</td>
<td>20</td>
<td>20</td>
<td>48</td>
</tr>
</tbody>
</table>

Mineralization of nitrogen and phosphorus into nitrites/nitrates and orthophosphates, respectively occurs as well as bioconversion of organic material into intermediate species of organic acids. The pH shift behavior is related to a different type of substrate, thus different intermediate species may appear that effect pH value. The electrical conductivity (EC) was increased for different feed mixtures after vermicomposting due to the fact that organic matter was lost and mineral salts are present i.e. ammonium, phosphate, potassium, etc. (Kaviraj and Sharma, 2003). In particular, the EC increased by 44% and 251% using 100% CD after 54 and 130 days by adding 5 and 10 earthworms Eisenia foetida, respectively. Moreover, the EC was increased up to 331% using the mixture of 90% CD – 10% AS2 with 10 earthworms.

Vermiconversion of sludges in different vermireactors was also monitored after nutrient analysis for nitrogen, phosphorus and potassium. In the 100% CD a gradual increase of nitrogen (119% and 165% after 54 and 130 days by adding 5 and 10 earthworm Eisenia foetida, respectively) and of phosphorus (107% after 130 days with 10 earthworms) was observed. The concentration of potassium exhibited a progressive increase by 64% and 186% after 54 and 130 days, respectively. Similar studies showed a same trend of TKN increase in cow dung mixture (Atiyeh et al., 2000). Despite the fact that the number of earthworms was not constant due to mortality of earthworms the monitoring of nutrients and earthworms biomass continued. Total nitrogen variations in vermicomposting of different wastes is mainly attributed to the quality of physicochemical composition of substrates tested in feeding earthworms that strongly affects mineralization of nitrogenous organic compounds and the amount of nitrogen released from these compounds (Bohlen et al., 1999).
Table 12-7: Physicochemical analysis of the initial feeds of experiments in different feed mixtures of cow dung with anaerobic sludges.

<table>
<thead>
<tr>
<th>A/A</th>
<th>Mixtures</th>
<th>Moisture (%)</th>
<th>VS (%)</th>
<th>pH</th>
<th>EC (dS/cm)</th>
<th>TKN a (mg/g dry matter)</th>
<th>TP b (mg/g dry matter)</th>
<th>TK c (mg/g dry matter)</th>
<th>No. of earthworms</th>
<th>Biomass growth rate (g/earthworms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100%CD</td>
<td>86.0</td>
<td>92.5</td>
<td>8.6</td>
<td>0.46</td>
<td>6.00</td>
<td>9.41</td>
<td>35.76</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>100%CD</td>
<td>71.8</td>
<td>94.0</td>
<td>9.0</td>
<td>0.94</td>
<td>4.94</td>
<td>10.19</td>
<td>18.06</td>
<td>5</td>
<td>0.45</td>
</tr>
<tr>
<td>3</td>
<td>100%CD*</td>
<td>64.5</td>
<td>91.0</td>
<td>9.5</td>
<td>1.07</td>
<td>10.82</td>
<td>6.24</td>
<td>32.89</td>
<td>10</td>
<td>0.45</td>
</tr>
<tr>
<td>4</td>
<td>100%CD</td>
<td>81.0</td>
<td>89.5</td>
<td>9.5</td>
<td>1.02</td>
<td>12.88</td>
<td>18.62</td>
<td>39.96</td>
<td>20</td>
<td>3.50</td>
</tr>
<tr>
<td>5</td>
<td>90%CD-10%AS1</td>
<td>68.0</td>
<td>91.0</td>
<td>9.1</td>
<td>1.20</td>
<td>12.74</td>
<td>10.4</td>
<td>18.39</td>
<td>5</td>
<td>0.51</td>
</tr>
<tr>
<td>6</td>
<td>90%CD-10%AS2</td>
<td>69.0</td>
<td>90.5</td>
<td>8.6</td>
<td>0.90</td>
<td>7.79</td>
<td>11.02</td>
<td>23.75</td>
<td>5</td>
<td>0.56</td>
</tr>
<tr>
<td>7</td>
<td>90%CD-10%AS1*</td>
<td>60</td>
<td>88.0</td>
<td>9.8</td>
<td>1.60</td>
<td>13.90</td>
<td>10.73</td>
<td>27.37</td>
<td>10</td>
<td>0.28</td>
</tr>
<tr>
<td>8</td>
<td>90%CD-10%AS2*</td>
<td>58.5</td>
<td>86.0</td>
<td>9.2</td>
<td>1.55</td>
<td>10.86</td>
<td>14.46</td>
<td>24.11</td>
<td>10</td>
<td>0.29</td>
</tr>
<tr>
<td>9</td>
<td>85%CD-15%AS1</td>
<td>83.0</td>
<td>92.0</td>
<td>9.1</td>
<td>0.73</td>
<td>13.15</td>
<td>9.75</td>
<td>17.95</td>
<td>20</td>
<td>0.41</td>
</tr>
<tr>
<td>10</td>
<td>85%CD-15%AS2</td>
<td>83.5</td>
<td>87.0</td>
<td>9.5</td>
<td>0.90</td>
<td>10.43</td>
<td>23.30</td>
<td>45.22</td>
<td>20</td>
<td>0.23</td>
</tr>
<tr>
<td>11</td>
<td>80%CD-20%AS1</td>
<td>78.0</td>
<td>88.6</td>
<td>9.6</td>
<td>1.53</td>
<td>15.74</td>
<td>12.36</td>
<td>18.95</td>
<td>20</td>
<td>0.25</td>
</tr>
<tr>
<td>12</td>
<td>80%CD-20%AS2</td>
<td>75.0</td>
<td>81.0</td>
<td>8.9</td>
<td>1.50</td>
<td>11.79</td>
<td>16.77</td>
<td>30.80</td>
<td>20</td>
<td>0.22</td>
</tr>
</tbody>
</table>

a total Kjeldahl nitrogen (TKN); b total phosphorus; c total potassium; * the experiments with 10 earthworms were occurred in continuation of experiments with 5 earthworms.
Comparing mixtures 90% CD-10% AS1 and 90% CD-10% AS2 a similar increase in N-P-K was observed, whereas reduced growth of earthworms was observed compared to 100% CD. In general, vermicomposting with mixtures of 10% anaerobic sludge (AS1 and AS2) and 90% CD showed satisfactory results on the survival of earthworms and conversion through them in a rich compost with high levels of N-P-K.

Insignificant (within error limitations) increase of TP content in the feed mixtures of vermiReactors (mixtures of 10% AS1/AS2 and 90% CD) was monitored. However TP of vermicompost of vermiReactor with 100% CD increased 64% and 239% using 5 and 10 earthworms, respectively. This corroborates with the findings of Kaushik and Garg (2004) where TP in cow dung mixture increased 48% after 77 days. The increase in TP is associated directly with the action of worm gut enzymes and indirectly by stimulation of the microflora (Garg et al., 2006). The physical breakdown of the material is attributed to the activity of earthworm ingestion.

Total potassium concentrations in all vermiReactors were also higher in the final product of vermicasts compared to the initial feed mixtures. For vermiReactor 100% CD with 10 earthworms, TK increased ~60% after 75 days in agreement with previous study (Garg et al., 2006). In contrast a decrease in TK was monitored (Elvira et al., 1998) due to leaching of the soluble elements i.e. potassium by excess water that drained through mass. However no excess of water was observed in studied vermicasts thus avoiding the leaching minerals with runoff water.

Comparing mixtures of 85% CD - 15% AS1 and 85% CD - 15% AS2, the former showed higher values of N-P-K concentrations (39%, 14% and 8% respectively) as the vermiexperiment lasted longer (63 and 32 days for 85% CD - 15% AS1 and 85% CD - 15% AS2, respectively). Comparing mixtures of 80% CD - 20% AS1 and 80% CD - 20% AS2 the former showed higher values of P-K concentrations (14% and 95%, respectively) as the vermiexperiment lasted longer (70 and 48 days for 80% CD - 20% AS1 and 80% CD - 20% AS2, respectively). In both cases the mixtures with AS1 gave better results in terms of increased N-P-K concentration values.

Finally, during vermicomposting the biomass production by *E. foetida* in different vermiReactors were measured and worm growth rate was recorded in terms of biomass over the observation period. The earthworms reared in different waste mixtures had significant difference in growth rate. The highest worm biomass attained was observed in vermiReactor 100% CD (1275 mg/earthworm). The tested mixtures of anaerobic sludges in the vermiReactors promoted a decrease in biomass gain by *Eisenia foetida*. Initial increase in biomass was followed by stabilization and then, biomass loss was observed in all the vermiReactors. The loss in worm biomass can be attributed to the exhaustion of mixture food. The lost weight rate depended upon the quantity and nature of its ingestible sludge substrates (Gupta and Garg, 2008). Using the different mixtures, the growth rate of earthworms was higher for the mixture of 85% CD - 15% AS1 and 80% CD - 20% AS1 with longer duration of vermicomposting (63 and 70 days for 85% CD - 15% AS1 and 80% CD - 20% AS1, respectively).
### Table 12-8: Physicochemical analysis of the vermicompost obtained at the termination of experiments in different feed mixtures of cow dung with anaerobic sludges. The duration (t) of earthworms’ survival

<table>
<thead>
<tr>
<th>A/A</th>
<th>Mixtures</th>
<th>VS (%)</th>
<th>pH</th>
<th>EC (dS/cm)</th>
<th>TKN&lt;sup&gt;a&lt;/sup&gt; (mg/g dry matter)</th>
<th>TP&lt;sup&gt;b&lt;/sup&gt; (mg/g dry matter)</th>
<th>TK&lt;sup&gt;c&lt;/sup&gt; (mg/g dry matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100%CD</td>
<td>91.3</td>
<td>9.10</td>
<td>1.87</td>
<td>5.47</td>
<td>10.42</td>
<td>32.10</td>
</tr>
<tr>
<td>2</td>
<td>100%CD</td>
<td>90.6</td>
<td>9.56</td>
<td>1.36</td>
<td>10.82</td>
<td>6.24</td>
<td>29.62</td>
</tr>
<tr>
<td>3</td>
<td>100%CD&lt;sup&gt;*&lt;/sup&gt;</td>
<td>84.3</td>
<td>7.39</td>
<td>3.30</td>
<td>13.09</td>
<td>21.13</td>
<td>51.72</td>
</tr>
<tr>
<td>4</td>
<td>100%CD</td>
<td>86.0</td>
<td>8.00</td>
<td>5.00</td>
<td>15.50</td>
<td>11.31</td>
<td>33.00</td>
</tr>
<tr>
<td>5</td>
<td>90%CD-10%AS1</td>
<td>89.9</td>
<td>9.80</td>
<td>1.77</td>
<td>11.20</td>
<td>10.73</td>
<td>27.37</td>
</tr>
<tr>
<td>6</td>
<td>90%CD-10%AS2</td>
<td>87.3</td>
<td>9.10</td>
<td>1.69</td>
<td>10.86</td>
<td>13.28</td>
<td>23.53</td>
</tr>
<tr>
<td>7</td>
<td>90%CD-10%AS1&lt;sup&gt;*&lt;/sup&gt;</td>
<td>94.7</td>
<td>8.08</td>
<td>3.02</td>
<td>16.97</td>
<td>16.29</td>
<td>28.04</td>
</tr>
<tr>
<td>8</td>
<td>90%CD-10%AS2&lt;sup&gt;*&lt;/sup&gt;</td>
<td>78.7</td>
<td>8.35</td>
<td>3.88</td>
<td>9.67</td>
<td>16.00</td>
<td>33.09</td>
</tr>
<tr>
<td>9</td>
<td>85%CD-15%AS1</td>
<td>87.8</td>
<td>8.50</td>
<td>3.15</td>
<td>18.24</td>
<td>11.12</td>
<td>19.41</td>
</tr>
<tr>
<td>10</td>
<td>85%CD-15%AS2</td>
<td>82.0</td>
<td>8.46</td>
<td>4.46</td>
<td>6.92</td>
<td>13.28</td>
<td>35.95</td>
</tr>
<tr>
<td>11</td>
<td>80%CD-20%AS1</td>
<td>81.4</td>
<td>8.62</td>
<td>4.20</td>
<td>6.25</td>
<td>14.09</td>
<td>37.02</td>
</tr>
<tr>
<td>12</td>
<td>80%CD-20%AS2</td>
<td>86.2</td>
<td>7.90</td>
<td>3.67</td>
<td>12.19</td>
<td>7.48</td>
<td>35.90</td>
</tr>
</tbody>
</table>

<sup>a</sup> total Kjeldahl nitrogen (TKN); <sup>b</sup> total phosphorus; <sup>c</sup> total potassium; * the experiments with 10 earthworms were occurred in continuation of experiments with 5 earthworms

### 12.4.4 Conclusions

Based on our results, vermicomversion of anaerobically treated sludges by *Eisenia foetida* worms may successfully produce good quality compost if mixed with cow dung, in appropriate quantities. However the vermicompost with cow manure endured (73 days) longer than the vermicompost duration with sludge mixtures. Vermicomposting of methanogenic anaerobic sludge of co-digested agro-industrial wastes (i.e. 55% OMW, 40% CW and 5% LCM) with cow dung resulted in pH and volatile solids decrease whereas electrical conductivity measurements were increased. The moisture of treated material was maintained at about 70% by water spraying the sludge surface every 2 days securing appropriate moisture levels for worm growth. Vermicomposting resulted into significant increase in critical quality parameters, such as TKN, TP and TK in most tested mixtures.
Chapter 12 | Post-treatment of anaerobic digestate

12.5 References


Chapter 13.

Techno-economic evaluation of a full-scale centralized anaerobic digestion plant

13.1 Introduction

Market conditions are looking increasingly favourable for the growth of the anaerobic digestion industry in the world. While this form of biogas production has historically been limited to agricultural and wastewater uses, it is growing in favour as a method of managing and extracting value from wastes. The feasibility of anaerobic digestion projects varies state-to-state, however advances in technological application and favourable legislative developments are driving investment interest in the space.

Anaerobic digestion is a natural process in which bacteria break down organic matter in an oxygen-free environment to form biogas and digestate. A broad range of organic inputs can be used including manure, food waste, and sewage, although the composition is determined by the industry, whether it is agriculture, industrial, wastewater treatment, or others. Anaerobic digesters can be designed for either mesophilic or thermophilic operation at 35°C or 55°C, respectively. Temperatures are carefully regulated during the digestion process to keep the mesophilic or thermophilic bacteria alive. The resulting biogas is combustible and can be used for heating and electricity generation, or can be upgraded to renewable natural gas and used to power vehicles or supplement the natural gas supply. Digestate can be used as fertilizer. Anaerobic digestion has a defined process flow that consists of four distinct phases: pre-treatment, digestion, biogas processing and utilization, and disposal or reuse of solid waste.
13.2 Biogas plant components

13.2.1 Feedstock storage

In an AD unit it is important to ensure a stable and continuous supply of feedstock, of suitable quality and quantities. If the biogas plant owner is at the same time the feedstock producer, then the high quality feedstock supply can be easily guaranteed. The feedstock storage units must have the volumetric capability to handle the predefined amount of wastes for the unit. The dimensioning of the storage facilities is determined by the quantities to be stored, delivery intervals and the daily amounts fed into the digester.

Feedstock storage facilities can be classified into bunker silos (Picture 13.1(a)) which is used for solid feedstock’s like maize or sweet sorghum silage, and storage tanks for liquid feedstock (slurries, liquid manure etc.) (Picture 13.1(b)). Usually bunker silos have the capacity to store feedstock more than one year as a result of the harvesting period. On the contrary storage tanks for liquid substrates have the capacity to store feedstock several days.

Whole crop of sorghum is harvesting twice a year and needs to be preserved in order to provide continuous operation of the anaerobic digester over the year. Therefore ensiling is commonly used to preserve the substrate. Compression of the material and the absent of oxygen promotes the growth of lactic acid bacteria which convert the soluble carbohydrates to lactic acid under anaerobic conditions and thus prevents the growth of undesired microorganisms. The most common sillage type that is used in AD facilities is the surface walled clamp silos seals with plastic sheeting.
13.2.2 Feeding systems for wet and dry substrates

The ideal situation for a stable AD process is a continuous flow of feedstock through the digester in order to avoid organic loading socks. In practice, the feedstock is added periodically to the digester, in several batches during the day. This saves energy as feeding aggregates are not in continuous operation. There are various feeding systems and their selection depends again on feedstock quality, herewith their pumpability and on feeding intervals. There are two general feeding techniques depends on the feedstock type and its pumpability.

Pumpable feedstock is transferred from storage tanks to the digester by pumps. The pumpable feedstock category includes animal slurries and a large number of liquid organic wastes (e.g. flotation sludge, dairy wastes, fish oil). On the other hand, non-pumpable (fibrous materials, grass, maize silage, manure with high straw content) can be tipped/ poured by a loader into the feeding system and then fed into the digester (e.g. by a screw pipe system). Both feedstock types (pumpable and non-pumpable) can be simultaneously fed into the digester. In this case it is preferable to feed the non-pumpable feedstock through by-passes. Two types of pumps are frequently used: the centrifugal and the displacement pumps.

Centrifugal (rotating) pumps are often submerged, but they can also be positioned in a dry shaft, next to the digester. For special applications, cutting pumps are available (Picture 13.2(a)), which are used for materials with long fibres (straw, feed leftovers, grass cuttings). Centrifugal pumps (Picture 13.2(b)) use one or more impellers which attach to and rotate with the shaft, providing the energy that moves liquid through the pump and pressurizes the liquid to move it through the piping system. They are usually quick to install, require less maintenance than other alternatives, and are generally easy to repair. They are usually the best choice for lower viscosity (thin) liquids and high flow rates. There are a wide variety of materials of construction, ranging from various plastics and cast iron or stamped stainless steel for lighter duties - to bronzes, stainless steels, exotic alloys, and specialty plastics for more corrosive, abrasive, hygienic, or other difficult applications. Finally, these pumps work very easily. First the pump directs the liquid in the system into the suction port of the pump and from there into the inlet of the impeller. The rotating impeller moves the liquid along the spinning vanes, which increases the velocity energy of the liquid. The liquid, then, leaves the impeller vanes and then moves into the pump volute or diffuser casing, where the high velocity of the fluid is converted into high pressure through a diffusion process. The fluid is then guided into the discharge port of the pump and from there out into the system, or on to the next stage in the case of a multi-stage pump.

The positive displacement pumps (Picture 13.3(a)), compared to centrifugal pumps, are not capable of high flow rate, but are able to produce much higher pressures and transport viscous liquids or liquids containing fragile solids. Positive displacement pumps (PD) move a liquid and pressurize it to allow it to pass through the system by drawing the liquid into a chamber, then contracting the chamber to force the liquid out
of the pump at the necessary pressure to move through the piping system. PD pumps do not have impellers, but rather rely on rotating or reciprocating parts to directly push the liquid in an enclosed movable volume, until enough pressure is built up to move the liquid into the discharge system. Because they don’t rely on raising the velocity of the fluid as the centrifugal pump does by moving the liquid through the impeller, the fluid velocity inside a PD pump is much lower than a centrifugal pump, which is often a desirable feature for certain applications such as pumping fragile solids. There are two main types of PD pumps, the rotary pumps (including gear, screw, vane, peristaltic, lobe, and progressive cavity pumps) and the reciprocating pumps (including plunger, diaphragm, piston, hydraulic, and many other).

Progressive cavity pumps (Picture 13.3(b)) are known for their ability to handle difficult liquids containing solids and highly viscous liquids and are the most popular pump type in biogas facilities due to its ability of pumping liquids with high suspended solid content. The progressive cavity pump uses a single threaded screw or rotor rotating inside a double threaded rubber stator to move liquid through the pump and build pressure to move liquid through the system. The pump rotor is shaped like a rounded helically shaped screw, rotating with an interference fit inside a rubber stator which is also helically shaped. As the rotor turns, the helical shape of the rotor and stator create a cavity that moves or progresses along the length of the stator as the fluid is drawn through. A progressive cavity pump is able to generate about 75 psi of pressure at the discharge, but multiple rotors and stators can be connected in series to achieve higher pressures. The rotor design makes this pump type somewhat longer than other rotary positive displacement pump types. Since there must be enough room to pull the rotor out of the stator for maintenance, the floor space required for this pump type is greater than for other rotary PD pump types.

Picture 13-2: (a) typical cutting pump and (b) typical centrifugal pump used in a biogas facility.
Stackable feedstock (non-pumpable) like grass, maize silage, manure with high straw content, vegetable residues etc. must to be transported from a storage facility (bunker silo) to the digester feeding system. This is usually done by loaders or tractors. The feeding system includes a container, where stackable feedstock is poured by tractor, and a transport system, which feeds the digester. The transport system is controlled automatically and consists of scraper floors, walking floors, pushing rods and conveyor screws. The insertion of the feedstock into the digester has to be air tight and should not allow leak of biogas. For this reason, the feeding system inserts the feedstock below the surface layer of digester. Three systems are commonly used: wash-in shaft, feed pistons and feed conveyor screw.

Feeding solids to the digester through wash-in shafts or sluices, using front or wheel loaders, allows large quantities of solids to be delivered any time, directly to the digester. When using feed pistons, the feedstock is inserted directly into the digester by hydraulic cylinders, which push the feedstock through an opening in the wall of the digester. Feeding co-substrates to the digester can be done by using feed screws or conveyor screws. In this case, the material is pressed under the level of the liquid in the digester, using plug screws.

**Picture 13-3:** (a) typical positive displacement pump and (b) design of a progressive cavity pump.

**Picture 13-4:** Main configurations for feeding the digester with solid feedstock.
13.2.3 Types of full-scale digesters

All anaerobic digesters perform the same basic function. They hold organic matter in the absence of oxygen and maintain the proper conditions for methane forming microorganisms to grow. Common characteristics of all digesters, apart from being air proof, are that they have a system of feedstock feeding as well as systems of biogas and digestate output. In European climates anaerobic digesters have to be insulated and heated. Centralized co-digestion is a concept based on digesting animal manure and slurries, collected from several farms, in a biogas plant centrally located in the manure collection area. The central location of the biogas plant aims to reduce costs, time and manpower for the transport of biomass to and from the biogas plant. A co-digestion biogas plant is capable of co-digest livestock manure with a variety of other suitable substrates where in the most of the cases are transported from various collection points (farms, industries etc.) in the plant according to an established schedule.

A complete mix digester (conventional anaerobic digester) is basically a tank in which the substrate is heated and mixed with an active mass of microorganisms. Incoming liquid displaces volume in the digester, and an equal amount of liquid flows out. The HRT for this type of digesters varies among 20 - 40 days. However the hydraulic retention times could be shorter for thermophilic anaerobic systems. The digester can be continuously mixed or the reactor is stirred during feeding and only occasionally between feedings.

Digesters can be made of concrete, stainless steel, brick or plastic, shaped like silos, troughs, basins or ponds, and they may be placed underground or on the surface. Normally centralized biogas plants are made by stainless steel (Picture 13.5(a)) or concrete (Picture 13.5(b)). The stainless steel made digesters has the advantage of be quicker in their construction. On the other hand concrete made digesters needs professional planning, high quality concrete and specialist personnel for their manufacture, in order to avoid an improper construction, which could result in leakages, cracking and corrosion on the digester. Digesters made of reinforced concrete are sufficiently gas tight due to water saturation of the concrete from the moisture contained in feedstock and biogas. The size of digesters determine the scale of biogas plants and varies from few cubic meters to several thousands of cubic meters, like in the case of large commercial plants, often with several digesters. The design of a biogas plant and the type of digestion are determined by the dry matter content of the digested substrate. Therefore AD distinguish is wet AD when the dry matter content is lower than 20% and dry AD when the DM content of the substrate is above this value (usually between 20-40 %).

There are two main categories that the biogas plants can be classified as regard operating conditions, either as batch plants or as continuous plants. Batch plants are filled and then emptied completely after a fixed retention time. Each design and each fermentation material is suitable for batch filling, but batch plants require high labor input. As a major disadvantage, their gas-output is not steady. Batch-type digesters are
the simplest to build and are usually used for dry digestion. Continuous plants are fed and emptied continuously. They empty automatically through the overflow whenever new material is filled in. Therefore, the substrate must be fluid and homogeneous. Gas production is constant, and higher than in batch plants. Today, nearly all biogas plants are operating on a continuous mode.

Continuous digesters can be vertical, horizontal or multiple tank systems. Depending on the solution chosen for stirring the substrate, continuous digesters can be completely mixed digesters and plug flow digesters. Completely mixed digesters are typically vertical digesters while plug-flow digesters are horizontal. Vertical digesters (Picture 13.6(a)) are the most common type of digesters, they built on-site round tanks of steel or reinforced concrete, often with a conic bottom, for easy stirring and removal of sand sediments. They are air proof, insulated, heated and outfitted with stirrers or pumps. The digesters are covered by a roof of concrete, steel or gas proof membrane and the produced biogas is piped and stored in an external storage facility, close to the digester or under the gas proof membrane. Horizontal digesters (Picture 13.6(b)) have a horizontal axis and a cylindrical shape. This type of digesters are usually manufactured and transported to the biogas plant site in one piece, so they are limited in size and volume. Moreover, they are usually used for feedstock like chicken manure, grass, maize silage or manure with a high straw content. The standard type for small scale solutions is a horizontal steel tank of 50-150 m³, which is used as the main digester for smaller biogas plants or as pre-digesters for larger plants.
13.2.4 Digester components

13.2.4.1 Stirring

A minimum stirring of biomass inside the digester takes place by passive stirring. Passive stirring includes the influent of the feedstock, as result of thermal differentiation inside the reactor and by the up flow of gas bubbles produces during the process. Although passive stirring is not sufficient for optimal operation of a digester and active stirring must be implemented. The stirring technology can be distinguished into mechanical, hydraulic or pneumatic stirring.

Stirring prevents formation of layers of sediments, prevent channeling, brings the microorganisms in contact with the raw feedstock, facilitates the up flow of gas bubbles and homogenizes the distribution of heat and nutrients through the mass of the substrate. Stirring frequency can be continuous or periodically. In most of the plants the agitation frequency is periodically as a result of energy saving reasons and maintenance requirements of the equipment. Moreover because of the uniqueness of every biogas plant as a result of digester size and geometry, feedstock quality, foam formation etc., stirring sequencing can be empirically and can be optimized and adapted to the needs of each specific plant. In most of the cases the optimum duration and frequency of stirring sequences and adjustment of stirrers are made during start up of the plant.

Mechanical stirring (Picture 13.7) is the most common type of stirring among the vertical digesters. Based on their rotation speeds can be classified in slow running stirrers, medium and fast running stirrers. In the medium and fast running stirrers are categorized the submersible stirrers driven by electric motor. They are cooled by the surrounding medium and are constructed with anticorrosive material or coatings. Usually it is easy their adjustments in high and side. In the slow running stirrers are classified the paddle stirrers. Paddle stirrers have a horizontal or curved axis and the driven motor is mounted waterproof outside of the reactor. The stirring effect should only provide vertical mixing of the feedstock and pressing forward the feedstock.

Picture 13-7: (a) vertical shaft agitator, (b) blade agitatorand (c) vortex fans.
Pneumatic stirring uses the produced biogas, which is blown from the bottom of the digester through the mass of the feedstock. The bubbles of rising gas cause a vertical movement and stir the feedstock. The main disadvantage of this type of mixing is foaming problems and the production of floating layers.

Hydraulically stirred systems have the advantage that the mechanical parts of the stirrers are placed outside the digester and can be easily maintained. Hydraulic mixing is only occasionally appropriate for destruction of floating layers and, like the pneumatic stirring, only used for substrates with low amounts of suspended solids.

13.2.4.2 Biogas Storage

Selection of an appropriate biogas storage system makes a significant contribution to the efficiency and safety of a biogas plant. There are two basic reasons for storing biogas: storage for later on-site usage and storage before and/or after transportation to off-site distribution points or systems. A biogas storage system also compensates fluctuations in the production and consumption of biogas as well as temperature-related changes in volume. There are two broad categories of biogas storage systems: Internal Biogas Storage Tanks are integrated into the anaerobic digester while External Biogas Holders are separated from the digester forming autonomous components of a biogas plant. The simplest solution is the biogas storage established on top of digesters, using a gas tight membrane, which has also the function of digester cover. For larger biogas plants, separate biogas storage facilities are established, either as standalone facility or included in storage buildings.

All biogas storage facilities must be gas tight and with validated pressure requirements. In case of storage facilities which are not protected by buildings, they must be UV, temperature and weather proof. During installation of a biogas storage facility, the gas storage tanks must be checked for gas tightness. For safety reasons, they must be equipped with safety valves to prevent damages and safety risks. Areas where biogas exists must be protected from sources of ignition. Equipment and protective systems intended to be used in biogas areas should be selected to meet ATEX specifications. Moreover for explosion protection reasons an emergency flare is required. The gas storage facility must have the minimum capacity corresponding to one fourth of the daily biogas production. Normally, a capacity of one or two day’s gas production is recommended.
13.2.4.3 Biogas flares

There are situations where more biogas is produced than it can be used for energy generation. This can happen due to extraordinary high gas production rates or through maintenance of the energy recovery system. Storage of biogas is possible for short periods without compression, but for periods of more than a few hours it is generally not feasible due to the large volume. For this reason, each biogas plant is equipped with a biogas flare. In situations where there is an excess of biogas, which cannot be stored or used, flaring is necessary to eliminate any safety risks and to protect the environment. Moreover flaring could be the solution for safe disposal of the biogas produced by AD processes, where energy recovery is not feasible.
### 13.2.5 Biogas Conditioning

The biogas is saturated with water vapors. It contains, in addition to methane (CH$_4$), carbon dioxide (CO$_2$), hydrogen (H$_2$) and various amounts of hydrogen sulphide (H$_2$S). Hydrogen sulphide is a toxic gas, with unpleasant odor, forming sulphuric acid in combination with the water vapors in biogas. The sulphuric acid is corrosive and can cause damage to the, gas pipelines, CHP engines, to exhaust pipes etc. To prevent these negative effects, biogas must be desulphurized and dried. Most CHP units have minimum requirements for the properties of the combustible gas. Desulphurization techniques are distinguished in biological desulphurization techniques and chemical desulphurization techniques. The biological desulphurization can be made either inside the digester via the injection of a small amount of air (2-8%) into the raw biogas to achieve biological oxidation, or outside the digester with biological desulphurization tanks (Picture 13.10(a)). On the other hand the chemical desulphurization can be made also either inside or outside the digester with the addition of chemicals and external scrubbers.

The biogas inside the digester is saturated with water vapors. To protect the energy conversion equipment, water must be removed from the produced biogas. A part of the water vapors is condensed by cooling of the gas during transporting with the gas pipelines from the digester to the CHP unit. However the best way to protect a CHP unit is by installing a biogas dehumidification system (Picture 13.10(b)). In a biogas dehumidification system the biogas enter the system chilled and the excess water removed in a condensate separator. In addition to the removed water vapours, condensation also removes some of the undesirable substances such as water soluble gases and aerosols.

![Picture 13-10](a) biological desulphurization and (b) typical biogas dehumidification systems.
13.2.6 Energy recovery from biogas.

Biogas with methane content of 50%, has heating value of 21 MJ/N·m³, density of 1.22 kg/N·m³ and the mass is similar to air (1.29 kg/N·m³). Biogas can be burned for heat production either on site, or transported by pipeline to the end users and can be combusted in directly in boilers or burners. However, biogas needs to undergo condensation and particulate removal in order to be avoided damages in the burning equipment. The majority of anaerobic digestion facilities across the world use combined heat and power (CHP) engines for the production of renewable electricity. CHP generation is the most efficient way to utilize the produces biogas for energy production. Most gas engines have maximum limits for the content of moisture content, hydrogen sulphide, halogenated hydrocarbons and siloxanes in biogas. Therefore the gas steam has to be pretreated before the combustion in the CHP unit. A typical CHP generator has an efficiency of up to 90% and produces 35% electricity and 65% heat. The most common types of CHP plants are block type thermal power plants (BTTP) with combustion motors that are coupled to a generator. Generators usually have a constant rotation of 1500 rpm in order to be compatible with the grid frequency.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane, CH₄</td>
<td>50-75</td>
</tr>
<tr>
<td>Carbon dioxide, CO₂</td>
<td>25-45</td>
</tr>
<tr>
<td>Water vapor, H₂O</td>
<td>2 (20°C) – 7 (40°C)</td>
</tr>
<tr>
<td>Oxygen, O₂</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Nitrogen, N₂</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Ammonia, NH₃</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Hydrogen, H₂</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Hydrogen sulphide H₂S</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Picture 13-11: Containerized CHP generator (Weltec Biopower GmbH).
13.2.7 Pipes and fittings

Pipelines used in anaerobic digestion facilities must be corrosion proof and designed to handle liquids like biomass, hot water or biogas. The pipeline type depends on the materials used, the working temperature and the working pressure.

Most common material used for pipelines installations includes PVC, HDPE, PP, stainless steel. All piping and valves should be accessible so it can be easily be maintained.

13.2.7.1 Biomass pipelines

The pipelines used for transportation of biomass in a AD facility, should be of suitable diameter in order to be avoided fouling problems. Commonly biomass pipelines used have a diameter of at least 300 mm. Most pipelines are constructed by PVC or HDPE as a result of their chemical resistance to acids, their low cost compare to steel pipes and their characteristics. Long and angled pipelines are avoided because of pressure loss and clogging problems.

13.2.7.2 Water pipelines

Commonly the heating of the digesters is made by the water heated by the CHP unit during biogas combustion. The hot water distributed with water circulators and used to maintain the temperature of the digestor in the desired values. Insulation of pipelines is necessary especially in countries with cold climate.

![Picture 13-12: Typical hot water pipeline circuit in an AD facility.](image)

13.2.7.3 Biogas pipelines

Biogas pipeline must be installed sloped and be outfitted with moisture traps and valves, in order to release the condensate. Normally the pressure values in the biogas pipeline ranging among 0-10 mbar. Most manufactures install flame arresters (preventing transmission of deflagration in case of uncontrolled ignition) and pressure relief valves in the gas circuit for safety issues. The biogas pipeline must be corrosion proof and must have excellent corrosion resistance against H2S or ammonia.
13.2.7.4 Diaphragm valves

Diaphragm valves (Picture 13.14(a)) have a round body with two cavities separated by a circular opening that is smaller than the pipe size. In general, the sizes available for diaphragm valves are from 50 mm to 200 mm. It can be used for precise throttling and control services in wastewater treatment applications, since they can easily be automated and are available with positioners, limit switches, and other accessories. They are relatively expensive, and have relatively low Cv and as a result are unable to handle slurries which causes high pressure drop across the valve.

13.2.7.5 Knife Gate valves

Knife gate valves (Picture 13.14(b)) have a wedge or disc that travels up and down to either block or allow the flow of water. They are mainly used for isolation (shut off) for wastewater applications. The knife edge of the gate can cut through accumulated solids. Knife gate valves are used in wastewater systems for handling abrasive slurries or sludge applications. They are available from standard cast configurations as small as 50 mm up to 1,800 mm. Knife gate valves can cut through slurries, scale, and surface builds ups. Since they have an unobstructed flow path, they provide high flow capacity. They also have small face-to-face dimensions, which assists with weigh reduction of the valve and facilitates piping design.
13.2.7.6 **Butterfly valves**

In butterfly valves (Picture 13.15(a)), the flow is regulated through a disc-type element held in place in the center of the valve by a rod. Similar to ball valves, valve operation time is short because the valving element is simply rotated a quarter turn (90°) to open or close the passageway. Their face-to-face dimension is often extremely small, making the pressure drop across a butterfly valve much smaller than globe valves (see below). In general, sizes available for butterfly valves range from 50 mm to 1200 mm. Although butterfly valves are economical, they tend to foul up when used for sludges with high solid content. Especially vulnerable are the cavities around the disc stem, which can potentially entrap fluids and slurries.

13.2.7.7 **Ball valves**

Ball valves (Picture 13.15(b)) offer very good shut-off capabilities. A simple quarter-turn (90°) completely opens or closes the valve. This characteristic minimizes valve operation time and decreases the likelihood of leakage due to wear from the gland seal. In general, the sizes available are from 25 mm to 300 mm. Ball valves are not suitable for slurry applications. Some of the pertinent features of ball valves are ease of operation, high pressure and temperature capacities, high flow capacity and the ability to handle various chemicals as a result of the manufacture materials. Ball valves are easy to find in various construction materials like PP, PTFE, PVC, HDPE, Stainless steel etc.

*Picture 13-15*: (a) butterfly valves and (b) ball valves.
13.3 Composting of digestate

Normally the effluent of the anaerobic digester has to be further treated. The digestate is separated to solid and liquid fraction. Main equipment used for the separation of the digestate is diaphragm filter press, decanter centrifuge, belt filter press etc. (Pictures 13.16).

The liquid fraction can be used directly as a liquid fertilizer and spread to cultivation fields in the surrounding of the plant. Although is some cases there are legislative limitations on the volume of leachates and the amount of the nitrogen content which can be applied per hectare in cultivation fields. The solid fraction can be further treated in composting facilities.

The co-composting of the digestates obtained by anaerobic digestion of animal manures, with agricultural residues, such as pruning, could constitute an efficient method to manage these wastes, not only to reduce the potential environmental problems associated, but also to recycle them. The compost obtained is a stabilised organic material that contains humic-like substances, which can be safely used as organic amendment in agricultural soils. Temperature is one of the main parameters used to monitor the composting process, since its evolution is related to many of the biological reactions that take place. The temperature in all the piles showed a rapid increase at the beginning of the composting process, reaching values higher than 50°C in the first week. Main types of industrial composting are the aerobic composting and the vermicomposting.

13.3.1 Aerobic composting

Aerobic composting can be defined as a process in which, under suitable environmental conditions, aerobic organisms utilize considerable amounts of oxygen in decomposing organic matter to fairly stable humus. Aerobic bacteria and fungi manage the chemical process by converting the inputs into heat, carbon dioxide and ammonium. The main equipment used for the maintenance and process
control in aerobic composting facilities is temperature and moisture probes which are installed in the mass in order to maintain the optimum aerobic decomposition conditions.

There are different types of industrial aerobic composting such as aerated static piles, in-vessel composting and aerated (turned) windrow composting. Aerated static piles (ASP) offer process control for rapid biodegradation and work well for facilities processing wet materials and large volumes of feedstock. ASP facilities can be under roof or outdoor windrow composting operations, or totally enclosed in-vessel composting, sometimes referred to tunnel composting. The aeration system uses fans to push air through the composting mass (Picture 13.17(a)). On the other hand, in-vessel composting generally describes a group of methods that which confine the composting materials within a box. In-vessel composting systems can consist of metal or plastic tanks or concrete bunkers in which air flow and temperature can be controlled, using the principles of a bioreactor (Picture 13.17(b)). Finally, aerated windrow composting consists of placing the mixture of raw materials in long narrow piles called windrows (Picture 13.18(a)) that are agitated or turned on a regular basis. The turning operation mixes the composting materials and enhances passive aeration. There are a number of specialized machines for turning windrows (Picture 13.18(b)) that reduce the time and labour involved considerably, mix the materials thoroughly, and produce more uniform compost. Some of these machines are attached to farm tractors or front-end loaders.

Picture 13-17: (a) aerated static piles and (b) in-vessel composting.

Picture 13-18: (a) aerated windrow composting and (b) compost turner for windrows.
13.3.2 Vermicomposting

Vermicompost is the product or process of composting using various worms, usually red wigglers, white worms, and other earthworms to create a heterogeneous mixture of decomposing vegetable or food waste, manures and other organic materials. There are two main methods of industrial vermicomposting. The first method uses a windrow and organic material is added to it. Although the windrow has no physical barriers to prevent worms from escaping and this is the main disadvantage of this type of composting. The second method is flow-through system. The worms are fed an inch of "worm chow" across the top of the bed, and an inch of castings are harvested from below by pulling a breaker bar across the large mesh screen which forms the base of the bed. Because red worms are moving towards continuous to the new food source, the flow-through system eliminates the need to separate worms from the castings before packaging.

13.4 Process monitoring

The main parameters affecting the process which can be monitored on or off line are: the quantity and the composition of the influent feedstock, the biogas production rate and the gas composition, the process temperature, the total solids of influent and effluent, the pH values and the ammonium nitrogen.

Sampling in a biogas facility is made directly from inside of the digester. The sampling tubes which can be used are typical vials made of plastic or glass. Generally 500 mL of homogenized sample is enough for all the necessary analysis that could be carried out. In order to ensure that the sample is of fresh digestate, the first amounts of liquid digestate should be wasted before collecting the sample because the material in the pipeline is usually not representative. The samples should be cooled to 4°C and should remain at 4°C for short-term storage. For longer storage times (>1 weeks) the samples should be frozen.

Many existing biogas plants use weighing equipment for measuring the input of solid feedstock. For liquids, often no special equipment for the quantification of the influent feedstock is used and the operators empirically are based on the capacity of the feeding pumps. As regard feedstock characteristics important parameters are the pH value, the COD, the total and volatile solids content and the TKN of the substrate. Total solids concentration should normally not exceed 10%, in order to ensure the ease of pumping and mixing of digester contents. Monitoring the solids in the digester can be useful to measure and compare total and volatile solids of feedstock and digestate to determine the proportion of feedstock degradation. The pH value is an indicator on the state of the fermentation process. pH changes take place only after process instability has started. Therefore the measurement of the pH value is important for process monitoring. pH value could be determined either off-line or on-line. The off-line measurement is made after taking sample from the digester with a laboratory pH-meter. The on-line
measurement is made with pH probes mounted on the reactors walls. However beside on-line pH measurement is more accurate that the off-line method, it can be problematic as a result of rapid fouling of the electrode and subsequent requirement for regular cleaning.

In general, gas flow meters should be placed in a way that enables easy removal and cleaning as a result of biogas characteristics (particulate matter, corrosive, saturated in water). Another important point is that the complexity of the sensor (data transfer, calculation effort, etc.) should suit the purpose of the plant. The most common type of biogas flowmeter used in biogas facilities are ultrasonic flow meters (Picture 13.19(a)). An ultrasonic flow meter measures the velocity of a fluid in a pipeline with ultrasound to calculate volume flow. Moreover, it is essential to control process temperature in the biogas digester, as a stable temperature is necessary for a high performance of the microbes. For temperature measurements, Pt100 thermometers are normally used which are common industrial thermometers applied in food or biotech industry (Picture 13.19(b)). It is recommended to measuring the temperature at different locations in a digester.

![Picture 13-19](a) ultrasonic flow meter and (b) Pt100 thermometer.

Many biogas plants install on-line measuring devices for gas composition, but portable gas composition measuring devices are also in use (Picture 13.20). Gas composition measurements include CH$_4$ and CO$_2$, which are measured by infrared or thermal conductivity sensors, as well as in most cases H$_2$S and O$_2$, which are determined by electrochemical sensors.

![Picture 13-20](a) on-line device and (b) portable device for gas composition measurement.
13.5 Troubleshooting

There are many possible technical problems resulting process instabilities namely unstable feed and organic overload, hydraulic overload, temperature changes, ammonia and hydrogen sulphide inhibition, trace elements limitations etc.

In particular, if large variations of the daily organic loading rate to the biogas occur, this will result in variable rates of gas production. An organic overload occurs when the amount of organic matter fed to the biogas plant exceeds the total degradation capacity of the microbes to produce biogas. As a consequence, the organic matter is only partially degraded to volatile fatty acids (VFA) which then accumulate in the reactor. If accumulated VFA exceeds the buffer capacity of the reactor, acidification of the digester occurs and the pH decreases resulting in process failure. Moreover, if the hydraulic retention time does not allow enough time for multiplication of the anaerobic microbes, their concentration will decline and they will gradually be washed out of the reactor. Methanogens have lower growth rates compare to the acidogenic bacteria involved to the process. Therefore hydraulic overload and biomass wash out could lead in VFAs accumulation to the system and as a result process failure.

Most of the AD facilities work under mesophilic (30–38°C) or thermophilic (49–57°C) conditions. Temperature variations have negative effect on process stability resulting in changes in biogas productivity. Microbes normally need a time span in order to be adapted in temperature changes. Therefore, the temperature of a digester must be kept stable.

In practice, high nitrogen feedstocks can frequently pose problems on process stability in a biogas plants. Rapid changes from low nitrogen feedstocks to high nitrogen feedstocks can be especially problematic. As this depends on the specific mixed culture and its adaptation to ammonia, it is very difficult to define stability limits. High ammonia concentrations could result rapid increase in pH values which could inhibit the process. The pH values are normally controlled by the addition of strong acids. Although the use of chemicals to maintain stable the process increases the operational cost of the unit and therefore feedstock changes via the decrease of high nitrogen feedstocks is preferred. Furthermore, hydrogen sulphide (H₂S) is produced by anaerobic degradation of sulphur compounds and can become problematic at concentrations of 40-400 ppm, especially when coupled with other inhibitory components such as ammonia.

Finally, a lack of trace elements can be responsible for decreasing performance in biogas plants. Trace elements are often necessary for the build-up of enzymes, and are therefore essential for the microbes. If a plant shows problems with process stability and VFA concentrations increases, the first and most obvious reasons for process imbalances is the organic or hydraulic overload. If the instability can not be attributed to the above reasons trace elements availability should be checked, so that the appropriate trace elements can be added. Essential trace elements in a biogas process can be Ni, Co, Mb, Se.
Parameters such as VFAs, alkalinity and redox potential are considered early indicators of process imbalance. For example, VFAs are intermediate metabolites in the anaerobic digestion process that are produced during the acidification stage. The accumulation of VFAs could result in inhibition of the methanogens. Normally, a moderate accumulation of acetic acid in the system is normal; due to acetic acid is the final metabolic product of acidogens and precursor of methanogens. On the contrary, accumulation of propionic acid, butyric and valeric acid is normally a sign of severe process instability. The alkalinity represents the bicarbonates and is a measure of the buffer capacity in a digester. The bicarbonate buffer capacity is important in the biogas process so that a moderate accumulation of volatile fatty acids does not cause a decrease in the pH which would result in process failure. Moreover, anaerobic microbes need a negative redox potential for their metabolism. In an anaerobic digester normally the redox potential should be lower than -300 mV. The redox value is measured by a redox electrode which determines the voltage between oxidizing substances (electron donors) and reducing substances (electron acceptors) that are dissolved in the digester.

### 13.6 Safety practices for anaerobic digestion systems

Hazards associated with biogas systems include drowning, electric shock, and noise exposure. However, biogas and its constituents, many of which are colorless and odorless, can unknowingly expose operators and visitors to hazards such as asphyxiation and burns due the flammable nature of methane. Workers must take proper precautions when handling and storing organic material and managing the production of electricity and combustible gases (EPA, 2011).

Liquid tanks and ponds for storage pose a drowning threat. Whenever a drowning potential exists, ring buoys, ropes, or ladders should be readily available for rescue purposes. Serious injuries can result from falls of any distance. When possible, employees should perform maintenance work from the ground. At most AD facilities, however, multiple elevated locations are present. For example, equipment on the top of aboveground AD tanks are 3-10 meters off the ground. According to the OSHA general industry standard “any time a worker is at a height of four feet or more, the worker is at risk and needs to be protected” (OSHA). When ladders are used to access elevated equipment, they should be secured and supervised at all times. On the other hand, pipes containing hot fluids or exhaust gas can pose potential burn hazards. Other potential sources of burns are heat exchangers, boilers, pumps, engine generators. Simply rubbing up against a heat exchanger or accidently placing a hand on a hot pipe can result in serious burns. All employees and visitors to the AD facility should be cautioned not to touch any equipment or pipelines. Hot surfaces should be identified as burn hazards. To reduce the entanglement risk, all equipment safety guards should be in place and individuals should tie back long hair and avoid wearing loose-fitting clothing and jewelry.
Feedstock and digestate should be carefully transferred and handled. In the event of a major feedstock or digestate spill, workers should exercise caution when containing the material. The first step should be to control the source causing the spill. Once this is achieved, workers should control the spill by constructing temporary containment structures around the affected area. Isolating the spill reduces potential damage to nearby buildings and contamination of surface waters and sensitive areas. The final step in spill response is site cleanup and restoration. In the event of a mechanical failure, workers should reference the vendor manuals to troubleshoot the issue. Vendor manuals for mechanical machinery should be organized and included in the emergency action plan. To avoid mechanical failures, with support from the technology provider, the system operator should develop a preventative maintenance manual for the site.

Before an employee services a piece of electrical equipment, the power supply should be turned off and the employee should place a padlock on the power supply. The padlock serves to prevent someone else from accidentally re-energizing the equipment being serviced. The lock should have a tag on it identifying the individual who locked out the equipment. Employees should follow this practice every time they service any electrical or electrically powered equipment.

Smoking and open flames should be prohibited in the general vicinity of the digester and a setback distance of 5 to 15 meters is suggested for all possible ignition sources to reduce the potential for fire or explosion. Ignition sources can include light switches, electric motors, pilot flames, and cell phones. Facilities should designate smoking areas at least 15 meters from the digester system to ensure that visitors and employees do not inadvertently create an ignition source. Signs should also be used to warn all individuals of the explosion or fire risk associated with the system.

Exposure to high levels of noise can result in discomfort or short-term hearing loss. In extreme cases, or if the noise exposure occurs over a long period of time, permanent hearing loss can occur. The main source of high noise levels is the engine generator set. Actual decibel (dB) levels produced at an AD facility will differ due to varying acoustical settings, but a gen set can produce between 100–140 dB. The facility is required to supply noise protection devices, such as earplugs, to employees and visitors who are exposed to high noise levels (OSHA).

Constituents of biogas, including carbon dioxide, methane, and hydrogen sulfide, present the potential for both asphyxiation and fire or explosion in confined spaces. It is important to remember that even a few gallons of manure or other organic material in a tank or confined space can pose a serious health risk under the right conditions. Moreover, methane is flammable when it mixes with air. When an ignition source is presented the lower explosive limit (LEL) are above 5% of methane and 15% by volume of air respectively.

If biogas is inhaled in sufficiently high concentration, it can result in poisoning or asphyxiation symptoms and even death. A simple asphyxiant displaces oxygen, and chemical asphyxiants ‘reduce the body’s ability to absorb, transport, or utilize inhaled oxygen. In a biogas plant asphyxiants could be caused by high amounts of carbon
dioxide and methane and chemical asphyxiants by hydrogen sulphide and ammonia even which could be extremely toxic even in low concentrations.

Any electrical source above 600 volts is considered high voltage. Typically, transmission lines from the transformer are the source of the highest voltage on a farm. A transformer is a piece of machinery used to increase the voltage, allowing for more efficient transport of the electricity. The main hazard is the contact with exposed leads, which could be fatal. All electrical sources less than 600 volts are considered low voltage. Switches, controllers, fuses, breakers, wall outlets, and electrical panels are considered low-voltage devices. One major hazard associated with electrical panels is arcing, which occurs when electricity from an energized source jumps a gap of air and discharges into an adjacent conductive surface, typically metal. If an individual happens to be in the pathway of the arc, they can be seriously burned or killed.

Finally, the chemical which are used for the maintenance and the operation of the biogas plant must be kept secure places based on the instructions of the manufactures.

13.7 Economic assessment of anaerobic digestion plant

Intentions for designing a biogas plant can vary from protecting the environment and reducing waste to produce renewable energy and may include financial and non-financial incentives. Basic conditions for the implementation of a biogas production project are the existence and availability of raw material. In addition, you must ensure the ability to sell or use the finished products plant biogas, i.e. biogas/biomethane, electricity, heat and compost. The next step is to assess whether the project can be carried out under the existing local conditions.

The first step in developing a project idea for a biogas plant is to make a critical inventory of available types and amounts of feedstock in the region. There are two main categories of biomass that can be used as raw material in a biogas plant. The first category includes products from farms such as animal manure and slurries, energy crops, vegetable residues, agricultural by-products and agricultural wastes. The second category consists of a range of suitable organic waste such as waste food, municipal solid waste and waste from pharmaceutical industries. The suitability of all types of raw material should be evaluated on their potential methane, their possible contamination and economically (market prices, costs of collection etc.).

The second step in the planning idea of a biogas project is to find a suitable site for the installation of the biogas plant. In particular, the plant should be located at a suitable distance from residential areas in order to avoid the smells carried by air. It should have easy access to infrastructure such as the electricity grid in order to facilitate the sale of electricity and the main roads in order to facilitate the transportation of raw material and compost. Moreover, the plant should be located relatively close to the production of agricultural raw materials, aiming at minimizing the distance, time and cost of transportation of raw material.
Chapter 13  Techno-economic evaluation of a full-scale centralized anaerobic digestion plant

To obtain an installation permit, the investor must demonstrate the compliance of the project with the national legislation on reporting issues related to the handling and recycling of manure and organic waste, the limit values for emissions, exhaust emissions, noise and odors, the impact on groundwater, landscape protection, safety at work and safety of buildings.

The biogas plant, for co-digestion of agro-industrial wastes such as olive mill wastewater, cheese whey and cow manure, consists of the following parts:

The storage tank of waste, which is largely buried in the ground, will have a rectangular shape and is made of concrete reinforced with steel bars, protected against corrosion. Because the sources of waste are three, the tank is divided into three unequal parts depending on the amount of waste from each source. 55% of the working volume of the tank receives olive mill waste, 40% cheese whey and 5% cow manure. The hydraulic retention time of the waste in the tank is two days. Fig. 13.1 shows the construction cost of the storage tank as a function of the waste feed.

For digesters, where the process of anaerobic digestion and biogas production is performed, cylindrical, vertical, above ground and continuous type (i.e. continuously fed with raw material) was selected. In particular, two different constructions were examined, from concrete or stainless steel. The hydraulic retention time in the digester was selected 30 days. If you choose digester made of stainless steel, particular company (Weltec Biopower) is responsible for their construction fully equipped with stirring, heating system, biogas membrane and thermal insulation. Fig. 13.2(a) depicts the manufacturing cost of the stainless steel reactor as a function of digester volume. In the case of digesters made of reinforced concrete, you will first need to build the framework of the digester and then add all the other parties. Moreover, anticorrosive protection with proofing materials (such as bentonite) should be occurred on the base of the

Figure 13-1: Construction cost of concrete storage tank as a function of waste feed.
digester with its walls, whereas crystallization epoxy paint should be applied across the internal surface (the same method is used for storage tank protection against corrosion). It should then be thermally insulated with polyurethane materials (width 10 cm), which should be placed on the outer surface of the frame, and the whole structure will be coated externally with protection sheet aluminum, thickness 1 mm. Finally, for the function of a fully equipped digester installation of heat exchanger, which provide the necessary thermal energy to maintain the substrate temperature at appropriate levels, stirrers and membrane for collecting the biogas should be done. Fig. 13.2(b) shows the calculated cost of the digester frame with insulation as a function of digester volume. However, the costs of **stirring**, **heat exchanger** and **biogas membrane** depend on the digester volume. Fig. 13.3(a), (b) and (c) show the calculated cost for each one, respectively.

![Figure 13-2: Cost of (a) stainless steel and (b) concrete digester as a function of the volume.](image)

The **pumps** are responsible for the transport of feedstock from the storage tank to the digesters. For the transport of olive mill waste and cheese whey submerged centrifugal pumps are selected where for cow manure a rotary screw pump was chosen. The prices of these three pumps were identified to 1328, 1771 and 2084 €, respectively. **Pipelines** are used for substrate transfer from the storage tank to the digesters, which are equipped with valves, manometers and flow meters for effective monitoring of flow and pressure and also for the transportation of biogas digesters to CHP engine. All pipes are made of stainless steel type SS-316. The estimation of pipelines cost was 128910 €. In an anaerobic digestion plant, units of **dehumidification** and **desulfurization** are used for biogas treatment in order to be suitable for the combustion engine cogeneration, avoiding the chances of erosion and complete destruction of pipes and machinery. The cost of these units is approximately 23462 and 100000 €, respectively.
The machine combined heat and power (CHP) is used for the combustion of the produced biogas with simultaneous production of electricity and thermal energy. A part of the production of electricity and thermal energy can be used for the needs of the unit, while the rest can be sold to make a profit. In Fig. 13.4(a), the relation of electricity production (KW) with waste feed is illustrated, whereas Fig. 13.4(b) presents the cost of providing CHP engine versus waste. The biogas flare is used in cases when the biogas production is greater than it can manage the CHP unit and it costs approximately 33868 €. In order to avoid release quantities of methane into the atmosphere, it is burned and is released as carbon dioxide (CO₂). Moreover, the settling tank is made of concrete aboveground, where the digested substrate is reached from the digesters a first separation of the liquid and solid part is carried out. Fig. 13.5 presents the construction cost of the settling tank of concrete as a function of waste amount.
Furthermore, a **solar drying** unit is used for treatment of sludge in order to loose moisture (up to 90%). The cost of this unit depends on the waste feed (Fig. 13.6(a)). The **pelletizer** can be used after the previous unit, as it receives the dry matter obtained of the solar drying unit, giving it a specific shape, size and packaging. Then the pellets are usually sold and used as a fuel replacing oil. The chosen pelletizer is capable of processing 3 tons/h. In Fig. 13.6(b) is given the amount of dry matter (tons/d) as a function of waste feed. The cost of pelletizing chamber is 129430 €. Finally, an **electrical panel** and software PLC-SCADA is necessary, through which the treatment plant is fully controlled. The cost of such panel is 49200 €.
After extensive market research and communication with company managers, the final construction and operation costs of anaerobic digesters were calculated. Calculations were obtained in two different cases. The first is the operation of one reactor, whereas the second one is the split of the waste feed in two different reactors with equal total volume. Fig. 13.7(a) presents the construction cost as a function of the concrete digester volume in the case of one or two reactors. The construction cost was calculated including the costs of stirring, heat exchanger and biogas membrane needed. On the other hand, Fig. 13.7(b) illustrates the cost of one or two stainless steel reactors in different volume of reactors. In the case of concrete reactors a negligible difference was observed between one or two reactors construction. However, a difference in cost was noticed, constructing stainless steel reactors. In particular, 160000 € more are needed in the case of two reactors’ construction, whereas in high waste feed the percent of increasing cost is approximately 18.2%.

Furthermore, using the above estimations, technical and economic evaluation was performed for an integrated biogas plant (1.0 MW) in terms of anaerobic digestion and composting plant. The goals were the record of the requirements in building facilities and the related equipment of the plant. Moreover, the amount of investment required, the operating costs, the income from electricity production and the sale of the produced compost were estimated.
This study was based on a previous scenario examined in Section 5.4 (55% OMW, 40% CW, 5% LCM, v/v/v) which resulted to high methane production. Knowing that the operation of a centralized anaerobic digestion plant cannot be achieved by treating just the specific mixture, since some of the wastes are seasonal, we assume that the replacement of these wastes with different substrates will not significantly affect the productivity. For the installation of a sustainable anaerobic digestion plant of 1.0 MW power, a production of 6,634 m³ CH₄/d should be obtained. According to scenario 55% OMW, 40% CW and 5% LCM (see Section 5.4), a production of 5.4 m³ CH₄/d (1.35 m³ CH₄/m³·d) was observed, in a two-stage system with HRTs of 3 d (acidogenesis) and 16 d (methanogenesis). Considering this production, an assumption that daily supply of the plant is 300 m³/d of the mixture used in order to achieve the satisfied methane production of 6,634 m³ CH₄/d. Taking into account the aforementioned, a construction of an acidogenic reactor was considered, with working volume 900 m³ (HRT of 3 d). Considering the reactor’s headspace equal to 10% of the total volume, the estimated final volume of the acidogenic reactor was 1,000 m³. On the other hand, for the 2nd stage of methanogenesis, two reactors were considered for construction with each working volume equal to 3300 m³. In particular, an HRT of 20 d was used (greater than 16 d with a degree of safety) and also a headspace volume of approximately 10% of the total volume. Tables 13.2, 13.3 and 13.4 present the estimations related to building infrastructure of the integrated waste management of agro-industrial wastes and biomass residues, which are after market research and communication with companies. The unit may be accommodate solid residues such as sweet sorghum or corn, thus feed systems for solid substrates were taken account. Moreover, the purchase of land was also taken account and was estimated at an average price of 2.5 € /m². The installation of the plant was considered to take place on a small development area outside the boundaries of the urban fabric (at least 1000 m).
### Table 13-2: The basic equipment of anaerobic digestion plant.

<table>
<thead>
<tr>
<th>Description</th>
<th>Units</th>
<th>Surface ($m^2$)</th>
<th>Total Cost (in €)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic digesters (stainless steel)</td>
<td>3</td>
<td>1,268</td>
<td>1,088,000</td>
</tr>
<tr>
<td>Feed system for liquid wastewaters (storage tanks and pumps)</td>
<td>3</td>
<td>176.2</td>
<td>221,014</td>
</tr>
<tr>
<td>Feed system for solid wastes (storage tanks and pumps)</td>
<td>2</td>
<td>20</td>
<td>38,709</td>
</tr>
<tr>
<td>Machine combined heat and power (CHP), 1.0 MW</td>
<td>2</td>
<td>80</td>
<td>1,556,000</td>
</tr>
<tr>
<td>Biogas flare</td>
<td>1</td>
<td>10</td>
<td>33,868</td>
</tr>
<tr>
<td>Control automation and security</td>
<td>1</td>
<td>-</td>
<td>49,200</td>
</tr>
<tr>
<td>Electrical panel and software</td>
<td>1</td>
<td>-</td>
<td>200,000</td>
</tr>
<tr>
<td>Building for electronic management</td>
<td>1</td>
<td>60</td>
<td>150,000</td>
</tr>
<tr>
<td>Tank for final digestate ($1000 m^3$)</td>
<td>1</td>
<td>250</td>
<td>54,009</td>
</tr>
<tr>
<td>Additional surface</td>
<td></td>
<td>3,135.8</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>5,000</strong></td>
<td><strong>3,390,800</strong></td>
</tr>
</tbody>
</table>

### Table 13-3: Basic infrastructures for the composting plant.

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit cost (in €)</th>
<th>Units</th>
<th>Surface ($m^2$)</th>
<th>Total cost (in €)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excavation works</td>
<td>5,000</td>
<td>10</td>
<td>-</td>
<td>50,000</td>
</tr>
<tr>
<td>Temporary storage of concrete materials</td>
<td>40</td>
<td>250</td>
<td>250</td>
<td>10,000</td>
</tr>
<tr>
<td>Temporary storage of sludge</td>
<td>500</td>
<td>100</td>
<td>100</td>
<td>50,000</td>
</tr>
<tr>
<td>Area for garden waste cutting</td>
<td>60</td>
<td>200</td>
<td>200</td>
<td>12,000</td>
</tr>
<tr>
<td>“Closed” compost plant</td>
<td>170</td>
<td>1500</td>
<td>1,500</td>
<td>255,000</td>
</tr>
<tr>
<td>Biofilter</td>
<td>160</td>
<td>200</td>
<td>200</td>
<td>32,000</td>
</tr>
<tr>
<td>“Open” maturation area</td>
<td>80</td>
<td>1100</td>
<td>1,100</td>
<td>88,000</td>
</tr>
<tr>
<td>Sieving area</td>
<td>80</td>
<td>300</td>
<td>300</td>
<td>24,000</td>
</tr>
<tr>
<td>Storage area for equipment</td>
<td>80</td>
<td>100</td>
<td>100</td>
<td>8,000</td>
</tr>
<tr>
<td>Unpredictable</td>
<td>47,000</td>
<td>1</td>
<td>-</td>
<td>47,000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>3,750</strong></td>
<td><strong>576,000</strong></td>
</tr>
</tbody>
</table>
Table 13-4: Basic infrastructures for the artificial wetland (disposal of liquid effluent).

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit cost (in €)</th>
<th>Units</th>
<th>Surface (m²)</th>
<th>Total cost (in €)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disposal of liquid effluent (wetland)</td>
<td>250,000</td>
<td>10</td>
<td>10,440</td>
<td>250,000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>10,440</strong></td>
<td><strong>250,000</strong></td>
</tr>
</tbody>
</table>

Taking into account the aforementioned amounts of surface needed for the installation of an integrated waste management an area of 19,190 m² has to be purchased which it costs 47,975 €. Moreover, Table 13.5 shows the costs of the equipments are mainly related to the composting plant and of some secondary equipment, useful for the plant operation. The total cost for the installation of such installation was calculated equal to 5,008,775 € taking into account the Tables 13.2-13.5 and also the cost of the land.

Table 13-5: Primary and secondary composting plant equipment and general operating support of plant.

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit cost (in €)</th>
<th>Units</th>
<th>Total cost (in €)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solar drying system</td>
<td>85,000</td>
<td>2</td>
<td>170,000</td>
</tr>
<tr>
<td>Shredder of agricultural residues</td>
<td>10,000</td>
<td>1</td>
<td>10,000</td>
</tr>
<tr>
<td>Equipment for “closed” compost plant</td>
<td>162,000</td>
<td>2</td>
<td>324,000</td>
</tr>
<tr>
<td>Loader</td>
<td>60,000</td>
<td>1</td>
<td>60,000</td>
</tr>
<tr>
<td>Sieve-Bagged</td>
<td>80,000</td>
<td>1</td>
<td>80,000</td>
</tr>
<tr>
<td>Equipment for laboratory-office</td>
<td>25,000</td>
<td>1</td>
<td>25,000</td>
</tr>
<tr>
<td>General equipment</td>
<td>25,000</td>
<td>1</td>
<td>25,000</td>
</tr>
<tr>
<td>Unpredictable</td>
<td>50,000</td>
<td>1</td>
<td>50,000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>744,000</strong></td>
</tr>
</tbody>
</table>

Furthermore, calculations were also obtained regarding the operating costs of such an integrated plant on an annual basis. The costs of the operation taken account were in terms of buliding infrastructure and plant equipment (Table 13.6) and employment of staff (Table 13.7).
Table 13-6: Maintenance and operating costs of building infrastructure and plant equipment.

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit cost (in €)</th>
<th>Units</th>
<th>Total cost (in €)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance of building infrastructures (1.0% of investment)</td>
<td>27,000</td>
<td>1</td>
<td>27,000</td>
</tr>
<tr>
<td>Maintenance of equipment (2.0% of investment)</td>
<td>47,000</td>
<td>1</td>
<td>47,000</td>
</tr>
<tr>
<td>Energy costs</td>
<td>50,000</td>
<td>1</td>
<td>50,000</td>
</tr>
<tr>
<td>Operation costs</td>
<td>15,000</td>
<td>1</td>
<td>15,000</td>
</tr>
<tr>
<td>Monitoring</td>
<td>10,000</td>
<td>1</td>
<td>10,000</td>
</tr>
<tr>
<td>Insurance (e.g. cars etc.)</td>
<td>35,000</td>
<td>1</td>
<td>35,000</td>
</tr>
<tr>
<td>Consumable costs</td>
<td>15,000</td>
<td>1</td>
<td>15,000</td>
</tr>
<tr>
<td>Unpredictable</td>
<td>25,000</td>
<td>1</td>
<td>25,000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>224,000</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 13-7: Staff costs on an annual basis (include insurance fees).

<table>
<thead>
<tr>
<th>Description</th>
<th>Working months</th>
<th>Unit cost (in €)</th>
<th>Units</th>
<th>Total cost (in €)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head of plant</td>
<td>14</td>
<td>4,000</td>
<td>1</td>
<td>56,000</td>
</tr>
<tr>
<td>Technical manager</td>
<td>14</td>
<td>3,500</td>
<td>1</td>
<td>49,000</td>
</tr>
<tr>
<td>Electrician/plumber</td>
<td>14</td>
<td>2,500</td>
<td>3</td>
<td>105,000</td>
</tr>
<tr>
<td>Engine driver</td>
<td>14</td>
<td>2,500</td>
<td>4</td>
<td>140,000</td>
</tr>
<tr>
<td>Workers</td>
<td>14</td>
<td>2,000</td>
<td>2</td>
<td>56,000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>406,000</strong></td>
</tr>
</tbody>
</table>

Beyond the annual costs (maintenance and operation), the plant has income derived from the sales of electricity and from the produced compost. In particular, the selling price according to the Regulatory Authority for Energy (RAE) is 220 €/MWh if there is investment subsidy, whereas 253 €/MWh if the investment takes place self-financing. However, the price of good quality compost was estimated to 50 €/ton. This value is relatively low for good quality materials but can not easily predict the reaction of the local rural community. Table 13.8 presents the income calculated in case of investment with subsidy, whereas in Table 13.9 the income of a self-financing investment is illustrated.
Table 13-8: Income from an integrated plant for waste management in case of investment with subsidy.

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit cost</th>
<th>Units</th>
<th>Total cost (in €)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sale of electric energy</td>
<td>220 €/KWh</td>
<td>8,770 KWh</td>
<td>1,929,400</td>
</tr>
<tr>
<td>Sale of compost</td>
<td>50 €/ton</td>
<td>5,212.6 tons</td>
<td>260,630</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>2,190,030</strong></td>
</tr>
</tbody>
</table>

Table 13-9: Income from an integrated plant for waste management with self-financing investment.

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit cost</th>
<th>Units</th>
<th>Total cost (in €)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sale of electric energy</td>
<td>253 €/KWh</td>
<td>8,770 KWh</td>
<td>2,218,810</td>
</tr>
<tr>
<td>Sale of compost</td>
<td>50 €/ton</td>
<td>5,212.6 tons</td>
<td>260,630</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>2,479,440</strong></td>
</tr>
</tbody>
</table>

In Tables 13.10 and 13.11, the financial informations obtained from the above calculations were presented for investment with subsidy and without, respectively.

Table 13-10: Financial information for the investment with subsidy.

<table>
<thead>
<tr>
<th>Description</th>
<th>Values with Subsidy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total investment</td>
<td>5,008,775 €</td>
</tr>
<tr>
<td>Subsidy</td>
<td>1,000,000 €</td>
</tr>
<tr>
<td>Loan</td>
<td>4,008,775 €</td>
</tr>
<tr>
<td>Interest rate</td>
<td>7 %</td>
</tr>
<tr>
<td>Operation cost</td>
<td>630,000 €</td>
</tr>
<tr>
<td>Price per MWh</td>
<td>220 €</td>
</tr>
<tr>
<td>Price per ton of compost</td>
<td>50 €</td>
</tr>
<tr>
<td>Income</td>
<td>2,190,030 €</td>
</tr>
<tr>
<td>Profits (before taxes)</td>
<td>1,560,030 €</td>
</tr>
<tr>
<td>Net present value</td>
<td>3,746,518.74 €</td>
</tr>
<tr>
<td>Payback time</td>
<td>3 years</td>
</tr>
</tbody>
</table>
As can be seen, the proposal for an integrated waste management plant treating agro-industrial wastewaters as described above (Tables 13.10 and 13.11) is a great scientific technological and especially environmental solution for the management of such wastes. It is also a very interesting investment in which you can manage approximately 109,000 tons of waste per year. It can be produced 9,230 MWh of electricity and 10,860 MWh of thermal energy annually and also 5,213 tons of good quality compost. The total investment accounts at 5,008,775 €, whereas the total cost for operation and maintenance of the plant was estimated to 630,000 € per year. Finally, the pay-back time of the investment was calculated between three and four years.

### 13.8 References

Occupational Safety & Health Administration (OSHA): [https://www.osha.gov/](https://www.osha.gov/)
14.1 Main Conclusions

The scope of this dissertation was to enhance our knowledge regarding the treatment of agro-industrial wastes, such as olive mill wastewater (OMW), cheese whey (CW) and liquid cow manure (LCM) via the anaerobic digestion. Large amounts of the above wastewaters lead to a significant problem in the worldwide economy because of the fact that they are in many cases totally unexploited and thus dangerous for the environment. For the construction and operation of a centralized anaerobic digestion plant, fed with such regional agro-industrial wastes, a different substrate is needed in order to simulate the annual operation of the plant, especially the periods that wastewaters such as OMW and CW are seasonal unavailable. Sweet sorghum is an energy crop that widely used for biofuels production due to its structure and composition. Ensiling method is a suitable storage method to preserve fresh crops, such as sweet sorghum, throughout the year. Thus, ensiled sweet sorghum is an attractive substrate for biogas production since it can be stored and then to replace other seasonal wastes.

Firstly, co-digestion of such agro-industrial wastes in a two-stage mesophilic (37°C) acidogenic-methanogenic CSTR system is a sustainable and environmentally- attractive method to treat these wastes and moreover converts them efficiently from a burden to society to a useful resource. The biogas produced can be used for the generation of heat and/or electricity. The use of LCM as co-substrate in the anaerobic digestion of OMW and/or CW exhibited many advantages: It provides stability to the process and minimization of nutrients addition, due to nitrogen and alkalinity supply to the digested mixture. Moreover, it contributes to a stable year-round operation of an efficient anaerobic digestion plant able to provide a feasible solution to the seasonal problem of OMW or CW management. Co-digestion improves the methane yield when compared to anaerobic digestion of mono-substrates, due to the positive synergisms established in
the digestion medium or diluting inhibitory and/or toxic compounds, such as the phenolics contained in OMW. Since OMW and CW are seasonally available, it may be also concluded that these substrates can be treated in existing facilities that already digest LCM. They can either be directly fed to a LCM digester or firstly acidified (to control odours and their degradation) and safely stored for quite a long period, in order to be appropriately treated in existing LCM-digesting plants. In any case, the addition rate of these wastes and its percentage in the mixture must not exceed critical values for their co-digestion.

Although the two-stage anaerobic treatment process has several advantages over the conventional single-stage process, treatment of CW and LCM as mono-substrates showed that it depends on the substrate type. In particular, using LCM as a feedstock negligible difference between single and two-stage process was observed, due to the fact that cow manure is a slowly-degradable substrate, whereas its lignocellulosic matrix is primarily responsible for its low degradability. On the other hand, CW is a substrate mostly composed of easily-degradable sugars, thus the two-stage process was considered as a better treatment system than single one, since was achieved the selection and enrichment of different bacteria in each digester. Specifically, in conventional single reactor, in which the acid- and methane-forming microorganisms are kept together, the system was led to instability with many control problems. With the two-stage AD process, these problems were overcome and therefore this system was considered as a better treatment system for CW wastewater.

The performance of an acidogenic reactor is of paramount importance especially during the two-phase anaerobic stabilization of wastes, since the acid reactor should provide the most appropriate substrate for the subsequent methanogenic one. Bio-hydrogen can be generated via different pathways since many different types of bacteria are involved in the fermentation of organic wastes, whereas volatile fatty acids (VFAs), alcohols, H₂, CO₂ and other intermediate products (i.e. lactic acid) can be produced. It is acknowledged that a series of operating parameters including pH, temperature and hydraulic retention time (HRT) influence the performance of fermentation and the formation of intermediate fermentative products such as hydrogen and organic acids. During the first set of anaerobic co-digestion experiments, performed in a two-stage system, the acidogenic and methanogenic reactor were operated under uncontrolled pH and with HRTs of 3 d and 16 d, respectively. The pH in the acidogenesis stage was decreased to low values (3.5 - 4.0) as a result of VFAs accumulation (mainly acetate), whereas no hydrogen production was observed. Nevertheless, further experiments were conducted using different mixtures of agro-wastes in order to investigate the impact of controlled pH in the production of hydrogen and metabolic products. Different pH value in the process led to different distribution of metabolic soluble end-products with simultaneous different hydrogen yield. For example, low and high pH values resulted in kinetic limitation of lactic acid bioconversion, which is regarded to as a key factor explaining the low hydrogen productivity. Moreover, it is well documented in the literature that production of acetic and butyric acid favors the production of hydrogen
(with the fermentation of glucose to acetic acid giving the highest theoretical yield), whereas the lactic acid is decomposed into propionic and acetic acid with no hydrogen production. Taking account not only series of experiments at different controlled pH values but also mass balance calculations, hydrogen production is associated exclusively with butyric acid production, whereas lactic acid degradation attributed mainly to butyric acid forming. The rest of lactic acid was converted to propionic acid formation with simultaneous acetic acid production. Different conditions in acidogenesis, such as pH or anaerobic culture pretreatment, can lead to different metabolic pathways and end-products. In particular, heat-treatment of anaerobic culture before the experiment contributed to different metabolic pathways for lactic acid production from sugars, thus different final butyric acid concentration and hydrogen. Heat-treatment of sludge may eradicate the coexistence of bacteria in inoculums, which have an adverse effect on hydrogen fermentation, and accelerate an enrichment of hydrogen producing bacteria, such as spore forming *Clostridium* sp., which are highly tolerant to extreme environments. Furthermore, using different mixtures, a different optimal pH value was obtained, in terms of maximum hydrogen yield, which depends on the type of substrates and their main characteristics. For instance, treating the mixture of 55% OMW, 40% CW and 5% LCM, bio-hydrogen yield was efficiently produced and maximized by adjusting the operating pH at 6.0. Altering the mixture to 55% sorghum, 40% CW and 5% LCM, the maximum hydrogen yield was achieved at controlled pH 5.5. However, increasing the percent of sorghum in the mixture (95% sorghum and 5% LCM) the optimum pH value was lower than the previous one and equal to 5.0.

Optimization of operational conditions (i.e. pH, temperature) for hydrogen fermenters results to high hydrogen yields by simultaneously preventing methanogens and acetogens from hydrogen consumption. Moreover, HRT is also one important parameter which significantly affecting microbial ecology in CSTR digesters and must be optimized. In continuous acidogenesis experiments, an increase in the hydrogen yield was observed with simultaneous decrease of HRT value, may be due to the kinetic limitation in low HRTs of hydrogen-consuming microorganisms, which are dominant at pH 5.0 or higher. In the same way, methane production is influenced by the HRT or equally the organic loading rate. Different mixtures’ treatments, under steady state, were operated at different HRT value. For example, following experiments in a two-stage system, HRT of 16 d was a satisfied value for methanogenesis of agro-industrial wastes and sweet sorghum stalks. However, in some cases the HRT 16 d was not enough for the degradation of volatile fatty acids and thereby the methanogenic microorganisms were inactive due to low pH conditions. It is worthy to notice that the same mixture of agro-industrial wastes (55% OMW, 40% CW and 5% LCM), which was treated in two different operating conditions, produced different amount of methane whilst steady state was obtained at different HRT value. In particular, both treatments were taken place in a two-stage system. The difference was in the acidogenic reactor, which the first time was operated at HRT 3 d without pH control whereas the second experiment was operated at
lower HRTs values with controlled pH at 6.0. Although the second time was operated under optimized conditions in the first stage and higher hydrogen yield was observed, the methane production in the second stage was significantly lower than the first experiment and higher HRT was needed for steady state of the system. A possible reason for this situation was the concentration of cations Na\(^+\) in the acidified mixture after controlling the pH at higher value of 6.0. However, a difference in characteristics of wastes such as the phenolic compounds in the OMW may affect the stability of microorganisms.

As mentioned previously, co-digestion improves biogas yield due to the positive synergism established in the digestion medium and the supply of missing nutrients by the co-digestion. Although an improvement of methane yield has been reported by the co-digestion, an increase of hydrogen yield was also observed. The effect of co-digestion in acidogenesis varies according to the feedstock. Specifically, using a mixture consisting of OMW, CW and LCM (at a ratio of 55:40:5, v/v/v) an enhancement of hydrogen yield was obtained compared to the theoretical calculated of the individual fractions. However, a mixture consisting of CW, LCM and sweet sorghum stalks instead of OMW (at the same ratio) gave the same results between experimental and calculated, indicating that the OMW use in the mixture contributes to higher yields or even that some compounds of OMW act differently in case of mixing with other wastes. The same trend was observed in the methanogenesis using the same mixtures after the acidification of the first step. In particular, experiments were performed in order to investigate the effect of substrate type and substrate concentration on methane production. In the first tested mixture with OMW, an increase of the initial substrate concentration led to the decrease of the COD removal and the methane yield. Especially, the methane production rate of the mixture was lower at high organic load, may be due to long-chain fatty acids (LCFA), tannins or most probably phenolic compounds contained in OMW, which are responsible for the toxicity of methanogenic bacteria. On the other hand, in the mixture with no OMW (it was replaced with sorghum stalks) a slight decrease of methane yield and COD degradation was obtained at high initial substrate concentration, whereas negligible difference in methane production rates between full-strength and half-strength mixtures was observed.

As an example of an energy crop residue, sweet sorghum (fresh or ensiled) was studied in this dissertation. In order to improve biogas production from lignocellulosic biomass, a pretreatment process is necessary to disrupt the naturally recalcitrant carbohydrate-lignin shields that impair the accessibility of enzymes and microbes to cellulose and hemicellulose. The beneficial effects of pretreatment have been recognized for a long time, but the choice of pretreatment technologies must consider several factors, e.g. the type of lignocellulosic biomass and the downstream biological conversion processes. Various pretreatment methods, such as (thermal)-chemical through alkali (NaOH) or acid (HCl, H\(_2\)SO\(_4\)) or enzymatic through the addition of commercial enzymes, were applied in different varieties of sorghum. Using fresh sorghum as a substrate, a slight increase of solubilization yield was observed after acid
pretreatment, whereas alkaline pretreatment led to a significant decrease in soluble carbohydrates may be due to Maillard reaction between amino acids and reducing sugars. On the other hand, ensiled sorghum presented a significant increased yield of glucose after alkaline pretreatment, compared to the initial amount of glucose during composition analysis. It is known that alkaline pretreatment improved lignin solubilization and the hydrolysis of cellulose and hemicellulose. It has been reported that the biodegradability of lignocellulosic biomass increased with decreasing lignin content, i.e., the higher the lignin content, the lower the biogas production.

Thermal-alkaline hydrolysis of ensiled sorghum using two different alkaline solutions, 0.5%NaOH-0.5%KOH and 1%NaOH-1%KOH, at 80°C and contact time 2 h led to an increase of sugars by 352.74% and 561.13%, whereas lignin was removed significantly by 36.87% and 42.33%, respectively. Alkaline pretreatment is efficient to delignify and remove partly lignin and hemicellulose, whereas cellulose is preserved. The latter pretreatments were used in BMP experiments in order to evaluate the performance on the methane yield. The pretreatment step did not cause inhibition of the anaerobic process because the amounts of Na\(^+\) and K\(^+\) remained lower than the concentrations causing inhibition. The alkali pretreatment with 0.5%NaOH-0.5%KOH and 1%NaOH-1%KOH solutions led to an increase in methane yield of 10.70% and 17.68% respectively, compared to untreated ensiled sorghum.

In addition, enzymatic hydrolysis was studied with combined levels of enzyme dosage, different pH, temperature and soaking time. Although a satisfied sugars production can be achieved using low concentrations of enzymes, further increase of enzymes concentration led to a high-cost process without equivalent increase of sugars. It is well documented that enzymatic saccharification following pretreatment is required for bioethanol production, but usually is not needed for anaerobic digestion because anaerobic microbes in the digester (e.g. Clostridium sp.) have their own hydrolytic enzyme system. Moreover, the effect of enzymes in enhancing biogas production was minimal, and the cost of enzymes was high, whereas the retention time is relatively high compared to chemical pretreatments. Taking account the aforementioned, in the present dissertation only a thermal-alkaline hydrolysis was selected, prior to anaerobic digestion experiments for biogas production.

The Anaerobic Digestion Model No. 1 (ADM1) was used to simulate the biochemical conversion of the process and to calculate biogas production and composition. In order to facilitate model application, an approach for a detailed inflow characterization of the substrate used and modifications of ADM1 were suggested. The present study focused on the anaerobic methane production from raw liquid cow manure or acidified mixtures of agro-industrial wastes and sweet sorghum that were the effluents of hydrogen producing bioreactors. The structure of the model was modified to include lactate, caproate and ethanol among the metabolites produced in the acidogenic stage. The obtained results indicate that the ADM1 is capable of simulating biogas production from such substrates, after precise characterization of the substrates and adjustment of the kinetic constants. In fact, gas flow and methane were predicted quite
well at steady-state periods, whereas the correlation between model and experimental values was satisfactory considering the slopes of the profiles.

Furthermore, with the anaerobic digestion process a percentage of pollution load remains in the treated effluent resulting in the necessity of subsequent processing. Apart from the energy potential, co-digestion results in liquid and solid effluents that are also valuable, as they retain most of their nutrient constituents (nitrogen, phosphorus, trace elements, etc.) and so they can be used as fertilizers and soil improvers. Further purification of the anaerobic effluent through coagulation/flocculation and treatment in membrane filtration system (UF and NF) can be achieved with encouraging results. In particular, high removal efficiency (COD reduction by 52.53%) was obtained using the polyelectrolyte poly-(diallyldimethylammonium chloride). The supernatant phase was initially treated with an UF unit and was then fed to a NF unit. UF led to the removal of 36.38% of the organic matter and 7.83% reduction of TS. With the use of NF, the organic matter was reduced further by 35.77%. Overall, the combined treatment with the anaerobic digester and the membrane units was shown to be more effective than anaerobic digestion alone or the direct membrane separation of raw wastes. On the other hand vermicomposting of anaerobically treated sludges by *Eisenia foetida* worms may successfully produce good quality compost if mixed with cow dung, in appropriate quantities. Vermicomposting of methanogenic anaerobic sludge of co-digested agro-industrial wastes (i.e. 55% OMW, 40% CW and 5% LCM) with cow dung resulted in pH and volatile solids decrease whereas electrical conductivity measurements were increased. The moisture of treated material was maintained at about 70% by water spraying the sludge surface every 2 days securing appropriate moisture levels for worm growth. Vermicomposting resulted into significant increase in critical quality parameters, such as TKN, TP and TK in most tested mixtures.

Finally, a techno-economic evaluation of a full-scale centralized anaerobic digestion plant was performed. The first step in developing a project idea for a biogas plant is to make a critical inventory of available types and amounts of feedstock in the region. The suitability of all types of raw material was evaluated in terms of their potential methane, their possible contamination and economically (market prices, costs of collection etc.). The second step in the planning idea of a biogas project is to find a suitable site for the installation of the biogas plant. The proposal for an integrated waste management plant treating agro-industrial wastewaters is a great scientific technological and especially environmental solution for the management of agro-industrial wastes. It is also a very interesting investment in which you can manage approximately 109,000 tons of waste per year. It can be produced 9,230 MWh of electricity and 10,860 MWh of thermal energy annually and also 5,213 tons of good quality compost. The total investment accounts at 5,008,775 €, whereas the total cost for operation and maintenance of the plant was estimated to 630,000 € per year. Finally, the pay-back time of the investment was calculated between three and four years.
14.2 Future Recommendations

The work presented here can serve the basis of future research on biogas production from agro-industrial wastes and biomass plant residues through anaerobic digestion. In the context of a rapidly maturing biogas industry, economic returns are increasingly important. For the future reactors design needs to increase methane yield from an increasing range of feedstocks using a multi-stage system. Such benefits also include reduction of pathogen content. The arguments and opportunities of developing a microbial community structure and kinetic perspective-based improvement of biogas production are promising. Primarily because evidence suggests that consortia of organisms work together to improve yield and reduce HRT, mixing has to be good but not too rigorous to disrupt syntrophic activity. Also an obvious way to increase yield and reduce HRT is to increase the microbial density by immobilisation; however mixing can become more critical as nutrient flows are reduced. Immobilising the microbial biomass has the potential advantages of improved reactor efficiency and reducing fermentation failure. Future research is needed to investigate the combination of anaerobic digestion with biofuel processes (bioethanol, biohydrogen, or biobutanol) in order to increase the energy efficiency of the biorefinery process, e.g. the by-products of bioethanol and biohydrogen production can produce biogas via anaerobic digestion.

A step forward could potentially be the investigation of the optimum ratio of the mixture or even different wastes such as fruits, vegetables, manures etc., or energy crops. Manures remain the primary substrates for co-digestion due to their abundance and their unique characteristics such as good buffer capacity and the presence of almost all the essential nutrients and trace elements. Moreover, careful process control by online measurements of important parameters will optimize the process and increase the biogas yield.

Microbial community adaptation is therefore recommended prior to inoculation. Although hydrogen production is strongly inhibited in presence of by-products, carbohydrates are nonetheless degraded during the process and converted into other metabolites such as propionate, ethanol and/or lactate. This metabolic shift is mainly due to microbial community changes from H₂-producers to H₂-consumers or competitors. In order to yield as much hydrogen as possible, a niche favorable hydrogen evolution has to be created by regulating microbial metabolism away from formation of alcohols and reduced acids (e.g. lactate) and towards production of acetate and butyrate. Some technical issues need to be addressed in the laboratory in view of efficient producing hydrogen before the scale-up and commercialization of the dark hydrogen fermentation process. Nevertheless, a more comprehensive study is essential in order to enhance our knowledge regarding the microbial communities through the use of molecular tools.

More investigations have to been performed to determine exactly the inhibitory concentration of each by-product (furfural, 5-HMF, etc.) on both dark fermentation and anaerobic digestion process. Possible synergistic effect between the different by-
products on the anaerobic processes represent also a challenging work for a future, as information are clearly missing in the literature. Moreover, few studies have shown the degradation of by-products in continuous process. These results raise the questions of the impact of reactor type (batch or continuous) and of inoculum adaptation.

It is also worth noting that an effective pretreatment method is needed that will reduce the obstacles or will produce multiple desirable effects (e.g. lignin removal and decrease of cellulose crystallinity) so that biomass can be efficiently degraded for high biogas yield. In order to improve biogas production from lignocellulosic biomass, a pretreatment process is necessary to disrupt the naturally recalcitrant carbohydrate-lignin shields that impair the accessibility of enzymes and microbes to cellulose and hemicellulose. First of all, particle size reduction should be optimized in conjunction with optimum microbial growth and methane yield in the anaerobic digestion process. Furthermore research is also needed, in the area of different pretreatment techniques by optimizing the conditions or even the effect of combining the alkali pretreatment with other pretreatment methods like microwave, ultrasound, heat, etc., in order to maximize biogas yield with simultaneous minimum cost.

Moreover, the ADM1 could be a valuable tool for process design even in the case of a two-stage anaerobic process, whereas further modifications could further improve the predictions for the methane production. In addition, research will focus on broadening the database and testing the transferability to industrial-sized biogas plants. Considerably more work will need to be done to improved post-treatment and separation technologies, aiming to overcome transport constraints. It would be interesting to be found and implemented new post-treatment technologies. Another significant option is the recirculation of the digestate with the aim of diluting the initial feed of the digester. Moreover, the majority of the studies no ecotoxicological evaluation is reported, and the success of treatment is only based upon the reduction of colour, COD, phenol content, etc. In some works, although the phenolics content was reduced, the toxicity remained constant or slightly increased, resulting on a wastewater with a less organic content but more toxic than before treatment.
Figure A-1: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v), at pH 4.5
Figure A-2: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v), at pH 5.0.
Figure A-3: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v), at pH 5.5.
Figure A-4: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v), at pH 6.5.
Figure A-5: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v), at pH 7.0.
Figure A-6: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of OMW/CW/LCM mixture (55/40/5, v/v/v), at pH 7.5.
Figure A-7: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and lactic acid and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of agro-waste mixture (in ratio of 55:40:5), at pH 5.0.
Figure A-8: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and lactic acid and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of agro-waste mixture (in ratio of 55:40:5), at pH 6.0.
Figure A-9: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids and lactic acid and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of agro-waste mixture (in ratio of 55:40:5), at pH 6.5.
Figure A-10: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of mixture (95% FS1 and 5% LCM), at pH 4.5.
Figure A-11: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of mixture (95% FS1 and 5% LCM), at pH 5.5.
Figure A-12: (a) Consumption of carbohydrates, (b) evolution of main volatile fatty acids, lactic acid and ethanol and (c) gaseous products (biogas and hydrogen) during batch acidogenesis of mixture (95% FS1 and 5%LCM), at pH 6.0.
## APPENDIX B

### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>anaerobic digestion</td>
</tr>
<tr>
<td>ADM1</td>
<td>anaerobic digestion model No. 1</td>
</tr>
<tr>
<td>AS</td>
<td>anaerobic sludge</td>
</tr>
<tr>
<td>BD</td>
<td>biodegradability</td>
</tr>
<tr>
<td>BMP</td>
<td>biochemical methane potential</td>
</tr>
<tr>
<td>BOD₅</td>
<td>biochemical oxygen demand, g/L</td>
</tr>
<tr>
<td>CD</td>
<td>cow dung</td>
</tr>
<tr>
<td>Cel</td>
<td>cost of electricity, €/KWh</td>
</tr>
<tr>
<td>COD</td>
<td>chemical oxygen demand, g/L</td>
</tr>
<tr>
<td>CSTR</td>
<td>continuous stirred tank reactor</td>
</tr>
<tr>
<td>CW</td>
<td>cheese whey</td>
</tr>
<tr>
<td>e</td>
<td>Euler’s number (2.71828)</td>
</tr>
<tr>
<td>EC</td>
<td>electrical conductivity, ds/cm</td>
</tr>
<tr>
<td>El</td>
<td>electrical energy, KWh</td>
</tr>
<tr>
<td>EMY</td>
<td>experimental methane yield, mL CH₄/g VS</td>
</tr>
<tr>
<td>ES</td>
<td>ensiled sorghum</td>
</tr>
<tr>
<td>fᵢ</td>
<td>stoichiometric coefficient of “ᵢ” component uptake</td>
</tr>
<tr>
<td>FID</td>
<td>flame ionization detector</td>
</tr>
<tr>
<td>FS</td>
<td>fresh sorghum</td>
</tr>
<tr>
<td>H</td>
<td>cumulative hydrogen production</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>HRT</td>
<td>hydraulic retention time, days</td>
</tr>
<tr>
<td>I</td>
<td>inhibition factor</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>$k_{\text{dec},i}$</td>
<td>first order decay rate of “$i$” component</td>
</tr>
<tr>
<td>$k_{\text{dis}}$</td>
<td>disintegration kinetic constant</td>
</tr>
<tr>
<td>$k_{\text{hyd},j}$</td>
<td>hydrolysis kinetic constant of “$j$” component</td>
</tr>
<tr>
<td>$k_{m,i}$</td>
<td>Monod maximum specific uptake rate of “$i$” component</td>
</tr>
<tr>
<td>$k_{s,i}$</td>
<td>half saturation constant “$i$” component</td>
</tr>
<tr>
<td>LCM</td>
<td>liquid cow manure</td>
</tr>
<tr>
<td>$M$</td>
<td>cumulative methane production</td>
</tr>
<tr>
<td>$M_G$</td>
<td>mass of glucose, g</td>
</tr>
<tr>
<td>$M_X$</td>
<td>mass of xylose, g</td>
</tr>
<tr>
<td>NF</td>
<td>nanofiltration</td>
</tr>
<tr>
<td>$n_i$</td>
<td>coefficient of “$i$” component uptake</td>
</tr>
<tr>
<td>OLR</td>
<td>organic loading rate, kg COD/m$^3$·d or kg VS/m$^3$·d</td>
</tr>
<tr>
<td>OMW</td>
<td>olive mill wastewater</td>
</tr>
<tr>
<td>$P$</td>
<td>maximum production potential, mL</td>
</tr>
<tr>
<td>P-DADMAC</td>
<td>poly-(diallyldimethylammonium chloride)</td>
</tr>
<tr>
<td>PES</td>
<td>polyethersulfone</td>
</tr>
<tr>
<td>$P_G$</td>
<td>market price of glucose, €/g</td>
</tr>
<tr>
<td>$P_X$</td>
<td>market price of xylose, €/g</td>
</tr>
<tr>
<td>$R_m$</td>
<td>maximum production rate, mL/h or mL/d</td>
</tr>
<tr>
<td>RO</td>
<td>reverse osmosis</td>
</tr>
<tr>
<td>SCOD</td>
<td>soluble chemical oxygen demand, g/L</td>
</tr>
<tr>
<td>SHPP</td>
<td>specific hydrogen production potential, mL/g COD</td>
</tr>
<tr>
<td>$\text{SHPR}_{m}$</td>
<td>specific hydrogen production rate, mL/g VSS·h</td>
</tr>
<tr>
<td>$S_i$</td>
<td>soluble “$i$” component</td>
</tr>
<tr>
<td>SOC</td>
<td>soluble organic carbon, g/L</td>
</tr>
<tr>
<td>SOCD</td>
<td>soluble organic carbon, g/L</td>
</tr>
<tr>
<td>STP</td>
<td>standard temperature and pressure</td>
</tr>
<tr>
<td>TC</td>
<td>total cost or profit, €</td>
</tr>
<tr>
<td>TCD</td>
<td>thermal conductivity detector</td>
</tr>
<tr>
<td>TCOD</td>
<td>total chemical oxygen demand, g/L</td>
</tr>
<tr>
<td>TK</td>
<td>total potassium</td>
</tr>
<tr>
<td>TKN</td>
<td>total kjeldahl nitrogen, g/L</td>
</tr>
<tr>
<td>TMP</td>
<td>transmembrane pressure, bar</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td>TMY</td>
<td>theoretical methane yield, mL CH\textsubscript{4}/g VS</td>
</tr>
<tr>
<td>TOC</td>
<td>total organic carbon, g/L</td>
</tr>
<tr>
<td>TOC</td>
<td>total organic carbon, g/L</td>
</tr>
<tr>
<td>TP</td>
<td>total phosphorus</td>
</tr>
<tr>
<td>TS</td>
<td>total solids, g/L</td>
</tr>
<tr>
<td>TSS</td>
<td>total suspended solids, g/L</td>
</tr>
<tr>
<td>TVFAs</td>
<td>total volatile fatty acids, g/L</td>
</tr>
<tr>
<td>UF</td>
<td>ultrafiltration</td>
</tr>
<tr>
<td>V\textsubscript{CTec2}</td>
<td>volume of CTec2, mL</td>
</tr>
<tr>
<td>VFAs</td>
<td>volatile fatty acids, g/L</td>
</tr>
<tr>
<td>V\textsubscript{HTec2}</td>
<td>volume of HTec2, mL</td>
</tr>
<tr>
<td>VS</td>
<td>volatile solids, g/L</td>
</tr>
<tr>
<td>VSS</td>
<td>volatile suspended solids, g/L</td>
</tr>
<tr>
<td>X\textsubscript{j}</td>
<td>particulate “j” component</td>
</tr>
<tr>
<td>Y\textsubscript{G}</td>
<td>glucose yield</td>
</tr>
<tr>
<td>Y\textsubscript{i}</td>
<td>yield of biomass on “i” component</td>
</tr>
<tr>
<td>ζ</td>
<td>potential ζ</td>
</tr>
<tr>
<td>λ</td>
<td>lag-phase duration, hours or days</td>
</tr>
</tbody>
</table>
Σύνοψη Διδακτορικής διατριβής

C.1. ΠΕΡΙΛΗΨΗ

Στη παρούσα διατριβή πραγματοποιήθηκαν πειράματα αναερόβιας συγχώνευσης χρησιμοποιώντας αγροτο-βιομηχανικά απόβλητα ή/και γλυκό σόργο. Τα αγροτο-βιομηχανικά απόβλητα, όπως είναι τα απόβλητα ελαιοτριβείου, τυροκομείου αλλά και βουστασίου, χαρακτηρίζονται από υψηλό υλικό φορτίο και συνεπώς θεωρούνται ακατάλληλα για απευθείας διάθεση σε περιβαλλοντικούς αποδέκτες. Το μεθανογόνο δυναμικό του τυροκομείου είναι μεγαλύτερο από τα υπόλοιπα ως εύκολα αποδομήσιμο υπόστρωμα. Σε αντίθεση, το υγρό απόβλητο βουστασίου χαρακτηρίζεται από χαμηλό μεθανογόνο δυναμικό και βιοαποδομησιμότητα. Συγχώνευση αυτών οδήγησε σε υψηλότερη απόδοση μεθανίου κάτι το οποίο οφείλεται σε συνεργιστικές επιδράσεις όπως η συμβολή επιπλέον αλκαλικότητας, ιχνοστοιχείων, θρεπτικών κτλ.

Πειράματα αναεροβικής συγχώνευσης πραγματοποιήθηκαν σε σύστημα δύο σταδίων με αντιδραστήρες συνεχούς λειτουργίας (CSTRs), υπό μεσοφιλικές συνθήκες (37°C) και με υδραυλικό χρόνο παραμονής (HRT) 19 ημέρες (3 d στην οξεογένεση και 16 d στη μεθανογένεση) χρησιμοποιώντας μίγμα αγροτο-βιομηχανικών αποβλήτων. Ο μέγιστος ρυθμός παραγωγής μεθανίου (1.9 L CH4/LR·d) παρατηρήθηκε χρησιμοποιώντας το μίγμα 55% ελαιοτριβείο, 40% τυροκομείο και 5% βουστάσιο, ενώ εξίσου υψηλός ρυθμός παραγωγής μεθανίου και ίσος με 1.33 L CH4/L·d μετρήθηκε χρησιμοποιώντας το μίγμα 90% τυροκομείο και 10% βουστάσιο με 79% απομάκρυνση ολικού COD. Παρόλο που η διαδικασία της αναερόβιας χώνευσης σε δύο στάδια υπερτερεί, εν γένει, σε σχέση με τη συμβατική διεργασία ενός σταδίου, περαιτέρω πειράματα διεξήχθηκαν χρησιμοποιώντας απόβλητα τυροκομείου και βουστασίου. Πραγματικά, η διεργασία δύο σταδίων απέδωσε καλύτερα αποτελέσματα σε σύγκριση με τη συμβατική του ενός σταδίου αλλά μόνο στην περίπτωση του τυροκομείου και όχι του βουστασίου.
Λαμβάνοντας υπόψη τα προαναφερθέντα, το μίγμα 55% ελαιοτριβείο, 40% τυροκομείο και 5% βουστάσιο επιλέχτηκε για περαιτέρω μελέτη, όπως επίσης και δύο επιπλέον μίγματα στα οποία γλυκό σόργο προστέθηκε με σκοπό την προσομοίωση λειτουργίας μίας κεντρικής μονάδας αναερόβιας χώνευσης, η οποία τροφοδοτείται με τοπικά απόβλητα τα οποία θα αντικατασταθούν σε περίοδο μη εποχικής διαθεσιμότητας. Στην περίπτωση χρήσης ενσιρωμένου σόργου, πραγματοποιήθηκε θερμο-αλκαλική υδρόλυση πριν τη χρήση του, με σκοπό τη διαλυτοποίηση των υδατανθράκων και την απομάκρυνση της λιγνίνης που λειτουργεί ως παρεμποδιστής στην πρόσβαση των ενζύμων στην κυτταρίνη.

Για την περαιτέρω μελέτη αυτών των μιγμάτων, δύο λειτουργικές παράμετροι (το pH και ο υδραυλικός χρόνος παραμονής, HRT) εξετάστηκαν. Πειράματα διαλείποντος έργου έγιναν προκειμένου να διερευνηθεί η επίδραση του pH στην παραγωγή υδρογόνου και μεταβολικών προϊόντων, ενώ πειράματα συνεχούς λειτουργίας διεξήχθηκαν για την επίδραση του HRT στην παραγωγή υδρογόνου και μεθανίου σε διβάθμιο σύστημα. Χρησιμοποιώντας το μίγμα 55% ελαιοτριβείο, 40% τυροκομείο και 5% βουστάσιο, η μέγιστη απόδοση παραγωγής υδρογόνου παρατηρήθηκε σε pH 6.0 (0.64 mol H2/mol καταν. υδαταν.), ενώ το γαλακτικό οξύ ανιχνεύτηκε ως κύριο μεταβολικό προϊόν που παρουσίασε μία έντονη συσσώρευση πριν από την περαιτέρω μετατροπή του σε βουτυρικό οξύ και υδρογόνο. Σε συνεχή λειτουργία, ο μέγιστος ρυθμός παραγωγής υδρογόνου (1.72 L/LR·d) επιτεύχθηκε σε HRT 0.5 d, ενώ ο μεθανογόνος αντιδραστήρας παρουσίασε σταθερότητα με παραγωγή μεθανίου 0.33 L CH4/LR·d σε HRT 25 d. Κατά την επεξεργασία του μίγματος 55% σόργο, 40% τυροκομείο και 5% βουστάσιο, η βέλτιστη τιμή του pH βρέθηκε να είναι ίση με 5.5 και με απόδοση υδρογόνου 0.52 mol H2/mol καταν. υδαταν., ενώ η μέγιστη παραγωγή υδρογόνου και μεθανίου σε διβάθμιο σύστημα συνεχούς λειτουργίας παρατηρήθηκε σε HRTs 0.5 d και 16 d και ήταν ίση με 2.14 L H2/LR·d και 0.90 L CH4/LR·d, αντίστοιχα. Τέλος, αυξάνοντας το ποσοστό του σόργου στο μίγμα (95% σόργο και 5% βουστάσιο), το βέλτιστο pH βρέθηκε να είναι μικρότερο και ίσο με 5.0 (0.92 mol H2/mol καταν. υδαταν.) σε σύγκριση με τα προηγούμενα μίγματα. Επιπλέον η μέγιστη απόδοση υδρογόνου λήφθηκε σε μεγαλύτερο HRT (5d), το οποίο μπορεί να οφείλεται στην ύπαρξη λιγνοκυτταρινού υλικού, ενώ η μέγιστη παραγωγή μεθανίου (0.44 L/LR·d) επιτυγχάνεται σε HRT 25 d.

Περαιτέρω αξιοποίηση του χωνευμένου υπολείμματος μελετήθηκε με χρήση συνδυασµένου συστήματος υπερδιήθησης/νανοδιήθησης επιτυγχάνοντας επιπρόσθετη μείωση του οργανικού φορτίου στο διήθημα. Η μετατροπή της αναερόβια χωνευμένης υλόσ σε λίπασμα αξιολογήθηκε μέσω κομποστοποίησης με γεωσκώληκες (vermi-composting) επιτυγχάνοντας ικανοποιητικά αποτελέσματα στην αύξηση των συγκέντρώσεων N-P-K. Επιπλέον, αναπτύχθηκε τροποποιημένο μοντέλο της αναερόβιας χώνευσης (ADM1) με στόχο την προσομοίωση της αναερόβιας συχνόνυσης διαφορετικών υποστρωμάτων. Τα αποτελέσματα που προέκυψαν έδειξαν ότι το μοντέλο ήταν σε θέση να προβλέψει στην κυτταρίνη την περιόδο των πειραματικών δεδομένων.
Είναι φανερό ότι οι ανανεώσιμες πηγές ενέργειας έχουν προσελκύσει τη διεθνή κοινότητα τις τελευταίες δεκαετίες καθώς διαδραματίζουν καθοριστικό ρόλο στην μείωση του CO₂. Η ενέργεια από βιομάζα και απόβλητα θεωρείται ως μία από τις πλέον κυρίαρχες ανανεώσιμες πηγές ενέργειας του μέλλοντος [1]. Έτσι, τα οργανικά απόβλητα όπως κτηνοτροφικά, λύματα, ενεργειακές καλλιέργειες, γεωργικά και βιομηχανικά υπολείμματα έχουν ιδιαίτερη σημασία, δεδομένου ότι οι πηγές αυτές δεν ανταγωνίζονται με τις καλλιέργειες τροφίμων της γεωργικής γης και ωστόσο μπορούν να χρησιμοποιηθούν για την παραγωγή ηλεκτρικής ενέργειας, θερμότητας και βιοκαυσίμων. Το αυξημένο ενδιαφέρον για τις διεργασίες που αφορούν στη μετατροπή της βιομάζας σε ανανεώσιμες πηγές ενέργειας, όπως είναι η αναερόβια χώνευση, τόνισε την έρευνα σε αυτόν τον τομέα ενώ επίσης σημαντικός αριθμός ερευνητικών έργων έχει αναπτυχθεί για να αξιολογηθούν οι ιδανικές συνθήκες χώνευσης διαφόρων υποστρωμάτων, όπως είναι τα αγροτο-βιομηχανικά απόβλητα και οι ενεργειακές καλλιέργειες.

Οι περισσότερες μεσογειακές χώρες, υφίστανται τα τελευταία χρόνια σημαντικές περιβαλλοντικές πιέσεις από την ανεξέλεγκτη διάθεση των ανεπεξέργαστων ή ελλιπώς επεξεργασμένων υγρών αποβλήτων από διάφορες αγροτικές και κτηνοτροφικές δραστηριότητες (π.χ. ελαιοτριβεία, τυροκομεία, βουστάσια κλπ.). Ο εκσυγχρονισμός των αγροτοκτηνοτροφικών μονάδων και η αύξηση της παραγωγής τους, με το πέρασμα των χρόνων, έχει σαν συνέπεια την παραγωγή αυξημένου όγκου υγρών αποβλήτων. Από την άλλη μεριά, η βελτίωση του βιοτικού επιπέδου και η δυνατότητα καλύτερης πληροφόρησης του αγροτοκτηνοτροφικού μονάδων και η αύξηση της παραγωγής τους, με την αλματώδη ανάπτυξη των Μέσων Ενημέρωσης, οδήγησαν σε αυξημένες απαιτήσεις για καλύτερες συνθήκες διαβίωσης και αυξημένη ημιαπόρτια για την προστασία του φυσικού περιβάλλοντος. Είναι λοιπόν εύκολο κατανοητό ότι η αναερόβια χώνευση προτείνεται ως η πλέον ενδεδειγμένη και ταυτόχρονα με ελάχιστο τεχνολογικό ρίσκο μέθοδος επεξεργασίας για απόβλητα με υψηλό οργανικό φορτίο, όπως είναι τα αγροτο-βιομηχανικά απόβλητα (Ευρωπαϊκή Οδηγία 96/61/ΕΚ). Ενδιάμεσα και τελικά προϊόντα της διεργασίας της αναερόβιας συγχώνευσης, όπως το υδρογόνο και το μεθάνιο, είναι προϊόντα υψηλής ενεργειακής αξίας, τα οποία μπορούν να χρησιμοποιηθούν για την κάλυψη ενεργειακών απαιτήσεων της μονάδας παράγοντας και αυξημένης ενέργειας για περαιτέρω αξιόποινη. Η συγχώνευση αποβλήτων μπορεί να συντελέσει στην αποτελεσματικότητα μιας τέτοιας κεντρικής μονάδας καθώς και να διασφαλίσει τη σταθερότητα στη...
λειτουργία της αφού η ανάμιξη των αποβλήτων μπορεί να συνεισφέρει στη δημιουργία ενός πιο «εξισορροπημένου» μίγματος κατάλληλου για χώνευση χωρίς την προσθήκη θρεπτικών ουσιών, όπως αζώτου και φωσφόρου [2]. Η συγχώνευση αποβλήτων σε μια κεντρική μονάδα επεξεργασίας μπορεί, επιπρόσθετα, να συνεισφέρει και στην επίλυση του προβλήματος της εποχικής φύσης κάποιων αποβλήτων (π.χ. απόβλητα ελαιοτριβείων και τυροκομείων) το οποίο μπορεί να ξεπεραστεί με την αντικατάσταση αυτών με άλλα διαθέσιμα οργανικά απόβλητα διαθέσιμα στην περιοχή ή με αγροτικά υπολείμματα και ενεργειακά φυτά, όπως είναι το γλυκό σόργο.

Το γλυκό σόργο είναι ένα μονοετές λιγνοκυτταρινούχο φυτό μεγάλης φωτοσυνθετικής ικανότητας. Χαρακτηρίζεται ως ενεργειακό φυτό λόγω των υψηλών αποδόσεων του σε βιομάζα και του υψηλού ποσοστού διαλυτών σακχάρων (π.χ. γλυκόζης, σακχαρόζης) που περιέχει [3]. Τα ζωμόσιμα διαλυτά σάκχαρα μπορεί να μετατραπούν σε υγρά (βιοαιθανόλη) και αέρια βιοκαύσιμα (υδρογόνο, μεθάνιο), ενώ η λιγνοκυτταρινή παραμένει ανεκμετάλλευτη. Οι χαμηλές απαιτήσεις της καλλιέργειας του σε άρδευση και λίπανση καθώς και η εύκολη προσαρμοστικότητα του σε διάφορα είδη εδαφών και σε ποικίλες κλιματικές συνθήκες το καθιστούν πολλά υποσχόμενο καλλιεργήσιμο φυτό στην Ελληνική γη [4]. Η συντήρηση του γλυκού σόργου επιτυγχάνεται είτε με την άμεση αποθήκευση του, μετά την συγκομιδή του, σε χαμηλή θερμοκρασία (−18°C) είτε με ενσίρωση. Καθώς η χρήση ψυκτικών θαλάμων είναι ενεργοβόρος διαδικασία και άρα απαγορευτική, λόγω κόστους και περιβαλλοντικών επιπτώσεων, η διατήρηση των θρεπτικών συστατικών του σόργου για περαιτέρω χρήση επιδιώκεται μέσω της ενσίρωσης. Η ενσίρωση του φρέσκου γλυκού σόργου είναι μια διεργασία στερεής ζύμωσης και αποτελεί μια μέθοδος συντήρησης των κύριων συστατικών του σόργου ώστε να καθίσταται δυνατή η χρήση του ως υπόστρωμα για μακρύ χρονικό διάστημα μετά την συγκομιδή του.

C.3. ΠΕΙΡΑΜΑΤΙΚΟ ΜΕΡΟΣ

C.3.1. Αναλυτικές μέθοδοι

Στην παρούσα εργασία προσδιορίστηκαν οι συγκεντρώσεις του χημικά απαιτούμενου οξυγόνου (COD), βιοχημικά απαιτούμενου οξυγόνου (BOD), ολικών και πτητικών στερεών, ολικού και αμμονιακού αζώτου, ολικού και διαλυτού φωσφόρου, λιπών και ελαιών καθώς και αλκαλικότητας [5]. Οι μετρήσεις pH και ηλεκτρικής αγωγιμότητας πραγματοποιήθηκαν θερμοκρασίας (18°C) είτε με ενσίρωση. Καθώς η χρήση ψυκτικών θαλάμων είναι ενεργοβόρος διαδικασία και άρα απαγορευτική, λόγω κόστους και περιβαλλοντικών επιπτώσεων, η διατήρηση των θρεπτικών συστατικών του σόργου για περαιτέρω χρήση επιδιώκεται μέσω της διαδικασίας της ενσίρωσης. Η ενσίρωση του φρέσκου γλυκού σόργου είναι μια διεργασία στερεής ζύμωσης και αποτελεί μια μέθοδος συντήρησης των κύριων συστατικών του σόργου ώστε να καθίσταται δυνατή η χρήση του ως υπόστρωμα για μακρύ χρονικό διάστημα μετά την συγκομιδή του.
βιοαερίου καταγράφοταν σε αυτόματη συσκευή κατασκευασμένη από ιδιώτη. Τέλος, ο προσδιορισμός της λιγνοκυτταρίνης έγινε σύμφωνα με τη μέθοδο οξινής υδρόλυσης του NREL [8].

C.3.2. Υποστρώματα

Αγροτο-βιομηχανικά απόβλητα

Τα υποστρώματα που χρησιμοποιήθηκαν στην παρούσα διατριβή ήταν υγρά απόβλητα ελαιοτριβείου, τυροκομείου και βουστασίου. Και τα τρία απόβλητα συλλέχθηκαν από μικρές τοπικές μονάδες στην περιοχή της Πάτρας. Πιο συγκεκριμένα, το απόβλητο ελαιοτριβείου προήλθε από ελαιοτριβείο τριφασικής φυγοκέντρισης ενώ το απόβλητο τυροκομείου από μονάδα που παράγει κυρίως τυρί φέτα με ημερήσια παραγωγή 30 m3 σε υγρά απόβλητα. Τέλος το υγρό απόβλητο βουστασίου συλλέχθηκε από μία φάρμα η οποία εκτρέφει 230 αγελάδες για γαλακτοπαραγωγή. Η εγκατάσταση αυτή είναι πλήρως αυτοματοποιημένη και τα παραγόμενα απόβλητα διαχωρίζονται μέσω διέλευσης τους μέσα από περιστρεφόμενο κόσκινο σε υγρά απόβλητα και στερεή κοπριά αγελάδων. Μετα την συλλογή τους τα υγρά απόβλητα αποθηκεύθηκαν αμέσως στους -18°C μέχρι την περαιτέρω χρήση τους στα πειράματα.

Γλυκό σόργο

Το γλυκό σόργο που χρησιμοποιήθηκε στη παρούσα διατριβή αντιστοιχούσε σε 6 διαφορετικές ποικιλίες. Χρησιμοποιήθηκαν 3 είδη φρέσκου σόργου καθώς και 3 είδη ενσιρωμένου. Τα δύο τελευταία ενσιρωμένα σόργα προήλθαν από ενσιρωμένα τον τελευταίο φρέσκο σόργο. Καλλιεργήθηκαν συμφωνώς με τον Ευρωπαϊκό Κανονισμό EC 092/91 και συλλέχθηκαν από διαφορετικά τοπικά τοπικά επικεφαλής (Δυτική Ελλάδα) σε διαφορετικές εποχές. Κατά τη συγκομιδή της βιομάζας του ενεργειακού φυτού συμπεριελήφθη το στέλεχος, η ψίχα και ο φλοιός. Για τη διαδικασία της ενσιρωμένης της βιομάζας του γλυκού σόργου συμπεριελήφθη στο στέλεχος. Επιπλέον μελετήθηκε η προεπεξεργασία με χρήση ενζύμων (Cellic®CTec2 and Cellic®HTec2), ως μία εναλλακτική μέθοδος επεξεργασίας των
ΑΠΟΘΕΛΕΣΜΑΤΑ

C.4.1. Ποιοτικά χαρακτηριστικά των αγροτο-βιομηχανικών αποβλήτων στη Δυτική Ελλάδα και το μεθανογόνο δυναμικό τους

Αρχικά, πραγματοποιήθηκε πλήρης προσδιορισμός των κυριότερων χαρακτηριστικών για το κάθε απόβλητο που χρησιμοποιήθηκε στα πειράματα. Ένα σημαντικό χαρακτηριστικό που τα καθιστά ρυπογόνα προς το περιβάλλον είναι το υψηλό οργανικό φορτίο (έως και 140 g COD/L στο απόβλητο ελαιοτριβείου). Πιο συγκεκριμένα, τα απόβλητα ελαιοτριβείων χαρακτηρίστηκαν από μεγάλη συγκέντρωση
πολυφαινόλων και υψηλή συγκέντρωση στερεών που σε συνδυασμό με το μαύρο χρώμα τους, τα καθιστούν ιδιαίτερα τοξικά (φυτοτοξικά) και συνεπώς ακατάλληλα για απευθείας διάθεση σε περιβαλλοντικούς αποδέκτες [9]. Τα απόβλητα τυροκομείου πέρα από το υψηλό οργανικό φορτίο τους χαρακτηρίζονται από υψηλή συγκέντρωση υδατανθράκων [10] ενώ τα κτηνοτροφικά απόβλητα από υψηλή συγκέντρωση αξιών υπολογίστηκαν στη συμπεριφορά τους [11]. Προσδιορίζοντας το μεθανογόνο δυναμικό για κάθε απόβλητο, βρέθηκε ότι το τυροκομείο ως ένα εύκολα αποδομήσιμο υπόστρωμα έχει το μεγαλύτερο μεθανογόνο δυναμικό (399.29 mL CH₄/g VS προστιθέμενο). Σε αντίθεση, το υγρό απόβλητο βουστάσιου χαρακτηρίζεται από χαμηλό μεθανογόνο δυναμικό (216.41 mL CH₄/g VS προστιθέμενο) και μικρή βιοαποδομησιμότητα (27.62%) εφόσον αποτελείται από υψηλό ποσοστό ινών. Συγχώνευση αυτών οδήγησε σε υψηλότερη απόδοση μεθανίου (Σχήμα 1) κάτι το οποίο οφείλεται σε συνεργατικές επιδράσεις, όπως η έκπλοκτική διαδικασία και οι χημικές αλλαγές [12].


C.4.2. Προεπεξεργασία γλυκού σόργου για παραγωγή βιοαερίου.

Το γλυκό σόργο είναι ένα λιγνοκυτταρινούχο υλικό το οποίο χαρακτηρίζεται από υψηλή απόδοση σε βιομάζα και υψηλό ποσοστό σακχάρων (γλυκόζης, σακχαρόζης). Η προεπεξεργασία της λιγνοκυτταρίνης (πολυσακχαρίτες) του γλυκού σόργου συντελεί στην περαιτέρω οξιοποίηση του υλικού για τη μετατροπή του σε βιοκαύσιμα. Στόχος της προεπεξεργασίας του γλυκού σόργου ήταν η υδρόλυση της λιγνοκυτταρινούχας βιομάζας σε μονομερή σάκχαρα (όπως γλυκόζη, ξυλόζη, κ.ά.).

Αρχικά, πραγματοποιήθηκε η χημική επεξεργασία φρέσκου και ενσιρωμένου σόργου (1% w/v) με διαλύματα οξέων (HCl, H₂SO₄) και βάσης (NaOH) τα οποία θεωρούνται τα πιο συνήθη για την υδρόλυση της λιγνοκυτταρίνης. Οι συνθήκες που επιλέχθηκαν να
μελετηθούν, για κάθε υλικό και διάλυμα, ήταν η συγκέντρωση του διαλύματος (0.5–1.5%), η θερμοκρασία (25-100°C) και ο χρόνος παραμονής (24-48 h για T=25°C και 30 min–120 min για T=25°C). Η χημική προεπεξεργασία του φρέσκου σόργου σε ένα διάλυμα (HCl, H2SO4) οδήγησε σε αύξηση των διαλυτών σακχάρων μέχρι και 50% αυξάνοντας την θερμοκρασία. Παρόλο αυτά, με χρήση αλκαλικού διαλύματος (NaOH) τα σάκχαρα αυξήθηκαν κατά 25% σε T=37°C, ενώ περαιτέρω αύξηση της θερμοκρασίας συνέβαλλε στην μείωση αυξήσεων (30% μείωση) πιθανότατα λόγω των αντιδράσεων Μαίλλαρ (13-14). Η χημική επεξεργασία του ενσιρωμένου σόργου οδήγησε σε διαφορετικά αποτελέσματα σε σύγκριση με το φρέσκο σόργο. Πιο συγκεκριμένα, η οξιές επεξεργασίας του είχε ως αποτέλεσμα την αύξηση των σακχάρων μέχρι και 200% σε θερμοκρασία 50°C και 3% CTec2 και 0.8% HTec2. Ωστόσο, εκτιμώντας το κόστος και τα αντίστοιχα έσοδα που προκύπτουν σε κάθε μία περίπτωση επεξεργασίας σε σοργό αυξήθηκαν κατά 580% ακόμα και σε χαμηλές θερμοκρασίες.

Στη συνέχεια, μελετήθηκε η επίδραση της ενζυμικής υδρόλυσης στην διαλυτοποίηση των σακχάρων του φρέσκου γλυκού σόργου. Η βέλτιστη ενζυμική υδρόλυση όσον αφορά την απόδοση σε αύξηση των σακχάρων παρατηρήθηκε σε T=50°C, pH 5.50, χρόνο επαφής 12 h και συγκέντρωση ενζύμων 3% and 0.8% CTec2 και HTec2. Ωστόσο, εκτιμώντας το κόστος και τα αντίστοιχα έσοδα που προκύπτουν σε κάθε μία περίπτωση επεξεργασίας σε σοργό αυξήθηκαν κατά 580% ακόμα και σε χαμηλές θερμοκρασίες.

Επόμενος στόχος ήταν η μελέτη προεπεξεργασίας ενσιρωμένου σόργου με σκοπό την επεξεργασία του σοργου σε αναερόβια συγχώνευση με αγροτο-βιομηχανικά απόβλητα. Βάσει των προηγούμενων αποτελεσμάτων και σύμφωνα με τη σχετική βιβλιογραφία [15-16] επιλεγμένη η αλκαλική επεξεργασία (8% NaOH-0.5% KOH and 1%NaOH-1% KOH (Σχήμα 2(α) και (β)), συγκέντρωση 1% σακχάρων, 0.5% NaOH και 0.15 M KCl, αντίστοιχα για να μην υπάρξει παρεμπόδιση στους μεθανογόνους [17].

Η επεξεργασία του ενσιρωμένου σόργου σε σύγκριση με το φρέσκο σόργο οδήγησε σε υδρόλυση των σακχάρων με μέγιστη απόδοση (352.74%) σε θερμοκρασία 80°C για 2 h. Στη συγκέντρωση 0.5% NaOH-0.5% KOH οδήγησε σε υδρόλυση των σακχάρων με μέγιστη απόδοση (352.74%) σε θερμοκρασία 80°C για 2 h. Στη συγκέντρωση 0.5% NaOH-0.5% KOH, αύξηση της διαλυτοποίησης επιτεύχθηκε κατά 561.13% ομοίως στους 80°C για 2 h. Σκοπός επίσης της επεξεργασίας είναι η αλλαγή των ιδιοτήτων του υλικού, κάτι για το οποίο η αλκαλική υδρόλυση είναι επιλέξιμη [15]. Σημαντική μείωση της λιγνίνης, της τάξης του 36.87% και 42.33%, παρατηρήθηκε έπειτα από την επεξεργασία του σόργου στους
80°C για 2 h και σε διάλυμα 0.5%NaOH-0.5%KOH and 1%NaOH-1%KOH, αντίστοιχα.

Η αύξηση των διαλυτών σακχάρων του σόργου σε συνδυασμό με την απολυγνοποίηση της δομής του συνέβαλε στην αύξηση του μεθανογόνου δυναμικού. Πιο συγκεκριμένα, το μεθανογόνο δυναμικό του ενσιρωμένου σόργου υπολογίστηκε ίσο με 301.26 mL CH₄/g VS προστιθέμενο. Έπειτα από την αλκαλική επεξεργασία του με τα διαλύματα 0.5%NaOH-0.5%KOH and 1%NaOH-1%KOH, το μεθανογόνο δυναμικό αυξήθηκε σε 333.48 και 354.51 mL CH₄/g VS προστιθέμενο, αντίστοιχα. Τέλος, ενσίρωση εργαστηριακής κλίμακας πραγματοποιήθηκε σε δύο διαφορετικές ποικιλίες φρέσκου σόργου, με σκοπό τη μελέτη της διεργασίας όσον αφορά τη μετατροπή των υδατανθράκων σε ζυμωτικά προϊόντα όπως αιθανόλη, γαλακτικό οξύ κτλ. Παρατηρήθηκε διαφορετική κατανομή μεταβολικών προϊόντων μεταξύ των δύο ποικιλιών. Πιο συγκεκριμένα, κατά την ενσίρωση της πρώτης ποικιλίας παράχθηκαν κυρίως αιθανόλη (90 mg/g ξηρού βάρους) και γαλακτικό οξύ (15 mg/g ξηρού βάρους) με ταυτόχρονη παραγωγή βιοαερίου (234 mL). Ωστόσο, κατά την ενσίρωση της δεύτερης ποικιλίας, παράχθηκε μόνο γαλακτικό οξύ (48.5 mg /g ξηρού βάρους) μέσω ομογαλακτικής ζύμωσης. Σημαντικό είναι να αναφερθεί, ότι η ενσίρωση ως μέθοδος προεπεξεργασίας, δεν επηρέασε το μεθανογόνο δυναμικό του υλικού.

![Graph](image.png)

**Σχήμα C.2**: Προσδιορισμός των διαλυτών σακχάρων ύστερα από αλκαλική επεξεργασία του ενσιρωμένου σόργου με (α) 0.5%NaOH-0.5%KOH και (β) 1%NaOH-1%KOH συναρτήσει της θερμοκρασίας και του χρόνου παραμονής.

**C.4.3. Παραγωγή βιοαερίου από αγροτο-βιομηχανικά απόβλητα και γλύκικό σόργο σε σύστημα δύο σταδίων:**

**C.4.3.1. Αναερόβια συγχώνευση αγροτο-βιομηχανικών αποβλήτων σε διφάσιμο σύστημα.**

Πειράματα αναεροβίας συγχώνευσης πραγματοποιήθηκαν χρησιμοποιώντας διαφορετικά μίγματα αγροτο-βιομηχανικών αποβλήτων. Τα πειράματα διεξήχθησαν σε
διβάθμιο σύστημα με αντιδραστήρες συνεχούς λειτουργίας (CSTRs), υπό μεσοφιλικά συνθήκες (37°C) και με συνολικό υδραυλικό χρόνο παραμονής 19 ημέρες (3 d για το στάδιο της οξεογένεσης και 16 d για το στάδιο της μεθανογένεσης). Τέσσερα μίγματα μελετήθηκαν: 55% ελαιοτριβείο-40% τυροκομείο-5% βουστάσιο, 90% τυροκομείο-10% βουστάσιο, 80% ελαιοτριβείο-20% τυροκομείο και 20% ελαιοτριβείο-80% βουστάσιο, τα οποία λειτούργησαν μέχρι μόνιμης κατάστασης. Στο πρώτο μίγμα (55% ελαιοτριβείο-40% τυροκομείο-5% βουστάσιο) παρατηρήθηκε ο μέγιστος ρυθμός παραγωγής μεθανίου (1.35 L CH4/LR·d), με απόδοση μεθανίου 467.53 mL CH4/g VS προστιθέμενο, ενώ το διαλυτό και ολικό COD μειώθηκαν κατά 75.5% και 64%, αντίστοιχα. Εξίσου υψηλός ρυθμός παραγωγής μεθανίου (1.33 L CH4/LR·d) προέκυψε από την χώνευση του μίγματος 90% τυροκομείο-10% βουστάσιο, με απόδοση μεθανίου 658.82 mL CH4/g VS προστιθέμενο, υψηλότερη από το προηγούμενο σενάριο πιθανόν λόγω της ευκολότερης αποδόμησης του αποβλήτου τυροκομείου. Η αποδόμηση του διαλυτού και ολικού COD ήταν εξίσου υψηλή και ίση με 85.2% και 79%, αντίστοιχα. Χρησιμοποιώντας το μίγμα 20% ελαιοτριβείο-80% βουστάσιο, σημειώθηκε παραγωγικότητα ίση με 0.91 L CH4/LR·d, επιτυγχάνοντας 50% μείωση ολικού COD. Λόγω του υψηλού ποσοστού βουστασίου στο μίγμα, η παραγωγικότητα μειώθηκε εφόσον το COD είναι αποδομήσιμο σε μικρότερο ποσοστό σε σύγκριση με τα προηγούμενα σενάρια. Τέλος, κατά την επεξεργασία του μίγματος 80% ελαιοτριβείο-20% τυροκομείο, συσσώρευση των πτητικών λιπαρών οξέων (VFAs) στο στάδιο της μεθανογένεσης ανιχνεύτηκε σε HRTs 16 και 20 d. Για το λόγο αυτό, το HRT αυξήθηκε σε 30 d με σκοπό την μείωση των VFAs και την σταθεροποίηση του αντιδραστήρα. Ο ρυθμός παραγωγής μεθανίου σταθεροποιήθηκε σε 0.67 L CH4/LR·d, ενώ η αποδόση ολικού COD υπολογίστηκε ίση με 291.31 mL CH4/g VS προστιθέμενο. Συμπερασματικά, το απόβλητο βουστασίου λειτούργησε σαν σταθεροποιητή στην αναερόβια συγχώνευση του ελαιοτριβείου και/ή τυροκομείου όσον αφορά την συνεισφορά του στην αλκαλικότητα και στα θρεπτικά.

C.4.3.2. Μελέτη αναερόβιας χώνευσης ενός και δύο σταδίων υγρού αποβλήτου βουστασίου και τυροκομείου.

Παρόλο που η διαδικασία της αναερόβιας χώνευσης σε δύο στάδια υπερτερεί σε σχέση με τη συμβατική διεργασία ενός σταδίου [18], περαιτέρω πειράματα διεξήχθηκαν κριτικά από την έκταση του ελαιοτριβείου και την παραγωγικότητα του αντιδραστήρα. Αρχικά, μελετήθηκε η επεξεργασία του υγρού αποβλήτου σε διβάθμιο σύστημα. Ο οξεογόνος αντιδραστήρας λειτούργησε σε HRT 3 d μόνο για λίγες ημέρες εφόσον η παραγωγικότητα ήταν αμελητέα χωρίς ιδιαίτερη παραγωγή μεταβολικών προϊόντων. Σαν αποτέλεσμα το σύστημα λειτούργησε σε ένα στάδιο μεθανογένεσης (σε HRTs 16 και 20 d) με μέγιστο ρυθμό παραγωγής μεθανίου 1.72 L CH4/LR·d. Στην συνέχεια, το σύστημα ενός σταδίου τροφοδοτήθηκε με απόβλητα τυροκομείου και κατά το πρώτο διάστημα λειτουργίας του αντιδραστήρα η παραγωγικότητα αυξήθηκε επιτυγχάνοντας την αποδόμηση ολικού COD υπολογίστηκε ίση με 1.72 L CH4/LR·d λόγω
της ύπαρξης του βουστασίου από το προηγούμενο σενάριο. Στην πορεία, καθώς ξεπλύθηκε το απόβλητο βουστασίου, παρατηρήθηκε μείωση της παραγωγής βιοαερίου ως αποτέλεσμα της συσσώρευσης οξικού και προπιονικού οξέος. Περαιτέρω αύξηση του HRT δεν βοήθησε στην σταθερότητα των μεθανογόνων μικροοργανισμών, ενώ το pH συνέχισε να μειώνεται κάτω από τα επιθυμητά επίπεδα. Αντίθετα, η λειτουργία ενός συστήματος δύο σταδίων τροφοδοτούμενου με το ίδιο απόβλητο βελτίωσε τη διεργασία και θεωρήθηκε ικανοποιητική για την επεξεργασία αποβλήτου τυροκομείου. Πιο συγκεκριμένα, το ήδη οξεογενοποιημένο τυροκομείο από το πρώτο στάδιο (HRT 3 d) τροφοδοτήθηκε στο μεθανογόνο αντιδραστήρα (HRT 20 d) ο οποίος λειτούργησε σταθερά με ρυθμό παραγωγής μεθανίου ίσο με 0.68 L CH4/L.R·d, ενώ το ολικό αποδοθέθηκε κατά 76%.

C.4.3.3. Μελέτη επίδρασης λειτουργικών παραμέτρων (pH, HRT) στην αναερόβια συγχώνευση αγροτο-βιομηχανικών αποβλήτων και γλυκού σόργου σε διβάθμιο σύστημα.

Το μίγμα 55% ελαιοτριβείο-40% τυροκομείο-5% βουστάσιο επιλέχτηκε ως ένα καλό μίγμα για περαιτέρω μελέτη εφόσον έδωσε ικανοποιητική παραγωγικότητα, σε σύγκριση με τα υπόλοιπα μίγματα που μελετήθηκαν. Στη συνέχεια, μελετήθηκαν δύο επιπλέον μίγματα όπου σόργο προστέθηκε με σκοπό την προσομοίωση λειτουργιών μίας κεντρικής μονάδας αναερόβιας χώνευσης, η οποία θα τροφοδοτείται με τοπικά απόβλητα τα οποία θα αντικατασταθούν σε περίοδο μη εποχικής διαθεσιμότητας. Τα μίγματα αντίστοιχων σε 55% σόργο-40% τυροκομείο-5% βουστάσιο και 95% σόργο-5% βουστάσιο.

Για την βελτιστοποίηση αυτών των μιγμάτων, δύο λειτουργικές παράμετροι (pH και HRT) εξετάστηκαν. Πιο συγκεκριμένα, πειράματα διαλείποντος έργου (batch) έγιναν προκειμένου να διερευνηθεί η επίδραση του pH στην παραγωγή υδρογόνου, ενώ πειράματα συνεχούς λειτουργίας (CSTR) διεξήχθηκαν για την επίδραση του HRT στην παραγωγή υδρογόνου και μεθανίου, υπό μεσοφιλικές συνθήκες (37°C). Για τα πειράματα επίδρασης pH χρησιμοποιήθηκε φρέσκο σόργο, ως διαθέσιμο την στιγμή των πειραμάτων, ενώ στα πειράματα συνεχούς λειτουργίας χρησιμοποιήθηκε επεξεργασμένο σόργο λόγω του ότι σε μία κεντρική μονάδα αναερόβιας επεξεργασίας το σόργο θα πρέπει να είναι ενσιρωμένο για να είναι εφικτή η αποθήκευση και η συντήρηση του. Η μεθόδος προεπεξεργασίας του ενσιρωμένου σόργου που χρησιμοποιήθηκε ήταν θερμο-αλκαλική υδρόλυση (όπως αναφέρθηκε στο Β) με σκοπό την διαλυτοποίηση των υδατανθράκων και την αποκατάσταση της σχηματιζομένης ισχύς που λειτουργεί σαν παρεμπόδιστη στην πρόσβαση των ενζυμών στην κυτταρίνη.

**Μίγμα 55% ελαιοτριβείο-40% τυροκομείο-5% βουστάσιο**

Αρχικά, διεξήχθηκαν batch πειράματα σε διαφορετικές τιμές pH (4.5 - 7.5), το οποίο ρυθμίζονταν να παραμένει σταθερό κατά την διάρκεια των πειράματος, με σκοπό την διερεύνηση της επίδρασης του pH στην παραγωγή υδρογόνου και στη συνεχισμένη προέλαση που προκύπτουν από την οξεογένεση της οξεογενοποίησης του συγκεκριμένου μίγματος. Τα κύρια
μεταβολικά προϊόντα που ανιχνεύτηκαν ήταν το οξικό, προπιονικό, βουτυρικό, γαλακτικό οξύ και η αιθανόλη. Η μέγιστη απόδοση υδρογόνου (Σχήμα 3(α)) παρατηρήθηκε σε pH 6.0 (0.642 mol H₂/mol καταναλ. ισοδ. γλυκόζης), ενώ η μέγιστη παραγωγή πτητικών λιπαρών οξέων (VFAs) σε pH 6.5 (Σχήμα 3(β)). Η παραγόμενη ποσότητα οξικού και βουτυρικού οξέος ήταν παρόμοια σε pH 6.0 και 6.5, μολονότι η συγκέντρωση του προπιονικού οξέος αυξήθηκε με την αύξηση του pH. Το γαλακτικό οξύ προσδιορίστηκε ως ένα κύριο μεταβολικό προϊόν το οποίο παρουσίασε μία έντονη συσσώρευση (μέχρι 11 g/L) σε pH 6.0, πριν από την περαιτέρω μετατροπή του σε βουτυρικό οξύ και υδρογόνο. Η παραγωγή βιοαερίου και κυρίως υδρογόνου συσχετίζεται με την παραγωγή πτητικών λιπαρών οξέων και ιδιαίτερα στην περίπτωση αυτή με την παραγωγή βουτυρικού οξέος.

Στην συνέχεια, διβάθμιο σύστημα χρησιμοποιήθηκε για τη μελέτη της επίδρασης του υδραυλικού χρόνου παραμονής (HRT) στην παραγωγή υδρογόνου και μεθανίου, αντίστοιχα. Ο οξεογόνος αντιδραστήρας τροφοδοτήθηκε με το μίγμα 55% ελαιοτριβείο-40% τυροκομείο-5% βουστάσιο, καθώς η επίδραση του υδραυλικού χρόνου παραμονής μετατρέπεται σε μεγαλύτερη αποδοτικότητα ήταν σαν προϊόν 0.75 d με μέγιστο ρυθμό παραγωγής υδρογόνου 1.72 L/LR·d και απόδοση 0.54 mol H₂/mol καταναλ. υδατανθράκων. Από την άλλη πλευρά, ο μεθανογόνος αντιδραστήρας προσφέρθηκε με το μήκος οξεογενοποιημένο μίγμα από το πρώτο στάδιο και λειτούργησε σε HRTs 20 και 25 d. Σταθερότητα του συστήματος επετεύχθη σε HRT 25 d, ενώ σε μικρότερο HRT 20 d παρατηρήθηκε συσσώρευση πτητικών λιπαρών οξέων, κυρίως οξικού οξέος (Σχήμα 4(β)). Ο ρυθμός παραγωγής μεθανίου σε μόνιμη κατάσταση σε HRT 25 d βρέθηκε ίσος με 0.33 L CH₄/LR·d (Σχήμα 4(α)).
Σχήμα C.4: Η παραγωγή (α) βιοαερίου και μεθανίου και (β) των κύριων μεταβολικών προϊόντων, στην αναερόβια συγχώνευση μίγματος 55% ελαιοτριβείο-40% τυροκομείο-5% βουστάσιο.

Μίγμα 55% σόργο-40% τυροκομείο-5% βουστάσιο

Σαν συνέχεια του προηγούμενου μίγματος μελετήθηκε το μίγμα 55% σόργο-40% τυροκομείο-5% βουστάσιο. Στην περίπτωση αυτή έγινε αντικατάσταση του αποβλήτου ελαιοτριβείου από γλυκό σόργο που αντιπροσωπεύει την περίοδο όπου το απόβλητο ελαιοτριβείου δεν είναι διαθέσιμο. Ομοίως, αρχικά πραγματοποιήθηκαν batch πειράματα με απότερο στόχο την μελέτη της επίδρασης του pH (5.0, 5.5, 6.0, 6.5) στην παραγωγή υδρογόνου και μεταβολικών προϊόντων. Στα συγκεκριμένα batch πειράματα χρησιμοποιήθηκε φρέσκο σόργο. Σύμφωνα με τα αποτελέσματα που προέκυψαν, το pH 5.5 θεωρήθηκε ως βέλτιστο με τη μέγιστη απόδοση υδρογόνου 0.52 mol H2/mol catanal. Ισοδ. γλυκόζης (Σχήμα 5(α)), ενώ η αποδόμηση των υδατανθράκων παρατηρήθηκε μέγιστη σε pH 6.5. Επιπλέον πείραμα στο βέλτιστο pH έδειξε ότι χρήση θερμικά επεξεργασμένης αναεροβικής μαγιάς είχε θετική επίδραση στην παραγωγή υδρογόνου (1.09 mol H2/mol καταναλ. Ισοδ. γλυκόζης) σαν αποτέλεσμα της εξάλειψης κυκλικών μεταβολικών προϊόντων για τις χαμηλές αποδόσεις σε υδρογόνο. Σε όλα τα πειράματα που έλαβαν χώρα, τα κύρια μεταβολικά διαλύτα προϊόντα ήταν τετρατοκατάλοιπο αεερίου (p.χ. αλκοόλ, προπιονικό και βουτυρικό οξύ), αθυαλόλη και γαλακτικό οξύ (Σχήμα 5(β)). Και σε αυτή την περίπτωση μίγματος, το γαλακτικό οξύ λειτουργεί σαν ενδιάμεσο μεταβολικό προϊόν με τελικά παραγόμενα προϊόντα το βουτυρικό οξύ και το υδρογόνο. Η διαδικασία θερμικής επεξεργασίας της αναεροβικής μαγιάς οδήγησε στην κυριαρχία διαφορετικών μεταβολικών μονοπατιών για τη παραγωγή γαλακτικού οξέος που συνέβαλλε σε υψηλότερη απόδοση υδρογόνου.
Σαν συνέχεια, και λαμβάνοντας υπόψη τα παραπάνω αποτελέσματα, πραγματοποιήθηκε η διερεύνηση της επίδρασης του ΗΤΡ σε διβάθμιο σύστημα. Στην συγκεκριμένη μελέτη, η απόδοση υδρογόνου και η παραγωγή μεταβολικών προϊόντων συναρτήθηκε κατά την διάρκεια των διαφόρων pH που μελετήθηκαν στο μίγμα 55% σόργο-40% τυροκομείο-5% βουστάσιο.

Σχήμα C.5: (α) Η απόδοση υδρογόνου και (β) τα κύρια μεταβολικά προϊόντα, συναρτήσει των διαφόρων pH που μελετήθηκαν στο μίγμα 55% σόργο-40% τυροκομείο-5% βουστάσιο.

Σε συγκεκριμένο μικρότερο pH, η παραγωγή υδρογόνου και η παραγωγή μεταβολικών προϊόντων επετεύχθη σε ΚΛΟΝΟ (C.5) με τη μέγιστη απόδοση υδρογόνου (0.90 mol H2/mol καταναλ. υδατανθράκων) και τη μέγιστη παραγωγή μεθανίου (0.90 L CH4/LR·d) σε ΚΛΟΝΟ (C.6). Επίσης, η διεργασία παρεμπόδιστη λόγω συσσώρευσης πτητικών λιπαρών οξέων (Σχήμα C.5(β)) με αποτέλεσμα την αποτυχία του συστήματος.

Σχήμα C.5 : (α) Η απόδοση υδρογόνου και (β) τα κύρια μεταβολικά προϊόντα, συναρτήσει των διαφόρων pH που μελετήθηκαν στο μίγμα 55% σόργο-40% τυροκομείο-5% βουστάσιο.
διαφορετικές τιμές του pH (4.5, 5.0, 5.5, 6.0), ενώ σε όλη τη διάρκεια του κάθε πειράματος καταγράφηκε η παραγωγή υδρογόνου και των μεταβολικών προϊόντων. Η μέγιστη απόδοση υδρογόνου (0.92 mol H₂/mol καταναλ. ισοδ. γλυκόζης) παρατηρήθηκε σε pH 5.0 (Σχήμα 7(a)), ενώ η μέγιστη αποδόμηση των υδατανθράκων επετεύχθη σε pH 6.0. Τα μεταβολικά προϊόντα που κυριάρχησαν ήταν τα ίδια με τα προηγούμενα πειράματα, ενώ η μέγιστη συγκέντρωση βουτυρικού οξέος παρατηρήθηκε σε pH 5.0 (Σχήμα 7(b)).

Σχήμα C.6 : Η παραγωγή (α) βιοαερίου και μεθανίου και (β) των κύριων μεταβολικών προϊόντων, στην αναερόβια συγχώνευση μίγματος 55% σόργο-40% τυροκομείο-5% βουστάσιο.

Σχήμα C.7 : (α) Η απόδοση υδρογόνου και (β) τα κύρια μεταβολικά προϊόντα, συναρτήσει των διαφόρων pH που μελετήθηκαν στο μίγμα 95% σόργο-5% τυροκομείο-5% βουστάσιο.

Στη συνέχεια, σε διβάθμιο σύστημα μελετήθηκε η επίδραση του HRT στην παραγωγή υδρογόνου και μεθανίου από την επεξεργασία του μίγματος 95% σόργο-5% βουστάσιο, όπου το σόργο στην περίπτωση αυτή ήταν ενσιρωμένο και επεξεργασμένο με διάλυμα 0.5%NaOH-0.5%KOH σε θερμοκρασία 80°C για 2 h (βλέπε
παράγραφο Β). Στην οξεογένεση μελετήθηκαν τρία HRTs (3, 5 και 8 d), όπου ο μέγιστος ρυθμός παραγωγής υδρογόνου παρατηρήθηκε σε HRT 5 d και ήταν ίσος με 0.13 L/LR·d ενώ η απόδοση υδρογόνου εκτιμήθηκε 1.68 mol H₂/mol καταναλ. υδατανθράκων. Από την άλλη πλευρά, στο στάδιο της μεθανογένεσης, ο αντιδραστήρας λειτούργησε σε HRTs 20 και 25 d. Ο μέγιστος ρυθμός παραγωγής μεθανίου (0.44 L CH₄/LR·d) επετεύχθη σε HRT 25 d (Σχήμα 8(α)) με απόδοση 295.3 mL/g VS προστιθέμενο, ενώ σε μικρότερο HRT 20 d η διεργασία παρεμπόδιστη λόγω συσσώρευσης πτητικών λιπαρών οξέων (κυρίως οξικού οξέος) (Σχήμα 8(β)) με αποτέλεσμα τη μείωση του pH με ταυτόχρονη μείωση της παραγωγικότητας (Σχήμα 8(α)).

Σχήμα C.8: Η παραγωγή (α) βιοαερίου και μεθανίου και (β) των κύριων μεταβολικών προϊόντων, στην ανεαρόβια συγχώνευση μίγματος 95% σόργο-5% βουστάσιο.

C.4.4. Επιπτώσεις της χρήσης του ελαιοτριβείου στην ανεαρόβια συγχώνευση.

Όπως έχει προαναφερθεί, η συγχώνευση αποβλήτων βελτιώνει την απόδοση σε βιοαέριο λόγω των θετικών συνεργιών που λαμβάνουν χώρα καθώς και με τη συνεισφορά θρεπτικών που υπάρχουν σε έλλειψη. Πειράματα διαλείποντος έργου πραγματοποιήθηκαν στο στάδιο οξεογένεσης και μεθανογένεσης, με σκοπό την μελέτη της συνεισφοράς της συγχώνευσης στην παραγωγή βιοαερίου καθώς και την επίδραση της χρήσης του αποβλήτου ελαιοτριβείου στην συγχώνευση.

Αρχικά, διεξήχθηκαν πειράματα με μεμονωμένα υποστρώματα ελαιοτριβείου, τυροκομείου και βουστασίου σε pH 6.0 με σκοπό την εκτίμηση μιας θεωρητικής απόδοσης και την σύγκριση της με την πειραματική απόδοση του μίγματος 55% ελαιοτριβείο-40% τυροκομείο-5% βουστάσιο (0.642 mol H₂/mol καταναλ. ισοδ. γλυκόζης). Το Σχήμα 9(α) παρουσιάζει την απόδοση υδρογόνου που προέκυψε για κάθε ένα απόβλητο καθώς και την εκτιμώμενη και πειραματική απόδοση του μίγματος. Η θεωρητική απόδοση του υπολογίστηκε λαμβάνοντας υπόψη τις αποδόσεις των μεμονωμένων πειραμάτων ήταν μικρότερη από την πειραματική και ίση με 0.401 mol H₂/mol καταναλ. ισοδ. γλυκόζης. Στην συνέχεια, πραγματοποιήθηκαν batch πειράματα μεμονωμένων υποστρώματων φρέσκου σόργου, τυροκομείου και βουστασίου σε pH 5.5
με σκοπό τη σύγκριση αυτή τη φορά με το μίγμα 55% φρέσκο σόργο-40% τυροκομείο-5% βουστάσιο. Στην συγκεκριμένη περίπτωση, η εκτιμώμενη απόδοση του μίγματος (0.51 mol H₂/mol καταν. ισοδ. γλυκόζης) ήταν παρόμοια με την πειραματική (0.52 mol H₂/mol καταν. ισοδ. γλυκόζης) θεωρώντας ότι καμία συνέργεια δεν λαμβάνει χώρα στο συγκεκριμένο μίγμα (Σχήμα 9(β)). Σε αντίθεση, το μίγμα αποτελούμενο από απόβλητο ελαιοτριβείου παρουσίασε σημαντική διαφορά μεταξύ εκτιμώμενης και πειραματικής απόδοσης και αυτό λογικά οφείλεται στα χαρακτηριστικά του ελαιοτριβείου.

Επειτα από το στάδιο της οξεογένεσης, πραγματοποιήθηκαν batch πειράματα μεθανογένεσης χρησιμοποιώντας τα δύο μίγματα που προαναφέρθηκαν, οξεογενοποιημένα αυτή τη φορά, σε δύο διαφορετικές αρχικές συγκέντρωσεις οργανικής φόρτισης. Χρησιμοποιώντας το οξεογενοποιημένο μίγμα 55% ελαιοτριβείο-40% τυροκομείο-5% βουστάσιο, διαφορά στον ρυθμό παραγωγής μεθανίου παρατηρήθηκε μεταξύ των δύο αρχικών συγκεντρώσεων φόρτισης. Πιο συγκεκριμένα, στην μισή αρχική φόρτιση ο ρυθμός παραγωγής μεθανίου ήταν μεγαλύτερος, ενώ ήταν εξίσου μεγαλύτερη η απόδοση μεθανίου (323.62 mL CH₄/g VS προστιθέμενο) και η αποδόμηση COD (50.2%), υποδηλώνοντας παρεμπόδιση υποστρώματος σε υψηλότερη συγκέντρωση οργανικής φόρτισης. Επίσης, σημαντική διαφορά παρουσίασε η αποδόμηση των φαινολών, όπου με μείωση της οργανικής φόρτισης στο μισό παρατηρήθηκε αύξηση της αποδόμησης φαινολών από 17.9% σε 40%. Πολύ πιθανόν η παρεμπόδιση σε υψηλή αρχική συγκέντρωση να οφείλεται στην μεγαλύτερη αρχική συγκέντρωση φαινολών στο μίγμα. Από την άλλη πλευρά, στην επεξεργασία του οξεογενοποιημένου μίγματος 55% φρέσκο σόργο-40% τυροκομείο-5% βουστάσιο, δεν παρατηρήθηκε ιδιαίτερη διαφορά στον ρυθμό παραγωγής μεθανίου. Ωστόσο, υπήρχε μικρή διαφορά στην απόδοση μεθανίου, όπου η μεγαλύτερη επετεύχθη στην μισή οργανική φόρτιση.

Σχήμα C.9 : Συγκριτική απόδοση υδρογόνου μεταξύ (α) ελαιοτριβείου, τυροκομείου, βουστασίου, εκτιμώμενου και πειραματικού μίγματος σε pH 6.0 και (β) φρέσκου σόργου, τυροκομείου, βουστασίου, εκτιμώμενου και πειραματικού μίγματος σε pH 5.5
C.4.5. Ανάπτυξη μαθηματικού μοντέλου για την προβλέψη παραγωγής μεθανίου.

Το μοντέλο αναερόβιας χώνευσης (ADM1) σχεδιάστηκε από μία ομάδα ερευνητών (International Water Association’s Task Group, 2012) με σκοπό τη μαθηματική μοντελοποίηση της διεργασίας της Αναερόβιας Χώνευσης. Είναι ένα δομημένο μαθηματικό μοντέλο, το οποίο περιλαμβάνει όλα τα στάδια της αναερόβιας χώνευσης (υδρόλυση, οξεογένεση, οξικογένεση και μεθανογένεση) που λαμβάνουν χώρα κατά την μετατροπή των σύνθετων οργανικών υποστρωμάτων σε μεθάνιο, διοξείδιο του άνθρακα και αδρανή προϊόντα [19].

Η εξωκυτταρική διαλυτοποίηση περιλαμβάνει την διάσπαση και την υδρόλυση, από τις οποίες η πρώτη είναι ένα μεγάλο μη βιολογικό βήμα που μετατρέπει το σύνθετο, σωματιδιακό, οργανικό υπόστρωμα σε αδρανή, υδατάνθρακες, πρωτεΐνες και λίπη. Το δεύτερο βήμα είναι η ενζυματική υδρόλυση των υδατανθράκων, πρωτεΐνων και λιπιδίων σε μονοσακχαρίτες, αμινοξέα και λιπαρά οξέα μεγάλου μοριακού βάρους (LCFA), αντίστοιχα. Όλα τα στάδια της διάσπασης και της υδρόλυσης περιγράφονται με κινητική πρώτης τάξης. Στη συνέχεια στο στάδιο της οξεογένεσης, οι μονοσακχαρίτες, τα αμινοξέα και τα λιπαρά οξέα μεγάλου μοριακού βάρους αποδομούνται από δυο ξεχωριστές ομάδες οξεογόνων, παράγοντας πτητικά λιπαρά οξέα μεγάλου μοριακού βάρους, υδρογόνο και διοξείδιο του άνθρακα. Ακολούθως στο στάδιο της οξικογένεσης τα οξικολυτικά μεθανογόνα βακτηρία (χρήστες οξικού), που καταναλώνουν οξικό οξύ και από μια ειδική ομάδα μεθανογόνων βακτηρίων (χρήστες υδρογόνου), που καταναλώνουν υδρογόνο [19]. Για τις ενδοκυτταρικές βιοχημικές αντιδράσεις που περιγράφουν την κατανάλωση των υποστρωμάτων χρησιμοποιείται κινητική τύπου Monod, ενώ ο θάνατος της βιομάζας αναπαρίσταται με κινητική πρώτης τάξης.

Στην συγκεκριμένη διατριβή, η εφαρμογή του μαθηματικού μοντέλου ADM1 έγινε με το λογισμικό AQUASIM 2.0 [20], ενώ χρησιμοποιήθηκε για την προσομοίωση μεθανίου από αναερόβια συγχώνευση σε μεσόφιλες συνθήκες. Δεδομένου ότι το μοντέλο δεν λαμβάνει υπόψη την παραγωγή διαφόρων σημαντικών μεταβολικών προϊόντων, όπως το γαλακτικό οξύ, η αιθανόλη και το καπροϊκό οξύ, η δομή του μοντέλου τροποποιήθηκε με σκοπό την βελτίωση της προσομοίωσης. Έπειτα από τις κατάλληλες τροποποιήσεις, το μοντέλο εφαρμόστηκε σε πειραματικά δεδομένα αντιδράσεων υποστρωμάτων σε μεταβολικές συνθήκες. Δεδομένου ότι το μοντέλο δεν λαμβάνει υπόψη την παραγωγή διαφόρων ομάδων μεθανογόνων βακτηρίων, η θάνατος της βιομάζας αναπαρίσταται με κινητική πρώτης τάξης. Τα τέσσερα μίγματα που χρησιμοποιήθηκαν για την προσομοίωση ήταν: 55% ελαιοτριβείο - 5% βουστάσιο, 90% τυροκομείο - 10% βουστάσιο, 20% ενσιρωμένο σόργο - 80% βουστάσιο, 55% ελαιοτριβείο - 40% βουστάσιο, 20% ενσιρωμένο σόργο - 80% βουστάσιο, 55% ελαιοτριβείο - 40% βουστάσιο. Τα τρία πρώτα σενάρια λειτουργίας με HRT 19 d, ενώ το τελευταίο μελετήθηκε σε τρία διαφορετικά HRTs 24, 16 και 12 d. Τα αποτελέσματα που προέκυψαν από την προσομοίωση έδειξαν ότι το τροποποιημένο
μοντέλο ήταν σε θέση να προβλέψει σε ικανοποιητικό επίπεδο την παραγωγικότητα σε βιοαέριο και μεθάνιο, καθώς και τις τιμές pH και πτητικών λιπαρών οξέων. Συγκεκριμένα, το μοντέλο προέβλεπε ικανοποιητικά και την μεταβολή της δυναμικής συμπεριφοράς του αντιδραστήρα κατά την επεξέργασία του μίγματος 55% ενσιρωμένο σόργο-40% τυροκομείο-5% βουστάσιο σε τρεις διαφορετικές οργανικές φορτίσεις, όπως την συσσώρευση των πτητικών λιπαρών οξέων (Σχήμα 10(γ)) σε HRT 12 d με αποτέλεσμα τη μείωση του pH (Σχήμα 10(β)) και της παραγωγικότητας σε μεθάνιο (Σχήμα 10(α)).

Σχήμα C.10: Τα πειραματικά δεδομένα και η πρόβλεψη του μοντέλου (α) στην παραγωγή βιοαερίου και μεθανίου, (β) στο pH και (γ) στην συγκέντρωση των κύριων πτητικών λιπαρών οξέων, κατά τη διάρκεια μεθανογένεσης του μίγματος 55% ενσιρωμένο σόργο-40% τυροκομείο-5% βουστάσιο.

C.4.6. Μετα-επεξεργασία της αναερόβιας απορροής μεθανογόνου αντιδραστήρα.

Είναι γνωστό ότι σημαντικό ρυπαντικό φορτίο παραμένει στην επεξεργασμένη απορροή έπειτα από την αναεροβική χώνευση με την ανάγκη περαιτέρω επεξεργασίας πριν τη διάθεση του στο περιβάλλον. Η αναεροβική συγχώνευση αγρο-βιομηχανικών αποβλήτων οδηγεί σε υγρά και στερεά παραπροϊόντα, τα οποία διατηρούν τα περισσότερα από τα θρεπτικά συστατικά τους (άζωτο, φώσφορος, ιχνοστοιχεία, κ.λπ.) και έτσι μπορούν να χρησιμοποιηθούν ως λιπάσματα και εδαφονευτικά.

Μία σημαντική μέθοδος μετα-επεξεργασίας αναερόβιας απορροής είναι ο διαχωρισμός με σύστημα μεμβρανών (υπερδιήθησης και νανοδιήθησης). Πριν το διαχωρισμό με μεμβράνες είναι απαραίτητο ένα στάδιο απομάκρυνσης των μεγάλων αιωρούμενων στερεών. Μια αποτελεσματική μέθοδος είναι η κροκίδωση με τη χρήση πολυηλεκτρολυτών, τεχνική που χρησιμοποιείται ευρέως για την επεξεργασία βιομηχανικών αποβλήτων [21]. Με την προσθήκη της κατάλληλης δόσης πολυηλεκτρολυτή, το COD και τα ολικά στερεά (TS) μπορούν να μειωθούν σημαντικά. Αρχικά εξετάστηκε η επίδραση της συγκέντρωσης του πολυηλεκτρολύτη poly-(diallyldimethylammonium chloride) στην κροκίδωση της χωνευμένης απορροής. Τα πειράματα πραγματοποιήθηκαν με μεταβαλλόμενη συγκέντρωση πολυηλεκτρολύτη (0.5-4.0 g/L). Η μέγιστη απομάκρυνση COD (52.53%) επετεύχθη σε συγκέντρωση του πολυηλεκτρολύτη 1.5 g/L. Στην συνέχεια, το διαλυτό μέρος από τη διαδικασία κροκίδωσης/καθίζησης χρησιμοποιήθηκε για περαιτέρω επεξεργασία σε σύστημα
μεμβρανών μικρής κλίμακας. Πιο συγκεκριμένα, έπειτα από την υπερδιήθηση το COD μειώθηκε κατά 36.38%, ενώ το διήθημα που προέκυψε μετά από τη μεμβράνη νανοδιήθησης χαρακτηρίστηκε από πιο χαμηλό COD (2.46 g/L) μειωμένο κατά 35.77%. Συμπερασματικά, η συνδυασμένη επεξεργασία αναεροβίας χόνευσης και μεμβρανών αποδείχτηκε αρκετά αποτελεσματική και σε σύνδεση με τις μεμονωμένες διεργασίες. Το τελικό διήθημα που προκύπτει θα μπορούσε να χρησιμοποιηθεί σε ανακυκλωσία με σκοπό την αραίωση των αποβλήτων πριν την αναεροβία χόνευση ή ακόμα και για αρδευτικούς σκοπούς.

Από την άλλη πλευρά, μελετήθηκε κομποστοποίηση με χρήση γαιοσκωλήκων Eisenia fetida για την παραγωγή καλής ποιότητας κομπόστ και με σκοπό τη διερεύνηση της βιοσταθεροποίησης αναεροβίας υλών προκύπτοντας από βιολογικό σταθμό και από αναεροβία και απορροή από μεθανογόνο αντιδραστήρα. Η διαδικασία πραγματοποιήθηκε σε στερεά κοπριά βουστασίου και σε μίγματα αυτών με αναεροβία υλών προκύπτοντας από μεθανογόνο αντιδραστήρα. Η διαδικασία πραγματοποιήθηκε με χρήση γαιοσκωλήκων συνεβαλλόταν στην μείωση του pH και των πτητικών στερεών του υποστρώματος και στις περισσότερες περιπτώσεις παρατηρήθηκε αύξηση των συγκεκριμένων παράγοντων κομπόστ.

C.4.7. Τεχνο-οικονομική εκτίμηση μίας κεντρικής μονάδας αναεροβίας επεξεργασίας.

Σαν τελευταίο κομμάτι για την ολοκλήρωση της διατριβής πραγματοποιήθηκε τεχνο-οικονομική μελέτη για την εγκατάσταση και λειτουργία μιας κεντρικής μονάδας αναεροβίας χόνευσης (ηλεκτρικής ισχύος 1.0 MW) η οποία θα τροφοδοτείται με αγροτο-βιομηχανικά απόβλητα και πιο συγκεκριμένα μίγμα αυτών σε αναλογία 55% ελαιοτριβείο-40% τυροκομείο-5% βουστάσιο. Η ολοκληρωμένη μονάδα διαχείρισης βασίστηκε σε δύο αλληλοσυμπληρώμενες τεχνολογίες: την αναεροβία χόνευσης, όπου παράγεται βιοαέριο και στην συνέχεια αξιοποιείται για παραγωγή ενέργειας (ηλεκτρικής και θερμότητας) και την κομποστοποίηση με στόχο την παραγωγή προϊόντων που μπορούν να αξιοποιηθούν στη γεωργία ως εδαφοβελτιωτικά.

Ο βασικός στόχος της μελέτης αυτής ήταν η καταγραφή των απαιτήσεων σε κτηριακές εγκαταστάσεις και σχετικό εξοπλισμό μιας μονάδας που να διαθέτει/συνδυάζει και τις δύο προαναφερθείσες τεχνολογίες, η εκτίμηση του ύψους των επενδύσεων που θα απαιτηθούν, του λειτουργικού κόστους καθώς και των εισροών από την παραγωγή ηλεκτρικής ενέργειας και πώλησης ανακυκλωμένων υλικών και παραγόμενου κομπόστ και τέλος ο βασικός προσδιορισμός των χαρακτηριστικών εκείνων που θα πρέπει να έχει ένα υπολογιστικό ιδιότητα επενδύσεως ώστε να μπορεί να φέρει σε πέρας το έργο, σε σχέση με τη νέα νομοθεσία για τις Ανανεώσιμες Πηγές Ενέργειας (ΑΠΕ).

Τα συμπεράσματα που προέκυψαν από αυτή τη μελέτη ήταν ότι μια ολοκληρωμένη μονάδα διαχείρισης αγροτο-βιομηχανικών αποβλήτων αποτελεί μια πολύ καλή
επιστημονικά, τεχνολογικά και κυρίως περιβαλλοντικά λύση στο θέμα της διαχείρισης των αποβλήτων αυτών. Μπορούν να διαχειριστούν 105,000 τόνοι αποβλήτων ανά έτος (εξαρτάται από τη σύσταση αυτών), με παραγωγή 9,230 MWh ηλεκτρικής ενέργειας και 10,860 MWh θερμικής ενέργειας σε ετήσια βάση, ενώ ταυτόχρονα παράγονται και 5,213 τόνοι καλής ποιότητας κομπόσ τ. Το συνολικό ύψος επένδυσής είναι 5,008,775 €, ενώ σε ετήσια βάση το κόστος λειτουργίας και συντήρησης είναι 630,000 €. Το συνολικό ύψος εσόδων από αξιοποίηση ενέργειας και υλικών εκτιμήθηκε 2,190,030 € ανά έτος (όταν υπάρχει επιδότηση) και 2,479,439 € ανά έτος (όταν δεν υπάρχει επιδότηση). Ο χρόνος αποπληρωμής της επένδυσης υπολογίστηκε μεταξύ τριών και τεσσάρων ετών.

C.5. ΣΥΜΠΕΡΑΣΜΑΤΑ

Στην παρούσα διατριβή μελετήθηκε η αναερόβια (συν)-χώνευση αγροβιομηχανικών αποβλήτων όπως είναι απόβλητα ελαιοτριβείου, τυροκομείου και βουστασίου. Τα συγκεκριμένα απόβλητα χαρακτηρίζονται από υψηλό οργανικό φορτίο το οποίο τα καθιστά ακατάλληλα για απευθείας διάθεση στο περιβάλλον. Τα απόβλητα ελαιοτριβείου χαρακτηρίζονται από υψηλή συγκέντρωση φαινολικών ενώσεων και στερεών που τα καθιστούν ιδιαίτερα τοξικά (φυτοτοξικά), τα απόβλητα τυροκομείου χαρακτηρίζονται από υψηλή συγκέντρωση υδατανθράκων, ενώ τα απόβλητα βουστασίου περιέχουν υψηλή συγκέντρωση αζώτου που αποτελεί σημαντικό περιβαλλοντικό ρύπο. Αναερόβια συγχώνευση αυτών οδήγησε σε υψηλές αποδόσεις μεθανίου, μεγαλύτερες από αυτές των μεμονωμένων αποβλήτων, κάτι το οποίο οφείλεται σε συνεργαστικές επιδράσεις όπως η συμβολή επιπλέον αλκαλικότητας, ιχνοστοιχείων, θρεπτικών κτλ.

Στη συνέχεια, πραγματοποιήθηκε μελέτη αναερόβιας συγχώνευσης τεσσάρων διαφορετικών σεναρίων (μίγμα 55% ελαιοτριβείο-40% τυροκομείο-5% βουστάσιο, μίγμα 90% τυροκομείο-10% βουστάσιο, μίγμα 20% ελαιοτριβείο-80% βουστάσιο και μίγμα 80% ελαιοτριβείο-20% τυροκομείο), σε διβάθμιο σύστημα οξεογένεσης και μεθανογένεσης. Σε όλα τα πειράματα παρατηρήθηκε αποδόμηση ολικών και διαλυτών υδατανθράκων με ταυτόχρονη παραγωγή πτητικών οξέων, κυρίως οξικό οξύ, ενώ δεν παρατηρήθηκε παραγωγή υδρογόνου κατά την οξεογένεση. Ωστόσο κατά την αναερόβια μεθανογένεση, η μέγιστη παραγωγικότητα σε μεθανίο παρατηρήθηκε στα μίγματα 55% ελαιοτριβείο-40% τυροκομείο-5% βουστάσιο και 90% τυροκομείο-10% βουστάσιο (1.35 και 1.33 L CH4/LR/d, αντίστοιχα). Αν και είναι γνωστό ότι η αναερόβια χώνευση σε δύο στάδια υπερτερεί σε σχέση με τη συμβατική διεργασία ενός σταδίου, περαιτέρω πειράματα διεξήχθησαν χρησιμοποιώντας απόβλητα τυροκομείου και βουστασίου. Η διεργασία δύο σταδίων οδήγησε σε καλύτερα αποτελέσματα σε σύγκριση με τη συμβατική του ενός σταδίου μόνο στην επεξεργασία του τυροκομείου και όχι του βουστασίου. Αυτό οφείλεται πολύ πιθανό στο γεγονός ότι το απόβλητο τυροκομείου είναι ένα εύκολο αποδομήσιμο υπόστρωμα και χρειάζεται διαχωρισμόν των μικροοργανισμών για μία πιο αποτελεσματική και σταθερή διεργασία.
Η συμπεριφορά του σταδίου της οξεογένεσης είναι αρκετά κρίσιμη και καθοριστική για το μετέπειτα στάδιο της μεθανογένεσης. Είναι γνωστό ότι λειτουργικές παράμετροι όπως pH, θερμοκρασία και υδραυλικός χρόνος παραμονής (HRT) επηρεάζουν σημαντικά την ζύμωση και την παραγωγή μεταβολικών προϊόντων. Επιλέχτηκε το μίγμα 55% ελαιοτριβείο-40% τυροκομείο-5% βουστάσιο για τη μελέτη της επίδρασης του pH και του HRT, καθώς επίσης και δύο ακόμα μίγματα 55% σόργο-40% τυροκομείο-5% βουστάσιο και 95% σόργο-5% βουστάσιο, στα οποία έχει γίνει η αντικατάσταση των εποχικών αποβλήτων από γλυκό σόργο. Αρχικά μελετήθηκε η επίδραση του pH στην παραγωγή υδρογόνου το οποίο είχε σημαντική επηρεαστεί στην κατανομή των μεταβολικών προϊόντων και κατά συνέπεια στην απόδοση υδρογόνου. Το γαλακτικό οξύ θεωρείται ως ενδιάμεσο προϊόν εφόσον πρώτα παράγεται και στη πορεία μεταβολίζεται κυρίως σε βουτυρικό οξύ με ταυτόχρονη παραγωγή υδρογόνου. Ισοζύγια μάζας πραγματοποιήθηκαν ωστόσο, για την διερεύνηση των μεταβολικών μονοπατιών που έλαβαν χώρα στη διεργασία. Η βέλτιστη τιμή του pH βρέθηκε να εξαρτάται από το υπόστρωμα και πιο συγκεκριμένα ίση με pH 6.0 για το μίγμα 55% ελαιοτριβείο-40% τυροκομείο-5% βουστάσιο, pH 5.5 για το μίγμα 55% σόργο-40% τυροκομείο-5% βουστάσιο και pH 5.0 για το μίγμα 95% σόργο-5% βουστάσιο. Στη συνέχεια, μελετήθηκε η επίδραση του HRT στην παραγωγή υδρογόνου και μεθανίου σε σύστημα δύο σταδίων, χρησιμοποιώντας και τα τρία μίγματα. Στο στάδιο της οξεογένεσης, η μέγιστη παραγωγή υδρογόνου παρατηρήθηκε σε HRT 0.75 d, ενώ η παραγωγή μεθανίου ήταν μέγιστη σε HRT 25 d. Επεξεργάζοντας το δεύτερο μίγμα, όπου γλυκό σόργο χρησιμοποιήθηκε στη θέση του ελαιοτριβείου, η μέγιστη παραγωγή υδρογόνου εκτιμήθηκε σε HRT 0.5 d, ενώ η παραγωγή μεθανίου σε HRT 16 d. Στο στάδιο της μεθανογένεσης, η μέγιστη παραγωγή υδρογόνου στο σύστημα κατέληξε σε αποτυχία λόγω συσσώρευσης πτητικών λιπαρών οξέων από την υψηλή οργανική φόρτιση του συστήματος. Τέλος, η μέγιστη παραγωγή υδρογόνου χρησιμοποιώντας το μίγμα 95% σόργο-5% βουστάσιο παρατηρήθηκε σε HRT 5 d, μεγαλύτερη τιμή από τα προηγούμενα σενάρια λόγω αυξημένου ποσοστού σόργου, ενώ κατά το στάδιο της μεθανογένεσης η παραγωγή μεθανίου αυξήθηκε από τον πρώτο σταδιοδρόμο λόγω αύξηματος μεθανοποιήσης στο σύστημα κατά το στάδιο της μεθανογένεσης. Είναι πολύ σημαντικό να αναφερθεί ότι η παραγωγή μεθανίου από την επεξεργασία του μίγματος 55% ελαιοτριβείο-40% τυροκομείο-5% βουστάσιο ήταν μικρότερη την δεύτερη φορά, επειδή η βελτιστοποίησή του σταδίου της οξεογένεσης επηρεάστηκε από την ανεπάρκεια του υποστρώματος στο στάδιο της μεθανογένεσης. Αυτό μπορεί να οφείλεται είτε σε κάποια περιορισμούς στην παραγωγή μεθανίου από την επεξεργασία του μίγματος 55% ελαιοτριβείο-40% τυροκομείο-5% βουστάσιο ήταν μικρότερη την δεύτερη φορά, επειδή η βελτιστοποίηση του σταδίου της μεθανογένεσης ήταν μικρότερη κατά την παραγωγή μεθανίου, ενώ κατά το στάδιο της μεθανογένεσης η παραγωγή μεθανίου ήταν μικρότερη την πρώτη φορά. Επιπλέον μελέτη πραγματοποιήθηκε σε σχέση με τη χρήση του γλυκού σόργου ως υπόστρωμα συγχώνευσης και στην προεπεξεργασία του, ως λιγνοκυτταρινούχο υλικό. Χημική επεξεργασία (όξινη και αλκαλική) πραγματοποιήθηκε σε φρέσκο και σε ενσιρωμένο σόργο. Οι μεγαλύτερες αποδόσεις διαλυτοποίησης πραγματοποιήθηκαν σε ενσιρωμένο σόργο χρησιμοποιώντας αλκαλικό διάλυμα. Από την άλλη πλευρά, η
ενζυμική υδρόλυση φρέσκου σόργου οδήγησε σε μικρές αυξήσεις διαλυτών σακχάρων, όλες οποίες επιπεδεύθηκαν σε μικρή δοσολογία ενζυμών. Περαιτέρω αύξηση της συγκέντρωσης αυτών οδήγησε σε υψηλό κόστος διεργασίας, μη επιθυμητό. Συνέχεια της μελέτης της χημικής επεξεργασίας του ενσιρωμένου σόργου πραγματοποιήθηκε με χρήση αλκαλικού διαλύματος με σκοπό την μετέπειτα χρήση του στην παραγωγή βιοαερίου. Υψηλό ποσοστό αύξησης διαλυτών σακχάρων παρατηρήθηκε με ταυτόχρονη μείωση της λιγνίνης, το οποίο στη συνέχεια λειτούργησε θετικά στην αύξηση του μεθανογόνου δυναμικού.

Η ανάγκη περαιτέρω επεξεργασίας της αναερόβιας μεθανογόνης απορροής οδήγησε σε μελέτη επεξεργασίας της με χρήση σύστημα μεμβρανών καθώς και κομποστοποίησης με χρήση γαιοσκωλήκων. Πιο συγκεκριμένα, η χρήση μεμβρανών (υπερδιήθησης και νανοδιήθησης) οδήγησε σε πολύ καλά αποτελέσματα με σημαντική μείωση του COD, έπειτα από διαχωρισμό της απορροής σε σύστημα κροκίδωσης/καθίζησης με χρήση πολυηλεκτρολύτη. Από την άλλη πλευρά, η διαδικασία κομποστοποίησης με χρήση γαιοσκωλήκων συνέβαλε στην μείωση του pH και των πτητικών στερεών του υποστρώματος και στις περισσότερες περιπτώσεις παρατηρήθηκε αύξηση των συγκεντρώσεων αζώτου, καλίου και φωσφόρου που είναι δείκτες καλής ποιότητας κομπόστ.

Μαθηματική μοντελοποίηση διεξήχθη επιπλέον προσομοίων προσομοίων της παραγωγής από τα υγρά απόβλητα βουστασίου, καθώς και από τέσσερα επιπλέον σενάρια που μελετήθηκαν παραπάνω. Τα τέσσερα σενάρια με χρήση γαλακτικού εκχυλίσματος, διαφορετικής αναλογίας και διαφορετικής διάρκειας (HRT). Τα αποτελέσματα που προέκυψαν από την προσομοίωση έδειξαν ότι το τροποποιημένο μοντέλο ήταν σε θέση να προβλέψει με ακρίβεια τις τιμές pH και πτητικών λιπαρών οξέων, αλλά και τις τιμές COD και άλλων διαφορετικών μεταβολικών προϊόντων (γαλακτοκομικά). Σημαντικές είναι οι αναφορές στην ανάγκη αναπτύξεως εξώφυλλων για την εκκαθάριση της παραγωγής απόβλητων, καθώς και στην ανάγκη αποτροπής της παραγωγής απόβλητων.

Τέλος, για την ολοκλήρωση της διατριβής πραγματοποιήθηκε οικονομική μελέτη για την επένδυση μιας κεντρικής μονάδας αναεροβικής μεθανόλησης (ηλεκτρικής ισχύος 1.0 MW) η οποία θα επεξεργάζεται με αγροβιομηχανικά απόβλητα και πιο οικονομικά με μίγμα αυτών σε αναλογία 55% ελαιοτριβείο-40% τυροκομείο-5% βουστάσιο. Τα συμπεράσματα που προέκυψαν από αυτή τη μελέτη ήταν ότι μια ολοκληρωμένη μονάδα αποτελεί μια πολύ καλή επιστημονικά, τεχνολογικά και κυρίως περιβαλλοντικά λύση στο σχέδιο της διαχείρισης των αποβλήτων αυτών.
C.6. ΒΙΒΛΙΟΓΡΑΦΙΑ


Curriculum Vitae

Personal Details

Name & Surname Margarita A. Dareioti
Date of birth 29 June 1984
Mobile +30 694 5079377
Current position Department of Chemical Engineering, University of Patras, Karatheodori 1, University Campus, GR 26500 Patras, Greece
E-mail margaritad@chemeng.upatras.gr
            m.dareioti@gmail.com

Education

2009 - present PhD. Candidate in Chemical Engineering
Laboratory of Biochemical Engineering and Environmental Technology, Department of Chemical Engineering, University of Patras, Greece

• PhD. Thesis title: “Energy valorization of agro-industrial wastes and sweet sorghum for the production of gaseous biofuels through anaerobic digestion”
• Research interests: anaerobic (co)-digestion of biomass, gaseous biofuels production, agro-industrial wastes, sweet sorghum, post-treatment of digestate, anaerobic digestion modeling, techno-economic evaluation.

2002 - 2009 B.Sc. (Diploma) in Chemical Engineering
University of Patras, Greece

• Grade: 7.14 of 10
• Diploma thesis title: “Biological Removal of Nitrogen using a pilot scale Sequencing Batch Reactor (SBR)”
• Academic Advisor: Associate Prof. Michael Kornaros
Research and Work Experience

2009 - present  
Research Scientist  
at Biochemical Engineering and Environmental Technology Laboratory

Focus on the development of innovative or improved environmental processes towards the anaerobic co-digestion of agro-industrial wastes (i.e. olive mill wastewaters, cheese whey, cow manure) and sweet sorghum. Development, mathematical modeling, optimization and control of biochemical processes and combined technologies integrating physicochemical and biological processes for the treatment of municipal and industrial wastewaters and solid wastes in order to biofuels production (i.e. bio-hydrogen, methane)

Supervision of Graduate Thesis  
in the Chemical Engineering Department, University of Patras

2007 - 2008

Participation in Technical Report  
Development of Enterpreneurship and Innovation in University of Patras–II. Department of Chemical Engineering

“Business plan for the exploitation of agro-industrial wastes in Kefalonia Island, Greece”

Teaching Experience

2009 fall  
Graduate Teaching Assistant  
Dept. of Chemical Engineering, University of Patras, Greece  
Tutorial of “Unit Operations I”

2010 spring  
Graduate Teaching Assistant  
Dept. of Chemical Engineering, University of Patras, Greece  
Laboratory course on “Chemical and Biochemical Engineering Laboratory”

Graduate Teaching Assistant  
Dept. of Materials Science, University of Patras, Greece  
Laboratory course on “Materials and Environment”
2010 fall  **Graduate Teaching Assistant**  
Dept. of Chemical Engineering, University of Patras, Greece  
Tutorial of “Unit Operations I”

2011 spring  **Graduate Teaching Assistant**  
Dept. of Materials Science, University of Patras, Greece  
Laboratory course on “Materials and Environment”

**Participation in Projects/ Fellowship**

09/2010 - present  “Education and Lifelong Learning” of the National Strategic Reference Framework (NSRF).  
Funded by: Heracleitus II. Investing knowledge society through the European Social Fund.

1/2010 - 8/2010  “Development of INTEGRated aGRoIndustrial wASTE management politics maximizing materials recovery and energy exploitation (INTEGRASTE)”  
Funded by: European Commission – LIFE+2008

09/2008 - 11/2008  “Environmental monitoring of Astakos harbor located in Platygiali, Greece”  
Funded by: AKAPORT S.A

05/2007 - 11/2008  “Complete treatment of agroindustrial wastewaters in the area of Greece-Italy (AGROENERGY)”  
Funded by: INTERREG III A/Greece – Italy, 2000-2006

10/2007 - 02/2008  “Biological Treatment of Landfill leachates using a SBR”  
Funded by: Municipal Wastes association of Zakynthos

05/2006 - 05/2008  “Biological Nitrogen Removal using a pilot scale sequencing batch reactor (SBR)”  
Funded by: General Secretary of Research and Technology in Greece (PRAXE)

**Affiliations/Memberships**

Since 2009  Member of the Technical Chamber of Greece

Since 2009  Member of the PanHellenic Chamber of Chemical Engineers of Greece
List of Publication in peer reviewed Journals


2. **Dareioti, M.A.**, Vavouraki, A.I., Dokianakis, S.N., Kornaros, M., 2015. Assessment of single vs. two-stage anaerobic digestion using liquid cow manure or cheese whey. *to be submitted*


## Participation in National and International Conferences


