TECHNICAL UNIVERSITY OF CRETE
SCHOOL OF ENVIRONMENTAL ENGINEERING
LABORATORY OF TOXIC AND HAZARDOUS WASTE MANAGEMENT

PhD Thesis

«INTEGRATED SOLID ORGANIC WASTE TREATMENT AND VALORIZATION IN THE MEDITERRANEAN AREA USING ANAEROBIC DIGESTION»

FRANTSESKA-MARIA PELLERA

Environmental Engineer, M.Sc.

Chania 2017

ΠΟΛΥΤΕΧΝΕΙΟ ΚΡΗΤΗΣ
ΣΧΟΛΗ ΜΗΧΑΝΙΚΩΝ ΠΕΡΙΒΑΛΛΟΝΤΟΣ
ΕΡΓΑΣΤΗΡΙΟ ΔΙΑΧΕΙΡΙΣΗΣ ΤΟΞΙΚΩΝ ΚΑΙ ΕΠΙΚΙΝΔΥΝΩΝ ΑΠΟΒΛΗΤΩΝ

ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

«ΟΛΟΚΛΗΡΩΜΕΝΗ ΕΠΕΞΕΡΓΑΣΙΑ ΚΑΙ ΑΞΙΟΠΟΙΗΣΗ ΣΤΕΡΕΩΝ ΟΡΓΑΝΙΚΩΝ ΑΠΟΒΛΗΤΩΝ ΣΤΗΝ ΠΕΡΙΟΧΗ ΤΗΣ ΜΕΣΟΓΕΙΟΥ ΜΕ ΧΡΗΣΗ ΑΝΑΕΡΟΒΙΑΣ ΧΩΝΕΥΣΗΣ»

ΦΡΑΝΤΣΕΣΚΑ – ΜΑΡΙΑ ΠΕΛΛΕΡΑ

ΜΗΧΑΝΙΚΟΣ ΠΕΡΙΒΑΛΛΟΝΤΟΣ, M.Sc.

ΧΑΝΙΑ 2017

Examination Committee

Evangelos Gidarakos – Supervisor
Professor
School of Environmental Engineering
Technical University of Crete

Evangelos Diamadopoulos – Advisory committee
Professor
School of Environmental Engineering
Technical University of Crete

Dimitrios Komilis – Advisory committee
Associate Professor
Department of Environmental Engineering
Democritus University of Thrace

George Karatzas
Professor
School of Environmental Engineering
Technical University of Crete

Konstantinos Komnitsas
Professor
School of Mineral Resources Engineering
Technical University of Crete

Danae Venieri
Assistant Professor
School of Environmental Engineering
Technical University of Crete

Nikolaos Xekoukoulotakis
Assistant Professor
School of Environmental Engineering
Technical University of Crete

ACKNOWLEDGEMENTS

After completing this thesis, I realize what an amazing journey this was, full of challenges, frustration and exhaustion, but also excitement, accomplishment and happiness. It took a lot of hard work to reach the end of this path, but this would not have been possible without all the people who helped me along the way.

Firstly, I would like to express my deepest gratitude to my supervisor, Professor Evangelos Gidarakos, who believed in me since my undergraduate years, when I joined his laboratory to conduct my diploma thesis. During all these years, he guided and supported me and always helped me in developing my ideas. This PhD thesis would not have been possible without his contribution.

Special thanks go to the members of my advisory committee Professor Evangelos Diamadopoulos and Associate Professor Dimitrios Komilis, who always offered me their valuable advice and help, whenever I needed them.

I would also like to thank the members of my examination committee, Professor George Karatzas, Professor Konstantinos Komnitsas, Assistant Professor Danae Venieri and Assistant Professor Nikolaos Xekoukoulotakis, for taking the time to evaluate this thesis and for their useful suggestions and comments.

Moreover, I would like to express my gratitude to Associate Professor Alessandra Polettini and Assistant Professor Raffaella Pomi, of the University of Rome "La Sapienza", for their valuable advice and insightful comments.

I am very grateful to the members of the Environmental Organic Chemistry and Micro-pollution Research Unit, as well as of the Biochemical Engineering & Environmental Biotechnology Laboratory and the Hydrogeochemical Engineering and Soil Remediation Laboratory of the School of Environmental Engineering of the Technical University of Crete, for provision of equipment during the experimental work of this thesis.

My sincere gratitude goes to all the undergraduate and graduate students I worked with during the period I spent at the Laboratory of Toxic and Hazardous Waste Management, and especially to Emmanouil Pasparakis, Sofia Santori, Ioannis Acheilas and Konstantina Dementi. I am particularly grateful to Ioannis Moukazis, with whom I shared not only an office, but also many laboratory hours and very interesting and constructive conversations.

Furthermore, I would like to sincerely thank the past and present members of the Laboratory of Toxic and Hazardous Waste Management, Apostolos Giannis,

Kalliopi Anastasiadou, Maria Aivalioti, Eleni Kastanaki, Ioannis Hahladakis, Fotini Simantiraki, Athanasia Kousaiti, Katerina Valouma, Vassiliki Savvilotidou and Chrysanthie Makri, for the exceptional collaboration we had all these years.

I must also thank the "Alexander S. Onassis" Public Benefit Foundation for the financial support that it provided me, through the scholarship that it awarded me for my doctoral studies at the School of Environmental Engineering of the Technical University of Crete. This scholarship was an important contribution to my studies, as well as a great honor for me personally.

Lastly, I could not have accomplished so much without the help and the support of my dearest friends and family. Most importantly, I would like to express my heart-felt gratitude to my parents. They always loved me, helped me and supported me, especially through the difficult moments of this journey. They have always been beside me, teaching me to always follow my dreams and I will always be grateful to them.

ABSTRACT

The purpose of the present doctoral thesis is to study the treatment and valorization of some of the most produced types of solid organic waste of the Mediterranean area and especially of Greece, using the anaerobic digestion technology. Specifically, four solid organic waste originating from agroindustrial activities, namely winery waste (WW), cotton gin waste (CGW), olive mill waste (olive pomace) (OP) and juice industry waste (JW) (orange waste), were studied.

The first step of this study was the determination of the methane potential of the investigated substrates in their raw form, by evaluating the influence of different substrate to inoculum ratios (SIR) and inoculum types. To this purpose, Biochemical Methane Potential (BMP) assays were conducted, in which four SIR, i.e. 0.25, 0.5, 1 and 2 (on a volatile solids (VS) basis), were tested and three different inocula, namely anaerobic sludge, landfill leachate and gravitationally thickened anaerobic sludge, were compared. Ultimately, anaerobic sludge was found to be the most adequate inoculum among tested samples, while landfill leachate and thickened anaerobic sludge showed lower efficiencies. The optimum SIR for determining the methane potential of the investigated substrates were of 0.5 for WW and JW, and of 0.25 for CGW and OP, yielding 446.2, 446.0, 268.0 and 258.7 $mLCH_{4,STP}/gVS_{substrate}$, respectively. The complexity of the anaerobic digestion of the investigated substrates was manifested by the association of different SIR with 2- and 3-parameter kinetic models, while a multiple-stages modeling approach, appeared to be suitable for describing the experimental data.

The next part of the study focused on the application of two pretreatment methods prior to the anaerobic digestion of the investigated substrates, namely microwave and chemical pretreatment. In both cases, the objective was to evaluate the effect of such pretreatments on the solubilization and the degradability of the substrates. The effect on substrate solubilization was evaluated by analyzing the liquid fractions obtained after pretreatment for soluble chemical oxygen demand (sCOD) and total phenols (TPH) concentrations, while the effect on substrate degradability was assessed through BMP assays performed on the respective solid fractions. The conditions adopted in these BMP assays were based on the results of the first part of the study. Microwave pretreatment was carried out using a laboratory scale microwave reaction system, and by investigating the variation of four operational parameters, i.e. solid to liquid ratio (50, 75 and 100 g/L), heating rate (2.5, 5 and 10

°C/min), holding time (5, 10, 15 and 30 min) and temperature (75, 125, 150, 175 and 200 °C). On the other hand, for chemical pretreatment the use of eight different chemical reagents i.e. sodium hydroxide (NaOH), sodium bicarbonate ($NaHCO_3$), sodium chloride (NaCl), citric acid (H_3Cit), acetic acid (AcOH), hydrogen peroxide (H_2O_2), acetone (Me_2CO) and ethanol (EtOH), was investigated, under three condition sets resulting in treatments of varying intensity, depending on process duration (16, 8 and 4 h), reagent dosage (0.25, 0.5 and 1 mmol/gVS) and temperature (25, 60 and 90 °C). Different reagents were used in order to also determine the impact of different reagent natures (alkaline, acidic, saline, oxidative, organic) on the final results.

The results obtained from microwave pretreatment showed that temperature had the most important effect among the four investigated operational parameters, while optimum solid to liquid ratio, heating rate and holding time were determined and correspond to 50 g/L, 10 °C/min, and 5 min, respectively. Microwave pretreatment appeared to have exerted different effects on each investigated substrate. More specifically, WW and JW were mainly affected regarding their solubilization, while in the cases of CGW and OP, pretreatment most likely induced structural changes on these materials. Ultimately, the obtained results indicated that microwave pretreatment at temperatures between 125 and 150 °C could eventually result in the generation of samples that are more suitable for methane production. On the other hand, the results of chemical pretreatment indicated that the application of more severe conditions for this kind of process, is more effective on the solubilization of substrates such as those investigated in this study, with H_3Cit, H_2O_2, and EtOH appearing as the most effective reagents for this scope. However, in terms of methane production, moderate to high severity conditions were found to generally be the most satisfactory. More specifically, maximum specific methane yield values were obtained for samples generated after moderate severity treatments using EtOH, H_3Cit and H_2O_2 for WW, OP and JW, while a high severity treatment using EtOH had an analogous result for CGW. Solid fractions obtained with both pretreatments had lower methane yields compared with untreated substrates. Nevertheless, chemical pretreatment was proved better than microwave pretreatment in three out of four cases, i.e. for WW, CGW and JW. On the contrary, in the fourth case, that of OP, microwave pretreatment showed a better methane efficiency than chemical pretreatment.

In the third part of the study the anaerobic digestion of the four agroindustrial substrates in semi-continuous mode was investigated. Each substrate was digested separately in mono-digestion assays, as well as in combination with a synthetic organic fraction (SOF) sample, which was used as a co-substrate, in co-digestion assays. Further division of the assays in two Groups aimed at studying the application of different operational conditions, in both mono- and co-digestion systems. More specifically, in the assays of Group I, the variation in two operational parameters, namely organic loading rate (OLR) and hydraulic retention time (HRT), was investigated, whereas in the assays of Group II, different feeding materials were fed to the reactors in a sequential order, based on their seasonality. It was ultimately

observed that co-digestion of the four agroindustrial waste with SOF, resulted in higher methane yields compared with mono-digestion. Maximum methane yields in the first group of assays were obtained after halving the HRT and setting the OLR to 1.0 gVS/L/d, while further reduction of the HRT coupled to an increase of the OLR, led to a significant decrease of methane yields, due to system overloading and possibly, washout phenomena. The latter was true for the majority of assays, except those being fed with OP-substrates. Severe system overloading, which eventually resulted in system failure, was observed only for the assays being fed with a JW-substrate in mono-digestion mode. Feeding the assays of the second group, with different substrates in a sequential order, led to a more equilibrated operation, especially for co-digestion systems. Moreover, higher methane yields were observed during the periods in which WW- and JW-substrates were being fed to the reactors. Characterization analyses on the digestates obtained from all semi-continuous assays, suggested a potential suitability of these materials for land application.

ΠΕΡΙΛΗΨΗ

Σκοπός της παρούσας διδακτορικής διατριβής είναι η μελέτη της επεξεργασίας και αξιοποίησης στερεών οργανικών αποβλήτων, από τα πλέον παραγόμενα είδη στην περιοχή της Μεσογείου και ιδιαίτερα της Ελλάδας, χρησιμοποιώντας την τεχνολογία της αναερόβιας χώνευσης. Συγκεκριμένα, μελετήθηκαν τέσσερα στερεά οργανικά απόβλητα προερχόμενα από αγροβιομηχανικές δραστηριότητες, δηλαδή απόβλητα οινοποιείου (winery waste, WW), απόβλητα εκκόκκισης βάμβακος (cotton gin waste, CGW), απόβλητα ελαιουργείου (ελαιοπυρήνα) (olive pomace, OP) και απόβλητα βιομηχανίας χυμών (juice industry waste, JW) (απόβλητα πορτοκαλιών).

Το πρώτο βήμα της παρούσας μελέτης ήταν ο προσδιορισμός του δυναμικού μεθανίου των υπό μελέτη υποστρωμάτων στην αρχική τους μορφή, μέσω αξιολόγησης της επίδρασης διαφορετικών λόγων υπόστρωμα προς εμβόλιο (substrate to inoculum ratio, SIR) και διαφορετικών ειδών εμβολίου. Γι' αυτόν τον σκοπό, διεξήχθησαν δοκιμές βιοχημικού δυναμικού μεθανίου (biochemical methane potential, BMP), στις οποίες εξετάστηκαν τέσσερις τιμές SIR, συγκεκριμένα 0.25, 0.5, 1 και 2 (σε βάση πτητικών στερεών (volatile solids, VS)), και συγκρίθηκαν τρία διαφορετικά είδη εμβολίου, συγκεκριμένα, αναερόβια ιλύς, διασταλάγματα χώρου υγειονομικής ταφής αποβλήτων (ΧΥΤΑ) και βαρυτικά πυκνωμένη αναερόβια ιλύς. Εν τέλει, η αναερόβια ιλύς βρέθηκε ως η πλέον κατάλληλη μεταξύ των εξεταζόμενων δειγμάτων, ενώ τα διασταλάγματα ΧΥΤΑ και η πυκνωμένη αναερόβια ιλύς έδειξαν χαμηλότερη αποδοτικότητα. Οι βέλτιστες τιμές SIR για τον προσδιορισμό του δυναμικού μεθανίου των υπό μελέτη υποστρωμάτων ήταν 0.5 για τα WW και JW και 0.25 για τα CGW και OP, παράγοντας 446.2, 446.0, 268.0 και 258.7 $mLCH_{4,STP}/gVS_{υποστρώματος}$, αντίστοιχα. Ο σύνθετος χαρακτήρας της αναερόβιας χώνευσης των υπό μελέτη υποστρωμάτων έγινε εμφανής μέσω της συσχέτισης διαφορετικών SIR με κινητικά μοντέλα δύο και τριών παραμέτρων, με την προσέγγιση που λάμβανε υπόψη πολλαπλά στάδια στην διεργασία, να φαίνεται κατάλληλη για την περιγραφή των πειραματικών δεδομένων.

Το επόμενο μέρος της μελέτης επικεντρώθηκε στην εφαρμογή δύο μεθόδων προεπεξεργασίας πριν την αναερόβια χώνευση των υπό μελέτη υποστρωμάτων. Συγκεκριμένα, αυτές οι μέθοδοι ήταν η προεπεξεργασία με χρήση μικροκυμάτων και η χημική προεπεξεργασία. Σε αμφότερες τις περιπτώσεις, στόχος ήταν η αξιολόγηση της επίδρασης τέτοιου είδους προεπεξεργασιών στην διαλυτοποίηση και στην αποδομησιμότητα των υποστρωμάτων. Η επίδραση στην διαλυτοποίηση των

υποστρωμάτων αξιολογήθηκε μέσω αναλύσεων των υγρών κλασμάτων που προέκυψαν μετά την προεπεξεργασία, ως προς τις συγκεντρώσεις τους σε διαλυτό χημικά απαιτούμενο οξυγόνο (soluble chemical oxygen demand, sCOD) και ολικές φαινόλες (total phenols, TPH), ενώ η επίδραση στην αποδομησιμότητα των υποστρωμάτων εκτιμήθηκε μέσω της διεξαγωγής δοκιμών BMP, οι οποίες πραγματοποιήθηκαν για τα αντίστοιχα στερεά κλάσματα. Οι συνθήκες που υιοθετήθηκαν σε αυτές τις δοκιμές BMP βασίστηκαν στα αποτελέσματα του πρώτου μέρους της μελέτης. Η προεπεξεργασία με μικροκύματα πραγματοποιήθηκε με χρήση ενός συστήματος αντίδρασης μικροκυμάτων εργαστηριακής κλίμακας και διερευνώντας την μεταβολή τεσσάρων λειτουργικών παραμέτρων, συγκεκριμένα, του λόγου στερεό προς υγρό (50, 75 και 100 g/L), του ρυθμού θέρμανσης (2.5, 5 και 10 °C/min), του χρόνου παραμονής (5, 10, 15 και 30 min) και της θερμοκρασίας (75, 125, 150, 175 και 200 °C). Από την άλλη, για την χημική προεπεξεργασία των υποστρωμάτων διερευνήθηκε η χρήση οκτώ διαφορετικών αντιδραστηρίων, συγκεκριμένα, υδροξειδίου του νατρίου (NaOH), όξινου ανθρακικού νατρίου ($NaHCO_3$), χλωριούχου νατρίου (NaCl), κιτρικού οξέος (H_3Cit), οξικού οξέος (AcOH), υπεροξειδίου του υδρογόνου (H_2O_2), ακετόνης (Me_2CO) και αιθανόλης (EtOH), σε τρεις ομάδες συνθηκών, οι οποίες κατέληξαν σε επεξεργασία μεταβαλλόμενης έντασης, εξαρτώμενη από την διάρκεια της διεργασίας (16, 8 και 4 h), την δοσολογία αντιδραστηρίου (0.25, 0.5 και 1 mmol/gVS) και την θερμοκρασία (25, 60 και 90 °C). Η χρήση διαφορετικών αντιδραστηρίων υιοθετήθηκε με σκοπό τον προσδιορισμό της επίδρασης της διαφορετικής φύσης των αντιδραστηρίων (βασική, όξινη, αλατώδης, οξειδωτική, οργανική) στα τελικά αποτελέσματα.

Τα αποτελέσματα που προέκυψαν από την προεπεξεργασία με μικροκύματα έδειξαν ότι η θερμοκρασία είχε την πλέον σημαντική επίδραση ανάμεσα στις εξεταζόμενες λειτουργικές παραμέτρους, ενώ προσδιορίστηκαν βέλτιστες τιμές λόγου στερεό προς υγρό, ρυθμού θέρμανσης και χρόνου παραμονής, οι οποίες αντιστοιχούν σε 50 g/L, 10 °C/min, και 5 min. Η προεπεξεργασία με μικροκύματα φαίνεται να άσκησε διαφορετική επίδραση στο κάθε εξεταζόμενο υπόστρωμα. Ειδικότερα, τα WW και JW επηρεάστηκαν κυρίως όσον αφορά στην διαλυτοποίησή τους, ενώ στις περιπτώσεις των CGW και OP, η προεπεξεργασία πιθανότατα προκάλεσε αλλαγές στη δομή αυτών των υλικών. Εν τέλει, τα αποτελέσματα υποδεικνύουν ότι η προεπεξεργασία με μικροκύματα σε θερμοκρασίες μεταξύ 125 και 150 °C, ενδεχομένως να έχει ως αποτέλεσμα την παραγωγή δειγμάτων, που είναι καταλληλότερα για παραγωγή μεθανίου. Από την άλλη, τα αποτελέσματα που προέκυψαν από την χημική προεπεξεργασία υποδεικνύουν, ότι η εφαρμογή περισσότερο έντονων συνθηκών γι' αυτού του είδους την διεργασία, είναι πιο αποτελεσματική στην διαλυτοποίηση υποστρωμάτων, όπως είναι αυτά που διερευνήθηκαν στην παρούσα μελέτη, με τα αντιδραστήρια H_3Cit, H_2O_2, και EtOH να εμφανίζονται ως τα πλέον αποτελεσματικά γι' αυτόν τον σκοπό. Παρόλα αυτά, όσον αφορά στην παραγωγή μεθανίου, συνθήκες μέτριας με υψηλής έντασης βρέθηκαν να είναι γενικά οι περισσότερο ικανοποιητικές. Ειδικότερα, μέγιστες αποδόσεις μεθανίου προέκυψαν για τα δείγματα που παρήχθησαν μέσω προεπεξεργασίας μέτριας έντασης με χρήση EtOH, H_3Cit και H_2O_2, για τα WW, OP και JW, ενώ

προεπεξεργασία υψηλής έντασης με EtOH είχε ανάλογο αποτέλεσμα για τα CGW. Τα στερεά κλάσματα που προέκυψαν από αμφότερες τις προεπεξεργασίες είχαν χαμηλότερες αποδόσεις μεθανίου συγκριτικά με τα ανεπεξέργαστα υποστρώματα. Παρόλα αυτά, η χημική προεπεξεργασία αποδείχθηκε καλύτερη από την προεπεξεργασία με μικροκύματα στις τρεις από τις τέσσερις περιπτώσεις, συγκεκριμένα για τα WW, CGW και JW. Αντιθέτως, στην τέταρτη περίπτωση, αυτή της OP, η προεπεξεργασία με μικροκύματα είχε ως αποτέλεσμα μια καλύτερη απόδοση μεθανίου σε σύγκριση με την χημική προεπεξεργασία.

Στο τρίτο μέρος της μελέτης, διερευνήθηκε η αναερόβια χώνευση των τεσσάρων αγροβιομηχανικών υποστρωμάτων σε ημι-συνεχείς συνθήκες. Κάθε υπόστρωμα υπέστη χώνευση ξεχωριστά σε δοκιμές απλής χώνευσης (μόνο-χώνευσης), καθώς και σε συνδυασμό με ένα συνθετικό οργανικό κλάσμα (synthetic organic fraction, SOF), το οποίο χρησιμοποιήθηκε ως δεύτερο (συν-) υπόστρωμα σε δοκιμές συν-χώνευσης. Ο περαιτέρω διαχωρισμός των δοκιμών σε δύο ομάδες είχε ως στόχο την μελέτη της εφαρμογής διαφορετικών λειτουργικών συνθηκών, σε αμφότερα τα συστήματα, απλής χώνευσης και συν-χώνευσης. Ειδικότερα, στις δοκιμές της Ομάδας I, διερευνήθηκε η μεταβολή δύο λειτουργικών παραμέτρων, δηλαδή του ρυθμού οργανικής φόρτισης (organic loading rate, OLR) και του υδραυλικού χρόνου παραμονής (hydraulic retention time, HRT), ενώ στις δοκιμές της Ομάδας II, οι αντιδραστήρες τροφοδοτούνταν με διαφορετικά υλικά σε διαδοχική σειρά, η οποία βασίστηκε στην εποχικότητά τους. Εν τέλει, παρατηρήθηκε πως η συν-χώνευση των τεσσάρων αγροβιομηχανικών αποβλήτων με συνθετικό οργανικό κλάσμα, είχε ως αποτέλεσμα την επίτευξη υψηλότερων αποδόσεων μεθανίου σε σύγκριση με την απλή χώνευση. Στην πρώτη ομάδα δοκιμών, μέγιστες τιμές απόδοσης μεθανίου επιτεύχθηκαν μετά από τον υποδιπλασιασμό του HRT και την ρύθμιση του OLR σε 1.0 gVS/L/d, ενώ περαιτέρω μείωση του HRT σε συνδυασμό με αύξηση του OLR, οδήγησε σε σημαντική μείωση των αποδόσεων μεθανίου, εξαιτίας υπερφόρτισης των συστημάτων και πιθανότατα, φαινομένων έκπλυσης. Αυτό ισχύει για την πλειοψηφία των δοκιμών, εκτός από αυτές που τροφοδοτούνταν με υποστρώματα που περιείχαν OP. Έντονα φαινόμενα υπερφόρτισης, τα οποία και κατέληξαν σε αστοχία του συστήματος, παρατηρήθηκαν μόνο για τις δοκιμές που τροφοδοτούνταν με υποστρώματα τα οποία περιείχαν JW και που λειτουργούσαν σε συνθήκες απλής χώνευσης. Η τροφοδοσία των αντιδραστήρων της δεύτερης ομάδας με διαφορετικά υλικά σε διαδοχική σειρά, οδήγησε σε μια πιο ισορροπημένη λειτουργία, ειδικά όσον αφορά στα συστήματα συν-χώνευσης. Επιπλέον, οι υψηλότερες αποδόσεις μεθανίου παρατηρήθηκαν κατά την διάρκεια των περιόδων στις οποίες οι αντιδραστήρες τροφοδοτούνταν με υποστρώματα που περιείχαν WW και JW. Αναλύσεις χαρακτηρισμού των χωνευμένων υπολειμμάτων που προέκυψαν από όλες τις ημι-συνεχείς δοκιμές, υποδήλωσαν την εν δυνάμει καταλληλότητα αυτών των υλικών για χρήση στο έδαφος.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PICTURES

NOMENCLATURE

AcOH	acetic acid [where, Ac: acetyl, i.e. CH_3CO-]
ADF	acid detergent fiber
ADL	acid detergent lignin
AS	anaerobic sludge
AW	agroindustrial waste
BI	biodegradability index (%)
BMP	biochemical methane potential
CGW	cotton gin waste
E_C	specific energy consumption (kJ/kg VS)
E_M	specific energy corresponding to the energy produced from the pretreated samples in the form of methane (kJ/kg VS)
E_Q	specific energy corresponding to the energy produced in the form of heat (kJ/kg VS)
E_T	specific energy profit of the pretreatment (kJ/kg VS)
EtOH	ethanol
FAN	free ammonia nitrogen (mg/L)
H_2O_2	hydrogen peroxide
H_3Cit	citric acid
HR	heating rate (°C/min)
HT	holding time (min)
JW	juice industry waste
LL	landfill leachate
Me_2CO	acetone
MSW	municipal solid waste
MW	microwave
NaCl	sodium chloride
$NaHCO_3$	sodium bicarbonate
NaOH	sodium hydroxide
NDF	neutral detergent fiber
OFMSW	organic fraction of municipal solid waste
OP	olive pomace
sCOD	soluble chemical oxygen demand (mg O_2/g VS)
SIR	substrate to inoculum ratio
SMY	specific methane yield expressed as volume of methane per gram of VS added to the reactor (mL $CH_{4,\ STP}$/g VS)

SMY_P	specific methane yield expressed as volume of methane per gram of VS of pretreated substrate (mL $CH_{4,\ STP}$/g VS_P)
SMY_{Raw}	specific methane yield expressed as volume of methane per gram of VS corresponding to Raw substrate (mL $CH_{4,\ STP}$/g VS_{Raw})
SOF	synthetic organic fraction
STP	standard temperature and pressure
t_{80}	time period required in order to achieve at least 80% (t_{80}) of the total CH_4 production
TA	total alkalinity (mg $CaCO_3$/L)
TAN	total ammonia nitrogen (mg/L)
TAS	thickened anaerobic sludge
TMP	theoretical methane potential (mL $CH_{4,\ STP}$/g VS)
TOD	theoretical oxygen demand (mg O_2/g VS)
TPH	total phenols (mg GAE/g VS and mg GAE/L)
TS	total solids (%)
VFA	volatile fatty acids
VS	volatile solids (%)
WW	winery waste
Y_{TS}	mass yield for the pretreatment process based on total solids ($gTS_{pretreated\ sample}$/$gTS_{raw\ sample}$)
Y_{VS}	mass yield for the pretreatment process based on volatile solids ($gVS_{pretreated\ sample}$/$gVS_{raw\ sample}$)
Y_{Wet}	mass yield for the pretreatment process based on wet sample ($gWet_{pretreated\ sample}$/$gWet_{raw\ sample}$)

CHAPTER 1

Introduction

1.1 Research topic

One of the most important issues concerning modern societies is waste generation. Among waste materials, municipal solid waste and especially their organic fractions, are those with the highest production rates on a global level (Hoornweg and Bhada-Tata, 2012). Nevertheless, in areas such as the Mediterranean, where the agricultural and agroindustrial sectors are widespread and represent major contributors of the economy, agricultural and agroindustrial waste also constitute a significant portion of the total waste production (Fountoulakis et al., 2008). Such waste materials can constitute potential causes of environmental and health problems, if not appropriately managed and treated (Nigam et al., 2009).

The anaerobic digestion technology has been recognized as an effective organic waste management and treatment option. However, in the last decades, it has also been adopted for energy production, due to the high energy potential of its main product, biogas, which is rich in methane. Therefore, the application of this technology serves a double purpose, by providing both a viable strategy for combined waste management and alternative energy generation (Ariunbaatar et al., 2014; Sawatdeenarunat et al., 2015). This is extremely important considering the need for reducing fossil fuel usage and greenhouse gas emissions (Aboudi et al., 2016)

Consequently, considering the high production rates and availability of waste originating from agricultural and agroindustrial activities, these materials seem to represent suitable candidates for anaerobic digestion feedstock (Aboudi et al., 2016; Pagés Díaz et al., 2011). Moreover, they have also been characterized as renewable and low cost resources (Fernández-Cegrí et al., 2012; Zhao et al., 2014). Nevertheless, such materials, being mainly composed of cellulose, hemicellulose and lignin, are often characterized by a complex structure, which may render them recalcitrant to biodegradation. Indeed, the degree of this recalcitrance can vary depending on the substrate, i.e. on the specific contents of these three main components, and particularly lignin, which functions as a barrier in lignocellulosic matrices, preventing degradation and limiting the effectiveness of biological processes (Chandra et al., 2012). Due to this particularity characterizing lignocellulosic materials, pretreatment methods are often adopted prior to anaerobic digestion, in order to disrupt their structure and eventually enhance their digestibility. These methods, depending on their basic mode of action, can primarily be categorized as physical, chemical and biological (Zheng et al., 2014).

Additionally to the issues related to potential substrate recalcitrance, the anaerobic digestion process may be compromised by other factors, mainly related to nutrient balance and organic load issues. In fact, if such environmental conditions are not found favorable for microbial action, instability phenomena may be manifested, often leading to system failure (Esposito et al., 2012; Mata-Alvarez et al., 2014). In order to avoid this kind of problems, co-digestion of two or more substrates is frequently implemented. The objective of such a practice is to appropriately select the different co-substrates and their mixing ratios, in order to obtain final feedstock materials with more appropriate nutrient balance and organic content, and to ultimately achieve improved methane production and digestate stability (Astals et al., 2014; Fitamo et al., 2016).

1.2 Objectives of PhD thesis

The main goal of this thesis is to study some of the most produced types of solid organic waste of the Mediterranean area and especially of Greece, using the anaerobic digestion technology. More specifically, four agroindustrial waste were studied, namely winery solid waste, cotton gin solid waste, olive mill solid waste (olive pomace) and juice industry solid waste (orange waste).

The objectives of this study were the following:

- To evaluate the most suitable conditions for determining the methane potential of the investigated substrates, in their raw form.
- To evaluate the effect of different pretreatment methods, such as microwave and chemical pretreatment, on the solubilization of the substrates and to determine the most suitable conditions for the achievement of their maximum solubilization, as well as to evaluate the effect of these processes on substrate degradability and methane production and to characterize the pretreated materials.
- To study the conditions for anaerobic digestion of the substrates in semi-continuous mode, through their mono-digestion and their co-digestion with the organic fraction of municipal solid waste, as well as to characterize the resulting digestates, in order to evaluate their eventual further use.

1.3 Structure of PhD thesis

The present PhD thesis is comprised of seven chapters. A brief description of the contents of each chapter is presented in this section.

Chapter 2 presents a theoretical background regarding the investigated types of solid organic waste produced in the Mediterranean region, as well as the anaerobic digestion process and pretreatment methods for lignocellulosic materials.

In Chapter 3, the effect of different substrate to inoculum ratios (SIR) and inoculum types on the methane potential of the four investigated solid agroindustrial waste, i.e. winery waste (WW), cotton gin waste (CGW), olive pomace (OP) and juice industry waste (JW), was studied. Specifically, the influence of these factors was evaluated by conducting Biochemical Methane Potential (BMP) assays, in which four SIR (0.25-2) were tested and three different inocula, i.e. anaerobic sludge, landfill leachate and thickened anaerobic sludge, were compared.

In Chapter 4, the application of microwave pretreatment of the four agroindustrial waste prior to anaerobic digestion was investigated, in order to evaluate its effect on their solubilization and degradability. To this purpose, microwave heating was performed at five different temperatures and by examining varying solid to liquid ratios, heating rates and holding times.

In Chapter 5 the effect of different chemical pretreatments on the solubilization and degradability of the four agroindustrial waste was studied. The use of eight different reagents, namely sodium hydroxide (NaOH), sodium bicarbonate ($NaHCO_3$), sodium chloride (NaCl), citric acid (H_3Cit), acetic acid (AcOH), hydrogen peroxide (H_2O_2), acetone (Me_2CO) and ethanol (EtOH), was studied. Moreover, three condition sets were evaluated, resulting in

treatments of varying intensity, depending on process duration, reagent dosage and temperature.

In Chapter 6 the anaerobic digestion of the four agroindustrial waste in semi-continuous mode was studied, by conducting both mono-digestion and co-digestion assays. In co-digestion assays, a synthetic organic fraction sample was used as a co-substrate. Additionally, the assays were divided into two groups, in order to study the application of different conditions. The assays of Group I aimed at investigating the variation in operational conditions, such as the organic loading rate and the hydraulic retention time, while in the assays of Group II, the objective was to evaluate the performance of anaerobic digestion systems being fed with different substrates in a sequential order.

Chapter 7 presents an overview of the results obtained in the previous Chapters (3, 4, 5 and 6), providing comparisons between the two different pretreatment methods applied and additional general considerations regarding substrate solubilization, methane production and energy. Moreover, further research topics are evaluated.

1.4 Contribution and novelty of PhD thesis

The anaerobic digestion of the four agroindustrial waste being considered in the present thesis had not been extensively studied before in terms of evaluating the most suitable substrate to inoculum ratio for determining their methane potential through biochemical methane potential assays. Moreover, no specific researches examining the effect of inoculum type have been conducted in relation to such materials. This thesis makes a significant contribution to these issues, especially considering the evaluation of landfill leachate and thickened anaerobic sludge as inocula. Additionally, the application of a kinetic modeling approach, including both single-modeling and multiple-stage modeling, to BMP data referring to different substrate to inoculum ratio and inoculum type, has not been studied before, especially for the investigated substrates.

As far as pretreatments are concerned, there is lack of research concerning the application of microwave and chemical pretreatment on the investigated substrates, therefore this thesis contributes to adding information to this topic. Specifically, it provides optimum conditions for microwave pretreatment of the substrates in question, as well as data concerning the use of less common reagents, such as acetic acid, sodium chloride, citric acid etc. for chemical pretreatment. Moreover, regarding the latter pretreatment, the application of the different condition sets being adopted for the purposes of the present thesis, had not been investigated before.

Lastly, there is lack of studies regarding mono- and co-digestion of CGW, WW, OP and JW, under the conditions evaluated in the present study. To this regard, the major novelty of this thesis, is feeding both mono- and co-digestion systems with the four substrates in a sequential order, based on their seasonality. The evaluation of such a feeding mode is important, considering that it would contribute to the controlled management and utilization of a variety of regional waste materials, by taking advantage of the seasonal variations in their availability. In addition, this strategy would also allow the operation and exploitation of such an anaerobic digestion system during longer periods of time, or even continuously.

1.5 Publications

Publications in scientific journals

- *Directly related to the PhD Thesis*

Pellera F.-M., Gidarakos, E. **(2016)**. Effect of substrate to inoculum ratio and inoculum type on the biochemical methane potential of solid agroindustrial waste. *Journal of Environmental Chemical Engineering*, *4*(3), 3217-3229.

Pellera, F.-M., Santori, S., Pomi, R., Polettini, A., Gidarakos, E. **(2016)**. Effect of alkaline pretreatment on anaerobic digestion of olive mill solid waste. *Waste Management*, *58*, 160-168.

Pellera F.-M., Gidarakos, E. **(2017)**. Microwave pretreatment of lignocellulosic agroindustrial waste for methane production. *Journal of Environmental Chemical Engineering*, *5*(1), 352-365.

- *Related to the PhD Thesis area of research*

Pellera, F.-M., Pasparakis, E., Gidarakos, E. **(2016)**. Consecutive anaerobic-aerobic treatment of the organic fraction of municipal solid waste and lignocellulosic materials in laboratory-scale landfill-bioreactors. *Waste Management*, *56*, 181-189.

Publications in international conferences

- *Directly related to the PhD Thesis*

Pellera F.-M., Gidarakos E. **(2013)**.Study on the biochemical methane potential of solid agroindustrial waste. *14th International Waste Management and Landfill Symposium, SARDINIA 2013*, S. Margherita di Pula (Cagliari), Sardinia, Italy, 30 September – 4 October.

Pellera F.-M., Gidarakos E. **(2014)**. Effect of substrate to inoculum ratio on the biochemical methane potential of solid agroindustrial waste. *4th International Conference of Industrial and Hazardous Waste Management, CRETE 2014*, Chania, Crete, Greece, 2 – 5 September.

Pellera F.-M., Santori S., Gidarakos E., Pomi R., Polettini A. **(2014)**. Effect of alkaline pretreatment on methane potential of olive mill solid waste. *4th International Conference of Industrial and Hazardous Waste Management, CRETE 2014*, Chania, Crete, Greece, 2 – 5 September.

Pellera F.-M., Gidarakos E. **(2014)**. Effect of microwave pretreatment on methane potential of lignocellulosic materials. *5th International Symposium on Energy from Biomass and Waste, VENICE 2014*, San Servolo, Venice, Italy, 17 – 20 November.

Pellera F.-M., Gidarakos E. **(2016)**. Microwave treatment of agroindustrial waste prior to anaerobic digestion. *5th International Conference of Industrial and Hazardous Waste Management, CRETE 2016*, Chania, Crete, Greece, 27 – 30 September.

Pellera F.-M., Gidarakos E. **(2016)**. Chemical pretreatment of agroindustrial waste for methane production. *6th International Symposium on Energy from Biomass and Waste, VENICE 2016*, Great School of St. John the Evangelist, Venice, Italy, 14 – 17 November.

- *Related to the PhD Thesis area of research*

Pellera F.-M., Acheilas I., Gidarakos E. **(2015)**. Low-temperature thermal treatment of lignocellulosic waste prior to anaerobic digestion. *14th International Conference on Environmental Science and Technology, CEST 2015*, Rhodes island, Greece, 3 – 5 September.

Pellera F.-M., Dementi K., Gidarakos E. **(2015)**. Study on the operational parameters for anaerobic digestion of the organic fraction of municipal solid waste. *15th International Waste Management and Landfill Symposium, SARDINIA 2015*, S. Margherita di Pula (Cagliari), Sardinia, Italy, 5 – 9 October.

Pasparakis E., **Pellera F.-M.**, Gidarakos E. **(2015)**. Sequential anaerobic-aerobic treatment of the organic fraction of municipal solid waste and lignocellulosic materials in laboratory-scale landfill-bioreactors. *15th International Waste Management and Landfill Symposium, SARDINIA 2015*, S. Margherita di Pula (Cagliari), Sardinia, Italy, 5 – 9 October.

Moukazis I., **Pellera F.-M.**, Gidarakos E. **(2016)**. Characterization and methane potential of slaughterhouse waste. *5th International Conference of Industrial and Hazardous Waste Management, CRETE 2016*, Chania, Crete, Greece, 27 – 30 September.

Moukazis I., **Pellera F.-M.**, Gidarakos E. **(2016)**. Semi-continuous anaerobic co-digestion of slaughterhouse waste and agroindustrial waste. *6th International Symposium on Energy from Biomass and Waste, VENICE 2016*, Great School of St. John the Evangelist, Venice, Italy, 14 – 17 November.

1.6 References

Aboudi, K., Álvarez-Gallego, C.J., Romero-García, L.I. (2016). Biomethanization of sugar beet byproduct by semi-continuous single digestion and co-digestion with cow manure. *Bioresource Technology*, *200*, 311–9.

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., Lens, P.N.L. (2014). Pretreatment methods to enhance anaerobic digestion of organic solid waste. *Applied Energy*, *123*, 143–156.

Astals, S., Batstone, D.J., Mata-Alvarez, J., Jensen, P.D. (2014). Identification of synergistic impacts during anaerobic co-digestion of organic wastes. *Bioresource Technology*, *169*, 421–7.

Chandra, R., Takeuchi, H., Hasegawa, T. (2012). Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. *Renewable and Sustainable Energy Reviews*, *16*(3), 1462–1476.

Esposito, G., Frunzo, L., Giordano, A., Liotta, F., Panico, A., Pirozzi, F. (2012). Anaerobic co-digestion of organic wastes. *Reviews in Environmental Science and Bio/Technology*, *11*(4), 325–341.

Fernández-Cegrí, V., De la Rubia, M.A., Raposo, F., Borja, R. (2012). Effect of hydrothermal pretreatment of sunflower oil cake on biomethane potential focusing on fibre composition. *Bioresource Technology*, *123*, 424–429.

Fitamo, T., Boldrin, A., Boe, K., Angelidaki, I., Scheutz, C. (2016). Co-digestion of food and garden waste with mixed sludge from wastewater treatment in continuously stirred tank reactors. *Bioresource Technology*, *206*, 245–254.

Fountoulakis, M.S., Drakopoulou, S., Terzakis, S., Georgaki, E., Manios, T. (2008). Potential for methane production from typical Mediterranean agro-industrial by-products. *Biomass and Bioenergy, 32(2),* 155–161.

Hoornweg, D., Bhada-Tata, P. (2012). What a Waste: A Global Review of Solid Waste Management. World Bank, Washington DC, USA.

Mata-Alvarez, J., Dosta, J., Romero-Güiza, M.S., Fonoll, X., Peces, M., Astals, S. (2014). A critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renewable and Sustainable Energy Reviews*, *36*, 412–427.

Nigam,P.S.N. Gupta, N., Anthwal, A. (2009). Pre-treatment of agro-industrial residues, P.S.N. Nigam, A. Pandey (Eds.), Biotechnology for agro-industrial residues utilisation, Springer Science and Business Media BV, Dordrecht, 13–32.

Pagés-Díaz, J., Pereda-Reyes, I., Taherzadeh, M.J., Sárvári-Horváth, I., Lundin, M. (2014). Anaerobic co-digestion of solid slaughterhouse wastes with agro-residues: Synergistic and antagonistic interactions determined in batch digestion assays. *Chemical Engineering Journal*, *245*, 89–98.

Sawatdeenarunat, C., Surendra, K. C., Takara, D., Oechsner, H., Kumar Khanal, S. (2015). Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. *Bioresource Technology*, *178*, 178–186.

Zhao, X., Wang, L., Lu, X., Zhang, S. (2014). Pretreatment of corn stover with diluted acetic acid for enhancement of acidogenic fermentation. *Bioresource Technology*, *158*, 12–18.

Zheng, Y., Zhao, J., Xu, F., Li, Y. (2014). Pretreatment of lignocellulosic biomass for enhanced biogas production. *Progress in Energy and Combustion Science*, *42*, 35–53.

CHAPTER 2

Theoretical Background

2.1 Solid organic waste in the Mediterranean area

2.1.1 Wine production waste

Grapes (*Vitis vinifera*) are one of the most important and most cultivated fruit crops in the world, while wine production is a major agroindustrial activity on a global level and especially in the Mediterranean region (Bustamante et al., 2008; Díaz et al., 2013; El Achkar et al., 2016; Nogales et al., 2005). The global production of grapes and wine in 2013 was more than 77 million tons and 27 million tons, respectively. Mediterranean countries account for 38 and 44% of those quantities, respectively, with France, Italy, Spain and Greece being the main wine producers of the region (Table 2-1). At the same time, China (mainland) was the leader in worldwide production of grapes (11.6 million tons), while the United States of America (USA) were third in wine production (3.2 million tons), after France and Italy (FAOSTAT, 2016).

Table 2-1: Grapes and Wine production in Mediterranean countries in 2013

Countries	Grapes (t)	Wine (t)
Albania	184,731 [a]	18,000 [b]
Algeria	570,840 [a]	49,800 [b]
Bosnia and Herzegovina	31,800 [a]	4,163 [a]
Croatia	181,096 [a]	46,000 [b]
Cyprus	24,560 [a]	11,183 [a]
Egypt	1,389,133 [c]	4,500 [b]
France	5,518,371 [a]	4,293,466 [a]
Greece	957,400 [a]	311,530 [a]
Israel	85,140 [a]	5,200 [b]
Italy	8,010,364 [a]	4,107,370 [a]
Lebanon	87,131 [c]	15,000 [b]
Libya	33,105 [c]	-
Malta	4,315 [a]	2,450 [d]
Montenegro	40,000 [b]	16,000 [b]
Morocco	436,315 [a]	34,500 [b]
Slovenia	68,378 [a]	25,000 [b]
Spain	7,480,000 [a]	3,200,000 [b]
Syrian Arab Republic	306,736 [a]	85 [b]
Tunisia	132,000 [a]	28,500 [b]
Turkey	4,011,409 [a]	30,000 [b]
Mediterranean area (total for twenty countries)	29,552,824	12,202,747
European Union	26,486,635 [e]	14,310,120 [e]
World	77,181,122 [e]	27,421,931 [e]

[a] official data, [b] FAO estimate, [c] FAO data based on imputation methodology, [d] calculated data, [e] aggregate

The wine production process includes all the procedures followed during the elaboration of wine from grapes, mainly being performed during autumn (Díaz et al., 2013). The main steps followed during this process are similar for both red and white wine production, albeit with some slight differentiations in the order of the steps. In fact, red wine production includes destemming, crushing, fermentation, maceration, pressing, malolactic fermentation, clarification, blending, maturation, filtration and bottling, while white wine production encompasses destemming, crushing, pressing, fermentation, malolactic fermentation, clarification, maturation, filtration and bottling (Grainger and Tattersall, 2007; Oliveira and Duarte, 2016).

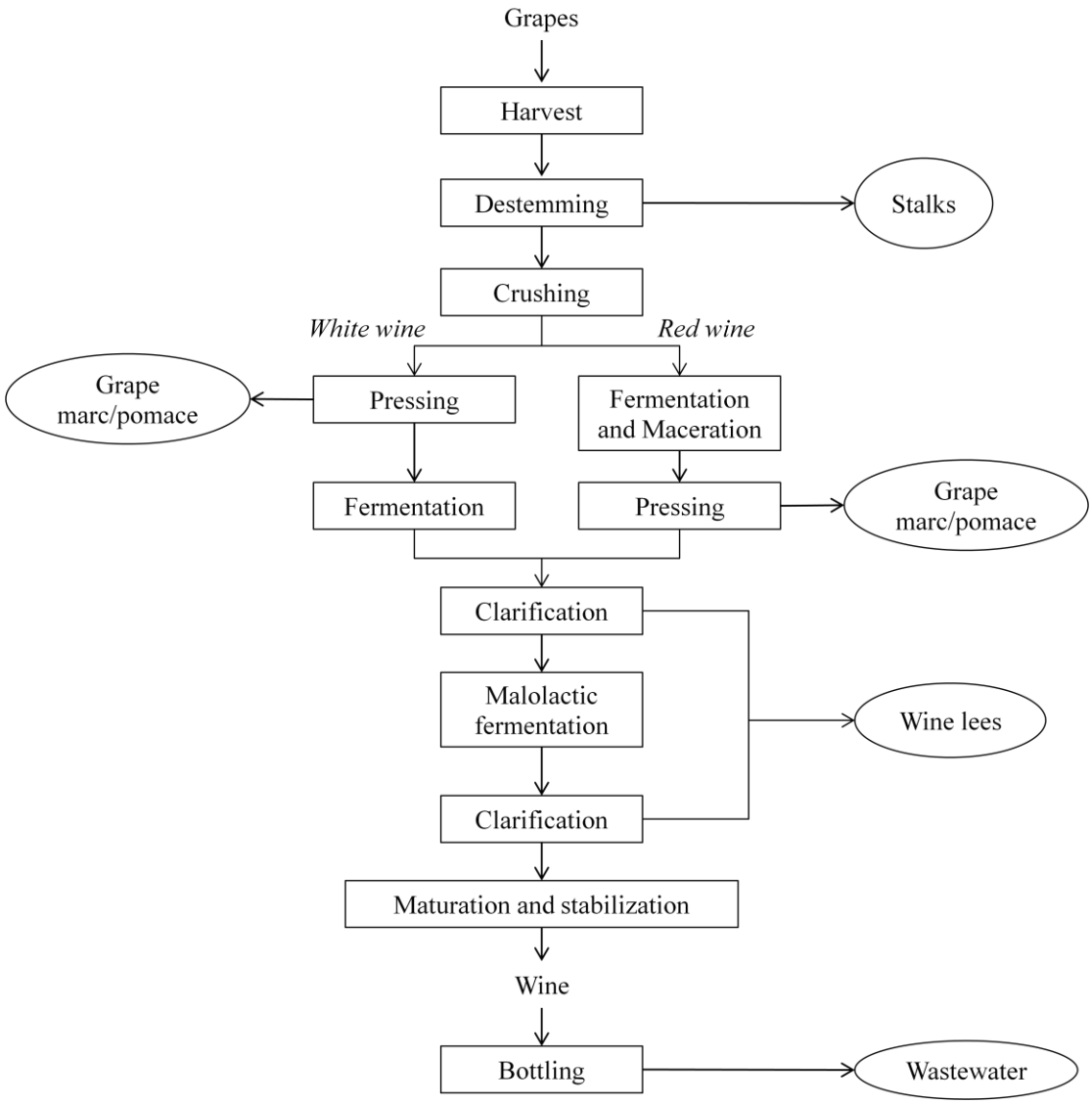


Figure 2-1: Diagram of the wine production process (adapted from Oliveira and Duarte (2016))

The waste streams generated from wineries include both solid waste and wastewater. A schematic diagram describing the vinification steps and the waste materials produced during this process is presented in Fig. 2-1. Wastewater mainly result from maintenance, washing and cleaning operations, which may be related to tank, floor and equipment washing, barrel cleaning, wine and product losses, bottling facilities, filtration units and rainwater (Da Ros et al., 2016; Fabbri et al., 2015; Oliveira and Duarte, 2016). On the other hand, solid waste are generated directly through wine production processes, specifically during destemming, pressing and settling and include three main types, namely stems or stalks, grape marc or pomace and wine lees (Bustamante et al., 2008; Fabbri et al., 2015; Oliveira and Duarte, 2016). All these materials account for more than 20% of the wet weight of the original fruit (Arvanitoyannis et al., 2006; Marculescu and Ciuta, 2013). Grape marc is the most abundant of the three types of solid winery waste and is composed of pressed skins, pulps and seeds and is characterized by a complex lignocellulosic nature (Díaz et al., 2013; El Achkar et al., 2016; Fabbri et al., 2015; Nogales et al., 2005; Oliveira and Duarte, 2016). This material represents approximately 12-14% of the fresh grape weight (Caceres et al., 2012; Oliveira and

Duarte, 2016). On the other hand, wine lees consist of the material that accumulates at the bottom of fermentation tanks (Nogales et al., 2005). The composition of such waste depends on the vinification process, although their main characteristics include an acidic pH (between 3 and 6), a COD greater than 30000 mg/L, potassium concentrations greater than 2500 mg/L and phenolic compounds concentrations up to 1000 mg/L (Da Ros et al., 2016). Both grape marc and wine lees are often used to recover added-value compounds. These include ethanol, tartrates and malates, citric acid, flavanols, tannins, polyphenols, tartaric acid and grape seeds oil. The by-product obtained after the recovery of all these compounds, mainly through distillation, may be used as fuel for heating or power-generation purposes, as soil mulches, as organic amendments prior to composting with other organic wastes, as well as animal feedstuff (Caceres et al., 2012; Nogales et al., 2005; Oliveira and Duarte, 2016).

2.1.2 Olive oil production waste

Olive tree (*Olea europaea*) cultivation and olive oil extraction have been among the most widespread activities in Mediterranean regions, since thousands of years (Azbar et al., 2004; Carlini et al., 2015). In fact, most of the global production of olives and olive oil is concentrated in the countries bordering the Mediterranean Sea (Battista et al., 2014; Kalderis and Diamadopoulos, 2010). In 2013, more than 22 million tons of olives and 2.8 million tonnes of olive oil were produced on a global level, of which almost 93% was provided from twenty Mediterranean countries. The leading country was Spain, followed in order by Italy and Greece (Table 2-2) (FAOSTAT, 2016).

Table 2-2: Olives and Olive oil production in Mediterranean countries in 2013

Countries	Olives (t)	Olive oil (t)
Albania	92,000 [a]	800 [c]
Algeria	578,740 [a]	64,700 [c]
Bosnia and Herzegovina	153 [a]	-
Croatia	34,269 [a]	1,000 [c]
Cyprus	12,728 [a]	1,680 [c]
Egypt	541,790 [a]	6,000 [c]
France	26,850 [a]	4,900 [c]
Greece	1,917,623 [a]	305,900 [c]
Israel	77,000 [a]	12,300 [c]
Italy	2,940,545 [a]	442,000 [c]
Lebanon	97,000 [b]	16,000 [b]
Libya	138,000 [b]	15,000 [c]
Malta	5 [b]	4 [d]
Montenegro	2,900 [b]	180 [d]
Morocco	1,181,675 [a]	114,100 [c]
Slovenia	1,479 [a]	400 [c]
Spain	9,250,000 [a]	1,110,000 [c]
Syrian Arab Republic	842,098 [a]	159,595 [a]
Tunisia	1,100,000 [a]	191,800 [c]
Turkey	1,676,000 [a]	187,900 [c]
Mediterranean area (total for twenty countries)	20,510,855	2,634,259
European Union	14,835,240 [e]	1,965,869 [e]
World	22,039,921 [e]	2,825,730 [e]

[a] official data, [b] FAO estimate, [c] unofficial data, [d] calculated data, [e] aggregate

The olive oil extraction process is conducted in olive mills and it involves the separation and collection of the oil from the olives, which are composed of 70–90% of pulp, 9–27% of stone and 2–3% of seed, on a total weight basis, with their two main constituents (water and oil) being concentrated in the pulp and seed (Alburquerque et al., 2004; Kalderis and Diamadopoulos, 2010). The oil extraction process is comprised of four main operations, namely fruit cleaning (defoliation, olive washing), preparation of the paste (crushing, malaxation), separation of the solid (pomace) and liquid phases (oily must and wastewater) and further separation of the liquid phases (oil/wastewater) (Petrakis, 2006). This process can be performed through the operation of discontinuous or continuous systems (Azbar et al., 2004; Carlini et al., 2015). Fig. 2-2 presents a schematic diagram of both these processes. Discontinuous systems consist of traditional pressing systems, a low cost and technically simple method, which includes washing, crushing, and kneading of the olives with the addition of hot water, as well as a pressing of the resulting paste to drain the oil. A vertical centrifugation or decanting step is finally adopted in order to separate the olive oil from the water. The waste materials produced through this type of extraction include a solid fraction, known as olive husk, and a liquid fraction consisting of a mixture of olive juice and added water, which also contains residual oil (Azbar et al., 2004; Carlini et al., 2015; Kalderis and Diamadopoulos, 2010; Roig et al., 2006). The traditional pressing system is characterized by disadvantages such as discontinuity and high cost (Carlini et al., 2015). Continuous extraction systems on the other hand, use centrifugation processes for separating the different phases. Two types of such processes exist, the three-phase and the two-phase processes, with both, having a similar olive oil yield, but differing in the amount and composition of their waste streams (Carlini et al., 2015; Kalderis and Diamadopoulos, 2010). In three-phase systems, hot water is added at the centrifugation step, resulting in the generation of three fractions, namely olive oil, wastewater and a wet solid waste (Azbar et al., 2004; Carlini et al., 2015; Kalderis and Diamadopoulos, 2010; Roig et al., 2006). The wastewater generated through this process is comprised of the water content of the fruit, usually defined as vegetation water, and the water used to wash and process the olives. This stream often contains soft tissues from olive pulp and a very stable oil emulsion (Borja et al., 2006). On the other hand, the respective solid waste, also called olive cake or olive pomace, consists of the seed and the spent olive mass (skin) (Kalderis and Diamadopoulos, 2010). These systems, compared with traditional pressing systems, are characterized by higher production, lower labor cost, smaller space requirement, better oil quality, improved process control and complete automation. Nevertheless, they also have some disadvantages, i.e. greater water and energy consumption, higher wastewater production and higher installation costs. In two-phase systems, two fractions are generated, namely oil and a mixed semi-solid stream composed of wastewater and olive cake, also known as wet pomace. These systems are often defined as “ecological”, due to the reduced water consumption (Azbar et al., 2004; Carlini et al., 2015; Kalderis and Diamadopoulos, 2010; Roig et al., 2006), i.e. 0.25 dm^3/kg processed olives compared with ~1.25 dm^3/kg processed olives (Borja et al., 2006). Nevertheless, their waste stream has a concentrated pollutant load, resulting in its difficult management (Carlini et al., 2015). Table 2-3 provides an estimation of the input and output data for the three types of olive oil extraction systems.

The physico-chemical characteristics of the waste materials generated through olive oil production are highly dependent on the method adopted for the extraction, while their qualitative and quantitative composition varies also according to soil cultivation, harvesting time, degree of ripening, olive variety, climatic conditions, use of pesticides and fertilizers and duration of aging (Azbar et al., 2004; Borja et al., 2006; Kalderis and Diamadopoulos,

2010; Roig et al., 2006). Both solid and liquid olive-mill waste are characterized by the following general properties: intense dark brown to black color, low pH (between 3 and 5.9) and strong acidic smell specific to olives, high content in organic (COD values up to 220 g/L) and phenolic (up to 80 g/L) compounds, presence of several complex substances, high solid content of the organic matter (total solids up to 20 g/L) and high electrical conductivity. The main organic compounds encountered in olive-mill-wastewater are sugars and phenolic compounds, while inorganics, such as metal cations and anions are also found together with a variable high number of bacteria, yeasts and fungi (Azbar et al., 2004; Battista et al., 2014; Borja et al., 2006; Carlini et al., 2015). As far as the olive-mill-solid-waste, i.e. olive pomace, is concerned, it consists of fragments of skin, pulp, pieces of kernels and some oil, with its major components being sugars (mainly polysaccharides), proteins, fatty acids (e.g. oleic acid and other C2-C7 fatty acids), polyalcohols, polyphenols and other pigments. Regarding its water and oil contents, they depend on the oil extraction process being applied, as well as on the operating conditions (Karantonis et al., 2008). In fact, in traditional pressing systems water content ranges from 25 to 30%, in three-phase systems it is around 30–50%, while in two-phase systems it is approximately between 60 and 70%. Moreover, oil content may vary between 2 and 4% (Azbar et al., 2004; Sánchez Moral and Ruiz Méndez, 2006). Olive pomace can further be processed in pomace treatment plants, in order to recover the oil that it contains, also called pomace oil. At present, pomace oil can be obtained through two types of methods, i.e. solvent extraction (traditional) and physical extraction or centrifugation (second centrifugation). The first method is used when dried pomace is available, while in the case of fresh or stored two-phase pomace, the second method is adopted. The solid fraction obtained after these processes is often used as fuel for heating purposes, or disposed of in landfills (Azbar et al., 2004; Kalderis and Diamadopoulos, 2010; Sánchez Moral and Ruiz Méndez, 2006).

Table 2-3: Input–output data for olive oil production processes (adapted from Azbar et al. (2004))

Production process	Input	Amount of input	Output	Amount of output
Traditional pressing systems	Olives	1 ton	Oil	~200
	Wash water	0.1-0.12 m^3	Solid waste (25% water + 6% oil)	~400
	Energy	40-63 kWh	Wastewater (88% water + solids and oil)	~600
Three-phase systems	Olives	1 ton	Oil	200
	Wash water	0.1-0.12 m^3	Solid waste (50% water + 4% oil)	500-600
	Fresh water for decanter	0.5-1 m^3	Wastewater (94% water + 1% oil)	1000-1200
	Water to polish the impure oil	~10 L		
	Energy	90-117 kWh		
Two-phase systems	Olives	1 ton	Oil	200
	Washing water	0.1-0.12 m^3	Solid + water waste (60% water + 3% oil)	800-950
	Energy	< 90-117 kWh		

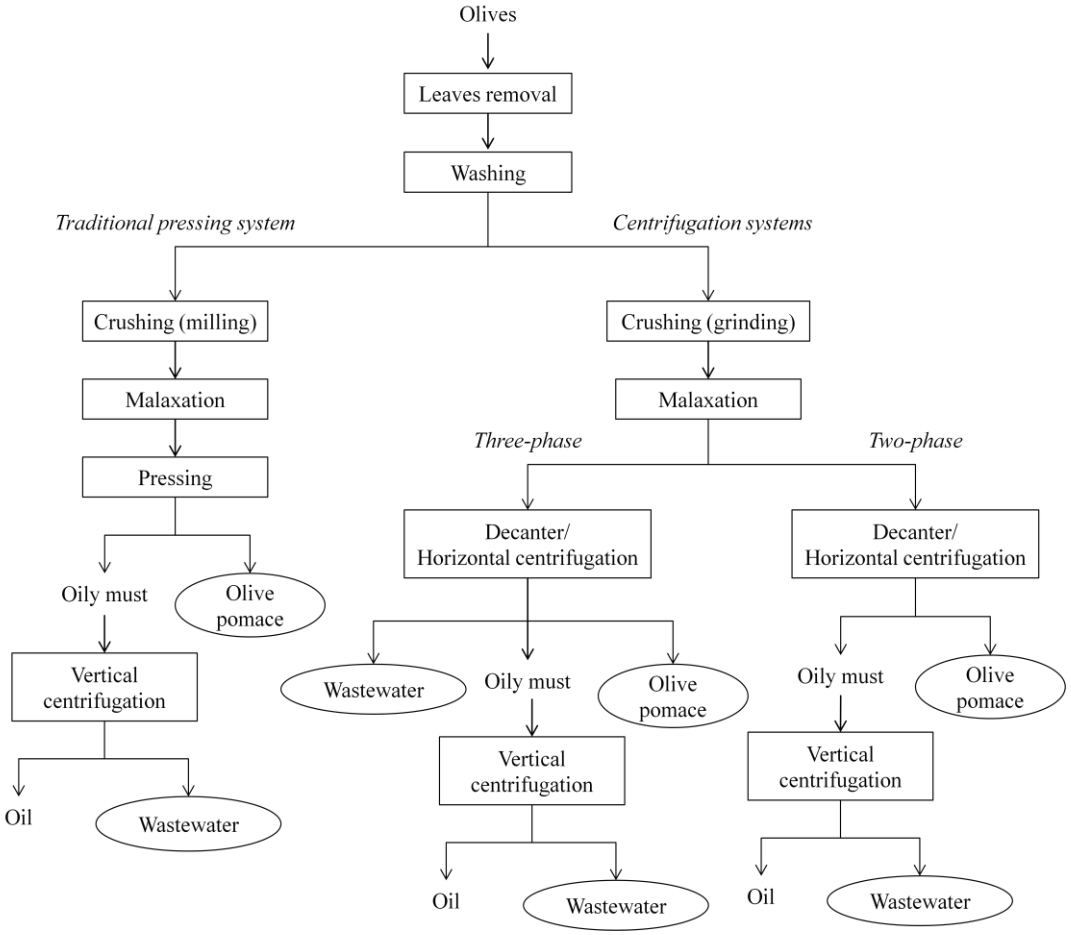


Figure 2-2: Diagram of the olive oil production processes (adapted from Kalderis and Diamadopoulos (2010) & Petrakis (2006))

2.1.3 Orange processing waste

Citrus fruits represent an important group of fruit crops on a global level, with oranges, and especially sweet oranges (*Citrus sinensis*) being among the most widespread (Boukroufa et al., 2015; Díaz et al., 2013; Sinha et al., 2012). The biochemical composition of oranges is rich in secondary metabolites, such as proteins, amines, polyamines, carbohydrates, organic acids, lipids, phenols, flavonoids, terpenoids, aromatic compounds, minerals, hormones, and vitamins, all of which add value to the fruit. Due to this added value, oranges are widely used for fresh consumption and juice processing, as well as essential oil extraction (Okino Delgado and Fleuri, 2016). Indeed, orange juice is one of the most widely consumed beverages, with oranges cultivation constituting a major agricultural activity and a significant economic sector in several countries, including Brazil the United States, Mexico, China, India, Iran and most Mediterranean countries (Martín et al., 2010). In fact, according to the Statistical Database of the Food and Agriculture Organization of the United Nations (FAOSTAT) (Table 2-4), 71.6 million tons of oranges were produced in 2013 on a global level with Brazil, the USA and China being the three major producers, accounting for 17.5, 7.6 and 7.3 million tons, respectively. On the other hand, on a Mediterranean level, the production of oranges reached almost 19% of the global amount, with Spain, Egypt, Turkey and Italy having produced the largest quantities, and Greece holding the sixth place after Algeria. During the same year, an orange juice production of nearly 371 thousand tons was recorded for Mediterranean

countries (FAOSTAT, 2015 & 2016). It has been estimated that approximately 70% of the total orange production is processed for juice or marmalade production (Martín et al., 2010), while it has also been reported that the quantity corresponding only to orange juice may range between 40 and 60% of the total amount (Okino Delgado and Fleuri, 2016; Wikandari et al., 2015).

Table 2-4: Oranges and Orange juice production in Mediterranean countries in 2013

Countries	Oranges (t)*	Orange Juice (single strength + concentrated) (t)**
Albania	7,382 [a]	-
Algeria	890,674 [a]	23,869
Bosnia and Herzegovina	130 [b]	-
Croatia	155 [a]	-
Cyprus	36,870 [a]	7,700
Egypt	2,886,015 [c]	-
France	4,117 [a]	941
Greece	805,500 [a]	43,520
Israel	90,220 [a]	22,983
Italy	1,708,337 [a]	27,720
Lebanon	124,146 [c]	1,864
Libya	50,191 [c]	-
Malta	815 [a]	150
Montenegro	9,100 [b]	-
Morocco	759,289 [a]	98,000
Slovenia	-	-
Spain	3,394,100 [a]	141,050
Syrian Arab Republic	792,227 [a]	-
Tunisia	130,000 [a]	1,160
Turkey	1,781,258 [a]	1,700
Mediterranean area (total for twenty countries)	13,470,526	370,657
European Union	6,186,694 [d]	-
World	71,579,503 [d]	-

[a] official data, [b] FAO estimate, [c] FAO data based on imputation methodology, [d] aggregate, * data accessed on 2016, ** data accessed on 2015

In order to produce orange juice, fresh oranges, after being harvested and received in the plant, are first subjected to washing, for impurities removal, prior to juice extraction through mechanical means. Other steps may include deoiling, deaeration, filtration, pasteurization and blending, while in certain cases chemical compounds may also be added to the juice. Fig. 2-3 presents a schematic diagram of the juice production process. Among the different types of orange juice available in the market are fresh juice, pasteurized juice, aseptic single-strength juice, single-strength juice from concentrate and frozen concentrated juice (Okino Delgado and Fleuri, 2016; Rezzadori et al., 2012; Sinha et al., 2012). It is estimated that during juice extraction approximately 50–60% of the processed fruit becomes waste (Wilkins et al., 2007). This material consists of peels, seeds, pulp, and segment membranes (Martín et al., 2010; Koppar and Pullammanappallil, 2013; Siles et al., 2016; Wikandari et al., 2015) and is usually characterized by a low pH (3–4), a high water content (around 80–90%) and a high organic matter content (around 95% of total solids) (Ruiz and Flotats, 2014). In addition to the solid waste, the juice production industry also generates significant amounts of wastewater, which apart from wash water may also contain condensate and press liquor. This

wastewater is commonly treated using lagoons or activated sludge processes (Koppar and Pullammanappallil, 2013). Apart from waste generated by the juice manufacturing industry, orange (and generally citrus) waste, may also include fruit discarded for commercial reasons (e.g. damaged fruit) or due to regulations that limit production. The exact quantity corresponding to this waste category is difficult to calculate, but it is estimated to range from 2 to 10% depending on the type of fruit and environmental (e.g. weather) conditions (Ruiz and Flotats, 2014).

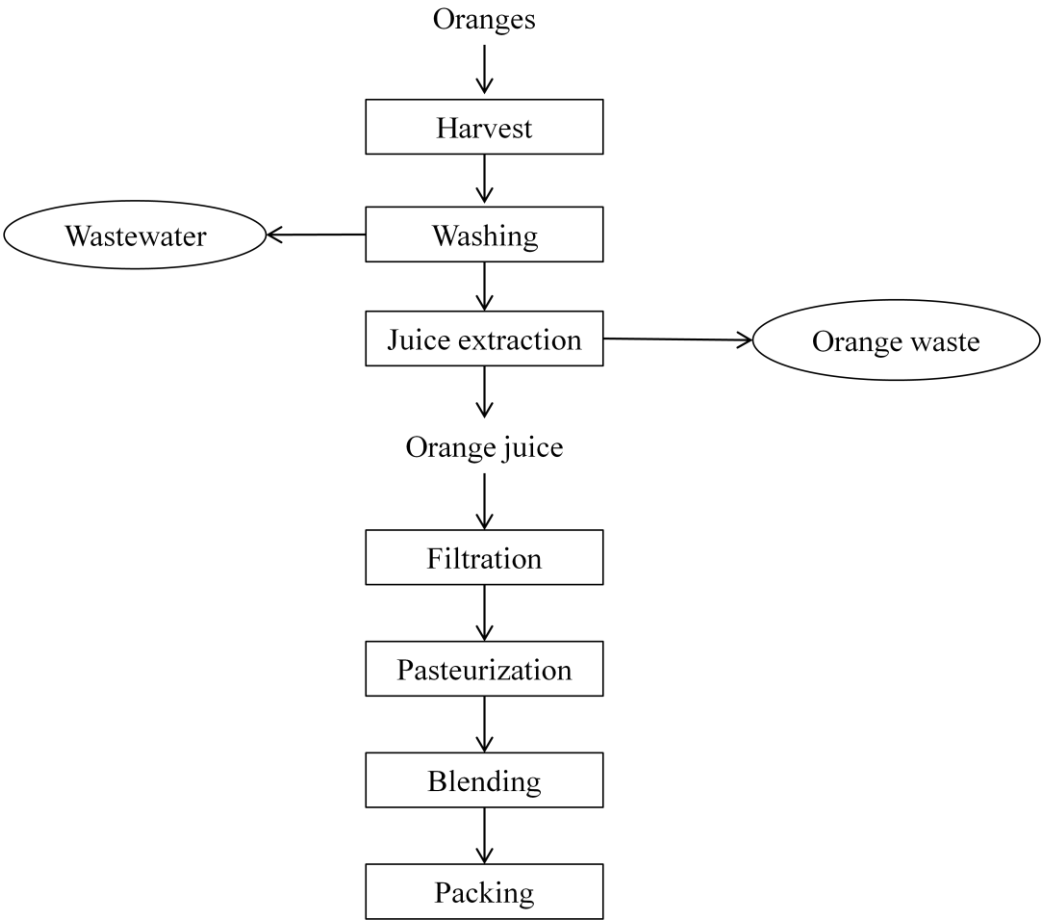


Figure 2-3: Diagram of the orange juice production process (adapted from Rezzadori et al., 2012)

Interestingly, orange peels are the major constituent of the waste material obtained from juice manufacturing, accounting for 60–65% of the entire waste (Negro et al., 2016; Wikandari et al., 2015). This results in a material with a very rich composition, which includes fat, sugars, acids (e.g. citric, malic and tartaric acid), insoluble carbohydrates, enzymes, flavonoids, essential oils (e.g. D-limonene), phenolic compounds, pectins and pigments (e.g. carotenoids) (Boukroufa et al., 2015). Due to this composition, orange waste are often further utilized to extract some of these added-value products (e.g. pectin, essential oils, flavonoids, molasses, D-limonene, fiber), which can be used in food products, pharmaceutical industries and other personal and home care products (Boukroufa et al., 2015; Díaz et al., 2013; Koppar and Pullammanappallil, 2013; Wikandari et al., 2015). Moreover, such waste materials can be used as livestock feed, mainly in pellets form, due to their high carbohydrate content, the significant proportion of cell wall components and their low degree of lignification (Díaz et al., 2013; Koppar and Pullammanappallil, 2013; Ruiz and Flotats, 2014).

2.1.4 Cotton processing waste

Cotton (*Gossypium spp.*) is one of the major fibre crops in the world with a high commercial value. It is grown in temperate and tropical regions, with specific areas of production including countries such as China, USA, India, Pakistan, Uzbekistan, Turkey, Australia, Greece, Brazil, Egypt etc. Most commercially cultivated cotton is derived from two species, *G. hirsutum* and *G. barbadense* (Govt. of India, 2011). China is the world leading producer of seed cotton (18.9 million tons in 2013), cotton lint (6.3 million tons) and cottonseed (12.6 million tons), while the global productions of these commodities in 2013, were almost 73, 24.5 and 45.5 million tons, respectively. As far as Mediterranean countries are concerned, they account for 5, 6 and 5% of the world's production of seed cotton, cotton lint and cottonseed, respectively, with Turkey, Greece and Egypt being the main producers (Table 2-5) (FAOSTAT, 2016).

Table 2-5: Seed cotton, Cotton lint and Cotton seed production in Mediterranean countries in 2013

Countries	Seed cotton (t)	Cotton lint (t)	Cottonseed (t)
Albania	820 [a]	230 [a]	540 [a]
Algeria	78 [a]	27 [a]	51 [a]
Bosnia and Herzegovina	-	-	-
Croatia	-	-	-
Cyprus	0 [a]	0 [a]	0 [a]
Egypt	435,000 [b]	106,000 [b]	140,000 [b]
France	-	-	-
Greece	870,178 [c]	280,000 [b]	475,000 [b]
Israel	28,600 [c]	14,000 [b]	18,000 [b]
Italy	-	-	-
Lebanon	-	-	-
Libya	-	-	-
Malta	-	-	-
Montenegro	-	-	-
Morocco	210 [a]	66 [a]	133 [a]
Slovenia	-	-	-
Spain	145,600 [c]	57,000 [b]	58,200 [b]
Syrian Arab Republic	169,094 [c]	99,000 [a]	109,911 [c]
Tunisia	2,000 [a]	660 [a]	1,260 [a]
Turkey	2,250,000 [c]	832,500 [b]	1,287,000 [c]
Mediterranean area (total)	3,901,580	1,389,483	2,090,095
European Union	1,016,015 [d]	337,065 [d]	533,330 [d]
World	73,019,723 [d]	24,543,551 [d]	45,466,502 [d]

[a] FAO estimate, [b] unofficial data, [c] official data, [d] aggregate

Cotton processing starts after the harvesting step, during which cotton bolls are removed from the plant, leaving behind cotton plant stalks, roots and leaves (Hamawand et al., 2016). Once removed from the field, seed cotton is transported to cotton gins, where the cotton fibers (lint) are separated from the cotton seed, while any foreign materials, such as leaves are also removed from the lint. The ginning process encompasses several steps, including opening of the cotton bolls, drying for moisture reduction, pre-cleaning, seed separation with circular saws, final lint cleaning and wrapping. Ultimately, cotton classing takes place, i.e. the

procedure through which cotton fibre is sorted into different quality-based grades (or classes), according to fiber strength, length, length uniformity, color, non-fiber content and fineness (Cotton Australia, 2016; National cotton council of America, 2016). Therefore, processing of seed cotton in ginning facilities, ultimately results in three products, namely cotton lint, cottonseed and waste (Figure 2-4). Cotton lint, which makes up approximately 35% of seed cotton, is ultimately turned into fabric, while cottonseed, which accounts for almost 55%, is used for manufacturing a variety of products, such as oil, plastics, explosives, stock feed, cosmetics, margarine and insecticides. On the other hand, waste materials account for the remaining 10% of seed cotton (Cotton Australia, 2016). These materials, often referred to as cotton gin waste or trash, are usually composed of burs and stems, fine particles (less than 5mm size), soil, mote, immature cottonseed, cotton lint, sticks, leaves and other plant materials (Hamawand et al., 2016; Placido and Capareda, 2013). Despite extensive research efforts regarding the use of cotton gin waste (i.e. manufacture of fire logs, pellet stove fuel, use as an energy source, use as livestock feed, raw material in asphalt roofing, direct use as a soil amendment), few methods have reached commercial acceptance (Macias-Corral et al., 2008). In fact, the most common disposal methods for this type of material include direct land application after composting and/or use as a low nutrient feeding material for beef cattle, with the low feeding value resulting from its high lignin and ash contents and its low crude protein and energy concentrations (Hamawand et al., 2016).

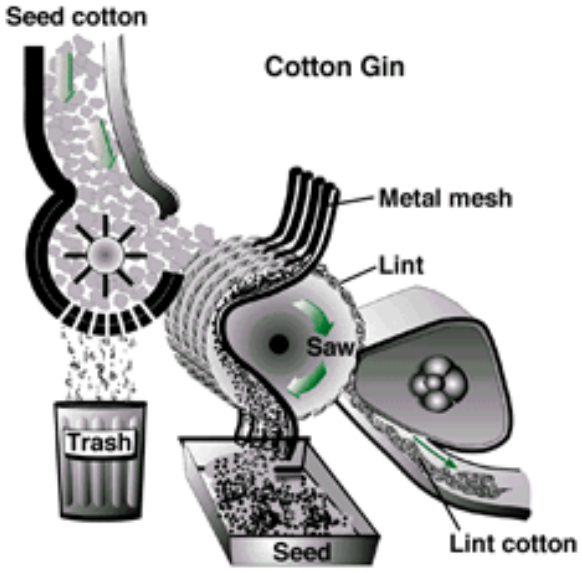


Figure 2-4: Operation of the cotton gin (McGraw-Hill Education, 2016)

2.1.5 Organic fraction of municipal solid waste

Municipal solid waste (MSW) is the waste that is generated from residential sources, such as households, as well as from institutional and commercial sources such as offices, schools, hotels etc. (Albanna, 2013). MSW generation on a global level has been increasing in the last decades, with an even more elevated increase being expected in the future. In fact, a decade ago, MSW generation was about 0.64 kg/capita/day, corresponding to 0.68 billion tonnes/year, while more recent levels reached approximately 1.2 kg/capita/day, corresponding to 1.3 billion tones/year. These levels are expected to increase to almost 1.42 kg/capita/day or

2.2 billion tons/year, by 2025. This progressive increment in waste production is a result of continuous population growth, improved economic development and higher urbanization rates (Hoornweg and Bhada-Tata, 2012).

Table 2-6 presents MSW production data for twenty Mediterranean countries. The total MSW production of this area reached approximately 179 million tones, with France, Turkey, Italy, Spain and Egypt having produced the top five quantities (Eurostat, 2016; Sweep-net, 2014; Waste Atlas, 2016).

Table 2-6: MSW production in Mediterranean countries

Countries	MSW production (t)
Albania	852,360
Algeria	10,300,000
Bosnia and Herzegovina	1,192,000
Croatia	1,721,000
Cyprus	533,000
Egypt	21,000,000
France	33,996,000
Greece	5,585,000
Israel	4,760,000
Italy	29,573,000
Lebanon	2,040,000
Libya	-
Malta	246,000
Montenegro	315,000
Morocco	6,852,000
Slovenia	853,000
Spain	21,184,000
Syrian Arab Republic	4,500,000
Tunisia	2,423,000
Turkey	30,920,000
Mediterranean area (total)	178,845,360
European Union (EU-28)	242,051,000

Data presented here correspond to the years 2012 or 2013, depending on the source

MSW typically contains a wide variety of materials and substances, with the main components including food scraps, garden (leaves, grass, brush) waste, wood and process residues, paper, plastic, glass, metal, textiles, leather, rubber, multi-laminates, e-waste, appliances, ash and other inert materials. The composition of MSW is highly dependent on a number of factors, such as culture, geographical location and regional habits, economic development (e.g. available population income and consumer behavior), climate, energy sources, waste collection and disposal practices, seasonality, lifestyle, industrial development, as well as demographics and legislation (Albanna, 2013; Campuzano and González-Martínez, 2016; Cesaro and Belgiorno, 2014; Gidarakos et al., 2006; Hoornweg and Bhada-Tata, 2012).

MSW composition in the Mediterranean area (Table 2-7) is characterized by high contents in organic and paper/cardboard materials, while metallic materials represent the smallest portion. The highest contents in organic materials (>50%) are mostly observed for countries situated in the Middle East and North Africa region (Sweep-net, 2014; Waste Atlas, 2016)

Table 2-7: MSW composition in Mediterranean countries

Countries	MSW composition (%)					
	Organic	Paper/ cardboard	Plastics	Metal	Glass	Others
Albania	48.8	13.5	13.2	1.1	5.8	17.5
Algeria	62.1	9.4	12.0	1.4	1.6	13.5
Bosnia and Herzegovina	-	-	-	-	-	-
Croatia	42.0	19.9	12.0	4.1	6.8	15.3
Cyprus	37.3	24.6	13.1	3.5	3.8	17.8
Egypt	56.0	10.0	13.0	2.0	4.0	15.0
France	32.0	20.0	9.0	3.0	10.0	26.0
Greece	46.0	19.0	9.0	5.0	5.0	16.0
Israel	40.0	24.0	13.0	3.0	3.0	17.0
Italy	39.5	25.8	14.6	2.7	5.8	11.6
Lebanon	52.5	16.0	11.5	5.5	3.5	11.0
Libya	-	-	-	-	-	-
Malta	52.8	16.5	12.5	4.0	6.1	8.1
Montenegro	-	-	-	-	-	-
Morocco	65.0	10.0	10.0	4.0	3.0	8.0
Slovenia	-	-	-	-	-	-
Spain	48.5	20.8	11.9	4.0	7.9	6.9
Syrian Arab Republic	57.0	7.0	7.0	4.0	3.0	22.0
Tunisia	68.0	10.0	11.0	4.0	2.0	5.0
Turkey	38.4	21.3	12.5	3.2	4.2	20.4
Mediterranean (average)	49.1	16.7	11.6	3.4	4.7	14.4

As also seen in Table 2-7, the highest proportion of MSW usually consists of the organic fraction (OFMSW). This fraction can be highly heterogeneous in terms of composition, source and structure, with many differences depending on the location. OFMSW mainly includes food waste and garden waste. Food waste usually represents the largest portion of OFMSW and it can originate from residential and commercial (e.g. restaurants, cafeterias) kitchens, markets, etc. On the other hand, garden waste consists of lignocellulosic materials, such as green grass clippings, leaves, weeds and tree prunings. The physico-chemical characteristics of OFMSW, such as moisture, as well as carbohydrates, proteins, lipids and lignin content are highly dependent on the specific materials present in the mixture. Moreover, the composition and the quality of OFMSW can be affected by collection and sorting strategies. For example, a high dry solid content characterizes mechanically sorted waste, as a result of the presence of inert materials, since they are not completely separable with this sorting approach. Ultimately, the composition and the quality of OFMSW determine the level of biodegradability of the material, and thus the performance of eventual downstream processes, as well as the quality of their end products (e.g. digestate from anaerobic digestion) (Albanna, 2013; Alibardi and Cossu, 2015; Campuzano and González-Martínez, 2016; Cesaro and Belgiorno, 2014).

The primary treatment and management options for MSW include landfilling, recycling, incineration, composting and open land disposal, with landfilling still representing the most adopted treatment method for several countries around the world (Albanna, 2013). Nevertheless, the remaining technologies other than landfilling have been increasingly gaining ground lately, as a result of the implementation of the European Landfill Directive (99/31/EC), which limits the quantity of biodegradable waste deposited on landfills (Alibardi

and Cossu, 2015). Statistical data regarding the Mediterranean region, for three different MSW management operations (Table 2-8) (Eurostat, 2016; Hoornweg and Bhada-Tata, 2012; Sweep-net, 2014; Waste Atlas, 2016), namely landfilling/disposal, material recycling and composting and digestion, confirm that landfilling still remains the most adopted option for waste management. In fact, most Mediterranean countries present much higher landfilling and much lower recycling rates, compared with the data referring to the European Union (EU) of 28, with the exceptions of France, Italy, Slovenia and Spain, which show more comparable values.

Table 2-8: MSW management operations in Mediterranean countries

Country	MSW management operations (%)		
	Landfill/ disposal	Material recycling	Composting and digestion
Albania	-	-	-
Algeria	30-40	7	1
Bosnia and Herzegovina	75.0	0.0	0.0
Croatia	82.1	13.2	1.7
Cyprus	79.4	13.1	1.5
Egypt	7.0	12.5	7.0
France	25.8	21.5	17.1
Greece	80.7	15.6	3.7
Israel	90.0	10.0	
Italy	36.9	24.8	14.6
Lebanon	48.0	8.0	15.0
Libya	-	-	-
Malta	79.7	7.7	4.9
Montenegro	88.3	1.0	0.0
Morocco	37.0	8.0	<1
Slovenia	26.3	28.0	6.8
Spain	55.7	15.5	17.0
Syrian Arab Republic	20.0	3.0	2.0
Tunisia	70.0	4.0	5.0
Turkey	81.7	0.0	0.5
Mediterranean (average)	57.9	10.7	6.1
European Union (EU-28)	29.9	26.9	15.4

2.1.6 Waste management issues

As far as appropriate management and disposal of the above mentioned solid waste are concerned, there are several problems that agro-industies and communities are confronted with.

Winery waste are usually characterized by low pH, high contents of suspended solids and biodegradable compounds, i.e. high levels of biochemical oxygen demand (BOD) and chemical oxygen demand (COD), as well as high contents of phytotoxic and antibacterial phenolic substances (Bustamante et al., 2008; Caceres et al., 2012; Díaz et al., 2013). Specifically in the case of grape marc, lack of treatment or even inappropriate treatment can lead to a number of environmental problems, including soil, surface and ground water pollution, foul odors, gathering of flies and pests and diseases spreading (Fabbri et al., 2015). Additionally, another common problem is related to the fact that large amounts of waste are

generated during a short period of the year, due to the seasonality of grapes (Bustamante et al., 2008). All the above make it difficult for producers to appropriately manage their waste, while the high cost of these operations is also a major issue (Fabbri et al., 2015).

Olive-mill waste also represent a serious environmental concern for major olive oil-producing countries and especially for Mediterranean countries. This is strongly related to their highly concentrated organic load and the high quantities being produced on a regular basis. In fact, the uncontrolled disposal of such huge amounts of waste could potentially result in severe land and water pollution (Battista et al., 2014; Carlini et al., 2015; Kalderis and Diamadopoulos, 2010).

Similarly, also in the case of orange juice manufacturing waste, their disposal constitutes a major problem for producers partially due to the market saturation and also because inappropriate management could cause pollution, as well as loss of valuable material for subsequent biorefinery processes (Ruiz and Flotats, 2014; Siles et al., 2016). However, their specific characteristics often limit possible management alternatives (Ruiz and Flotats, 2016). In fact, according to European regulations (Directive 2008/98/EC), such materials are inappropriate for landfill disposal, while composting is also not acceptable, due to their low pH and the presence of essential oils, which could inhibit the composting process. Moreover, the fast biodegradation of these materials may lead to anaerobiosis problems in compost piles. As far as thermal treatment is concerned (e.g. incineration, gasification and pyrolysis), these methods cannot be applied to orange waste either, due to the high water content of these materials. In fact, such an option would require a previous drying step, which would make the process energetically and economically inefficient (Ruiz and Flotats, 2014). On the other hand, the option of bioethanol production from orange waste, although technically feasible, is usually limited by the high investment requirements, while it is less energy efficient than methane (biogas) production through anaerobic digestion (Ruiz and Flotats, 2016).

Management options for cotton gin waste are limited mostly to composting, since this material can be neither incinerated nor directly returned to the field, due to the potential hazards resulting from these operations. In fact, cotton gin waste may be hazardous not only due to the soil borne viruses (*verticillium wilt*) that it may contain, but also due to pesticide contamination. Indeed, approximately 70 chemicals have been registered for use in cotton farming, which can contaminate cotton gin waste, since they have the ability of binding to leaf and soil material associated with the cotton lint (Hamawand et al., 2016; Macias-Corral et al., 2008).

The continuous increase in MSW production represents a major issue of concern, especially in relation to their management practices. In fact, inappropriate waste management poses numerous threats to the environment and to public health, while at the same time it could also have a significant impact on the economy, if not energy efficient (Hoornweg and Bhada-Tata, 2012; Pellera et al., 2016). More specifically, landfilling of OFMSW could potentially result in adverse impacts, such as odours, fires, VOC's, groundwater contamination by leachate etc. On the other hand, thermal treatment of OFMSW not always represents a feasible option, due to eventual low heating values of these materials, while composting is often accompanied by disadvantages, related to energy consumption and compost market issues (Alibardi and Cossu, 2015).

In order to prevent all the above mentioned problems related to the disposal of such waste materials and limit environmental concerns, the selection and application of appropriate management strategies, are mandatory.

2.2 Anaerobic digestion

2.2.1 Anaerobic digestion process

Anaerobic digestion is a biological process which involves the decomposition of organic matter by a microbial consortium in the absence of oxygen. Such a process can also be found in nature, specifically in naturally occurring anoxic environments, such as watercourses, sediments, waterlogged soils and mammalian guts (Khalid et al., 2011; Ward et al., 2008). The anaerobic digestion technology had initially been developed for waste stabilization. In fact, it has been successfully implemented in the treatment of a wide variety of organic waste substrates, including not only municipal and industrial wastewater and sludge, but also high solid feedstocks, such as agricultural and other lignocellulosic waste, animal manure, food waste and municipal solid waste. However, in the last decades, the research interests have shifted towards the application of this technology for energy purposes, since apart from being capable of reducing chemical oxygen demand (COD) and biological oxygen demand (BOD) from waste streams, it can also generate significant amounts of renewable energy. The end products of anaerobic digestion include energy-rich biogas and an organic residue rich in nitrogen (Li et al., 2011; Sawatdeenarunat et al., 2016). Biogas is composed of 40–70% (by volume) of methane gas, with the rest being carbon dioxide and traces of ammonia, hydrogen sulfide and hydrogen, and precisely due to its methane content, it is considered as a promising means of addressing global energy needs. Indeed, it is a convenient and clean fuel, which can either be used directly, or be converted into electricity (Abbasi et al., 2012; Mao et al., 2015). Anaerobic digestion is known to have limited environmental impacts (Ariunbaatar et al., 2014), while its environmental benefits essentially consist in two facts: the potentially harmful methane gas being produced from the decomposition of organic matter is prevented from being released to the atmosphere, and by burning this methane, carbon-neutral carbon dioxide will be released (Ward et al., 2008).

Anaerobic digestion processes can be classified in different categories according to a series of operational parameters, such as mode of operation (batch or continuous), temperature (psychrophilic, mesophilic or thermophilic), reactor design (plug-flow, complete-mix or covered lagoons) and solids content (liquid or solid-state). Specifically regarding the latter parameter, the process is characterized as solid-state anaerobic digestion when the solids content of the feedstock is greater than 15% (Li et al., 2011).

As mentioned earlier, a variety of microbes is involved in the anaerobic digestion process, with them being classified depending on the metabolic pathways they follow mainly as hydrolytic, fermentative, acetogenic, and methanogenic. Based on this classification, the anaerobic digestion process is divided accordingly into four phases, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 2-5) (Abbasi et al., 2012; Chandra et al., 2012; Li et al., 2011):

- *Hydrolysis*

During this phase, complex organic polymers are decomposed to simple water soluble monomers, i.e. proteins, fats and carbohydrates are hydrolyzed into amino acids, long-chain fatty acids and sugars, respectively. This process is carried out by extracellular enzymes or exoenzymes (hydrolase) of facultative and obligatorily anaerobic hydrolytic bacteria. More specifically, hydrolysis involves the breakage of covalent bonds in a chemical reaction with water, with this reaction requiring less time for carbohydrates (a few hours) and more time for proteins and lipids (a few days).

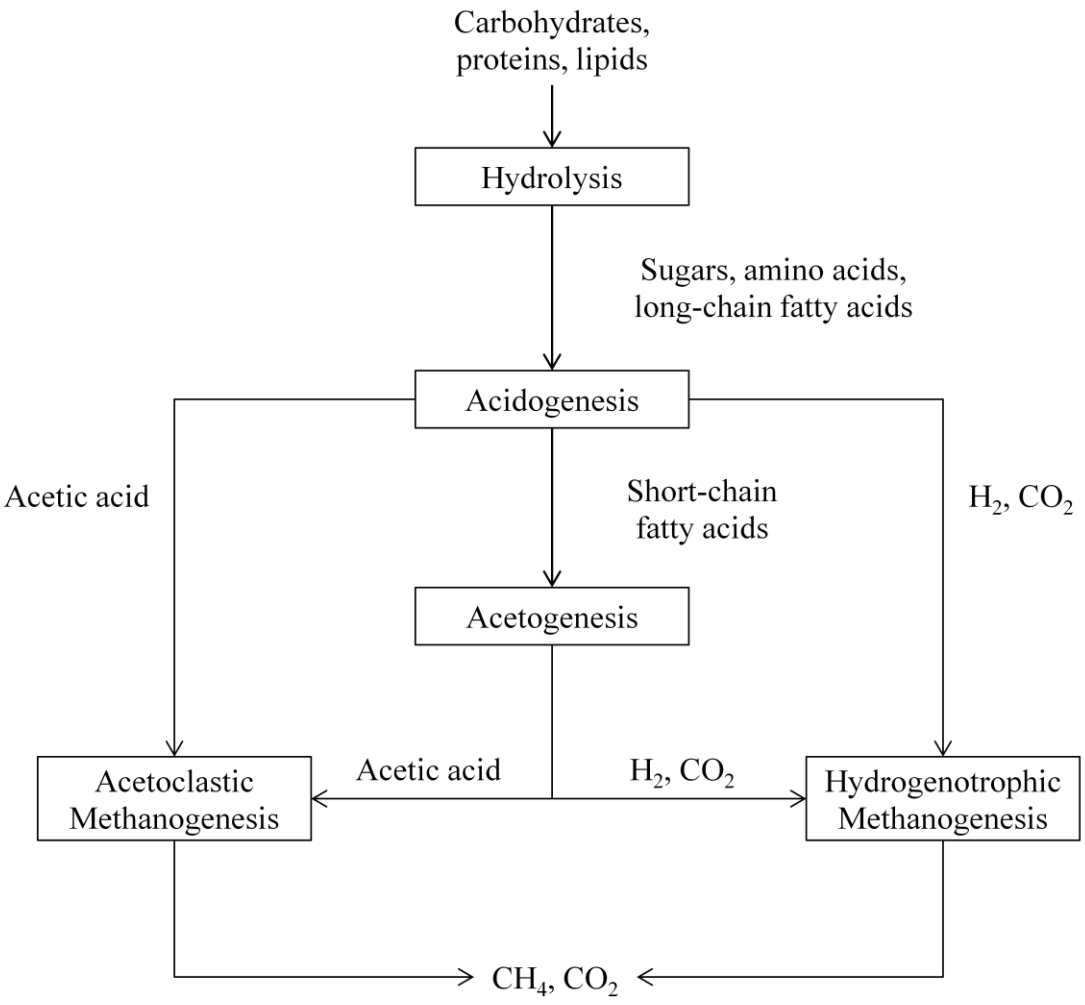


Figure 2-5: Diagram of the anaerobic digestion process (adapted from Li et al., 2011 & Zheng et al., 2014)

- *Acidogenesis*

The acidogenesis phase involves the action of fermentative bacteria, which consists in the degradation of the monomers obtained through hydrolysis into short-chain (C1–C5) organic acids (e.g. butyric acid, lactic acid, valeric acid, propionic acid, acetate, and acetic acid), alcohols, hydrogen and carbon dioxide.

- *Acetogenesis*

The fermentation products resulting from the acidogenesis phase are subsequently consumed as substrates by acetogenic bacteria (or acetogens) who further convert them to acetic acid, acetate, carbon dioxide and hydrogen.

- *Methanogenesis*

Methanogenesis is the final step of the anaerobic digestion process, during which acetate, carbon dioxide, hydrogen and methanol are consumed under strict anaerobic conditions by methanogens to produce methane. These methane producing microorganisms are in a

symbiotic relationship with acetogens, which produce methane precursors. Each methane precursor is degraded by specific methanogenic species.

In fact, three biochemical pathways are distinguished depending on the substrate being used for methanogenesis (Abbasi et al., 2012; Chandra et al., 2012):

I) Acetotrophic pathway (Acetoclastic methanogenesis): $CH_3COOH \rightarrow CO_2 + CH_4$

II) Hydrogenotrophic pathway (Hydogenotrophic methanogenesis): $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$

III) Methylotrophic pathway (Methyltrophic methanogenesis): $CH_3OH + H_2 \rightarrow CH_4 + H_2O$

It has been reported in literature that the rate-limiting step for complex organic substrates is the hydrolysis step, while in the case of easily biodegradable substrates, the rate-limiting step is methanogenesis (Ariunbaatar et al., 2014).

2.2.2 Factors affecting anaerobic digestion

There are certain factors which tend to affect the operation of an anaerobic digestion system, which are specifically associated with the requirements for the optimum function of the anaerobic microorganisms taking part in this process. The main factors include pH, temperature, substrate composition, C/N, organic loading rate, retention time, presence of inhibitors, etc.

2.2.2.1 pH

In the anaerobic digestion process, pH is one of the most important parameters being able to affect its performance, since it influences the growth of the microorganisms involved in the process (Chandra et al., 2012). For instance, hydrolysis and acidogenesis have been found to occur at pH values around 5.5 and 6.5 (Khalid et al., 2011; Ward et al., 2008). On the other hand, it has been reported that methane formation can take place within a pH interval ranging from 6.5 to 8.5, while values between 7.0 and 8.0 are considered as optimum (Weiland, 2010). Nevertheless, slightly different optimum ranges have also been mentioned in literature. Ward et al. (2008) reported an ideal pH range for anaerobic digestion between 6.8 and 7.2, while according to Chandra et al. (2012) the optimum pH range for methane production is even narrower, between 7.0 and 7.2. pH values outside the range 6.0–8.5 cause severe inhibition to the anaerobic digestion process (Weiland, 2010). In fact, values lower than 6.6 reduce the activity of methanogens, while as soon as they drop below 6.2 toxicity is manifested. On the other hand, values exceeding 8.5 may result in disintegration of microbial granules and subsequent failure of the process (Chandra et al., 2012; Ward et al., 2008). Such extreme values are usually attributed to volatile fatty acids accumulation or ammonia accumulation due to protein degradation, respectively (Weiland, 2010).

2.2.2.2 Temperature

There are three different temperature ranges at which different species of methanogens can function optimally, i.e. the psychrophilic range (10–20 °C), the mesophilic range (20–40 °C) and the thermophilic range (50–65 °C). Usually, mesophilic and thermophilic temperatures are preferred for anaerobic digestion, since most methanogens are active in these ranges. In fact, while large-scale applications are carried out mostly at mesophilic and thermophilic

conditions, even if the latter are applied to a lower extent, psychrophilic temperatures are usually confined to small-scale applications, such as Imhoff tanks, septic tanks, and sludge lagoons. This is probably related to the fact that a lower quantity of volatile solids is converted to methane under these conditions. Optimal performance under mesophilic and thermophilic conditions is usually obtained at temperatures of 35 and 55 °C, respectively, while methanogens inhibition can be observed in the range of 40–50 °C. Considering the potential negative effects on biogas production due to temperature changes, it is important that a constant temperature is maintained during anaerobic digestion. More specifically, while mesophilic methanogens can tolerate fluctuations even of +/−3 °C without significant impacts on methane production, thermophilic methanogens are more sensitive to such changes. In fact, although thermophilic operation results in a faster and more efficient process, with a good pathogen reduction and a high gas production, it is more difficult to control, due to these sensitivities (Abbasi et al., 2012; Chandra et al., 2012; Weiland, 2010).

2.2.2.3 Substrate composition

In the process of anaerobic digestion, the resulting methane yields and the composition of the obtained biogas, are highly dependent on the origin of the substrates, as well as on their composition (Chandra et al., 2012; Weiland, 2010). Indeed, the carbohydrate, fat and protein contents of a substrate would in turn affect the types of carbon source and the nitrogen quantities that will be available for use by microbes during the anaerobic digestion process. This is of high importance, since different groups of microbes are supported by different types of carbon sources, and nitrogen is required for reactions resulting in the production of new cell mass (Khalid et al., 2011). In other words, variations in the composition of different substrates in terms of carbohydrate, fat and protein contents, could lead to variations in the quantity of degradable matter that is ultimately converted to methane, thus affecting the methane content of biogas, which can range from 40 to 70% (by volume) (Abbasi et al., 2012; Chandra et al., 2012; Weiland, 2010). In fact, fats and proteins generally yield larger biogas and methane yields compared with carbohydrates. Biogas yields of 1535, 587 and 886 L/kg of VS destroyed, with respective methane contents around 70, 84 and 50% have been reported for fats, proteins and carbohydrates, respectively (Chandra et al., 2012). Nevertheless, although fats provide the highest yields, they tend to require longer retention times, as a result of their poor bioavailability, while the relatively lower gas yields of carbohydrates and proteins are usually associated with faster conversion rates (Weiland, 2010).

2.2.2.4 C/N ratio

Apart from the individual quantities of C and N present in a substrate, their relative proportion is also of great importance and is expressed as the carbon/nitrogen (C/N) ratio (Abbasi et al., 2012). The optimum C/N ratio tends to vary depending on the type of substrate intended for digestion (Li et al., 2011). Most of the literature proposes a C/N range between 20 and 30 as the most appropriate for anaerobic digestion, with a value of at least 25 being recommended as optimum (Chandra et al., 2012; Li et al., 2011). However, a wider range of values between 15 and 35 has also been reported as suitable (Kayhanian, 1999; Khalid et al., 2011; Weiland, 2010). The existence of an imbalance could inhibit the anaerobic digestion process by decreasing the activity of methanogens (Khalid et al., 2011; Li et al., 2011). In fact, if the C/N ratio is too high, nitrogen will be rapidly consumed by methanogens, in order to meet their protein requirements, thus no longer leaving enough quantities to react with the

left-over carbon content of the material. This, in turn will result in a reduced biogas production. On the other hand, if the C/N ratio is too low, nitrogen will be liberated and accumulated in the form of ammonium ions. This will lead to an increase in the pH inside the digester, with a toxic effect being exerted on methanogens, when values exceed 8.5 (Abbasi et al., 2012; Chandra et al., 2012).

2.2.2.5 Water content/Consistency

The water content of a substrate is another factor that influences anaerobic digestion. More specifically, the digestion slurry should have such a consistency that makes it neither too thick nor too thin. In fact, if the water content is too low and the slurry is too thick, adequate mixing may be difficult, thus impeding the gas flow to the upper part of the digester. Additionally, such a situation may lead to improper degradation of the organic matter and potential acetic acid accumulation, which in turn would result in reduced biogas quantities being obtained. On the other hand, if the water content of a substrate is too high, the solid particles may settle down into the digester, again resulting in improper degradation and in a reduced gas production (Abbasi et al., 2012; Chandra et al., 2012).

2.2.2.6 Organic loading rate

The organic loading rate (OLR) is defined as the amount of volatile solids (VS) or chemical oxygen demand (COD) being fed into a digester per day per volume unit. An increase in the OLR could allow for a reduction in the digester's size with a consequent reduction in the capital cost (Chandra et al., 2012). However, care should be taken when varying this parameter, since an excessive increase could lead to the disturbance of the equilibrium and productivity of the digestion process. In fact, while a small increase in OLR may initially increase the biogas yield of the digester, exceeding a threshold value, which usually is substrate-specific, may ultimately inhibit the microbial activity, due to an imbalance between the rate of hydrolysis/acidogenesis and methanogenesis, as a result of overloading. This imbalance is often manifested by increased VFA production and subsequent acidification phenomena (Mao et al., 2015). In other words, the amount of substrate being fed to the digester should be carefully selected, in order to allow the microbes to have enough time to effectively degrade the organic matter, without being overloaded (Chandra et al., 2012).

2.2.2.7 Retention time

The retention time is defined as the time required for achieving the desired extent of degradation of organic matter by microbes (Abbasi et al., 2012). This parameter is usually associated with the microbial growth rate and depends on the temperature at which the process is carried out, the OLR and the substrate composition (Mao et al., 2015). At higher retention times, higher biogas yields are usually obtained, since the volatile mass removal capacity is maximized. In fact, retention time is an important parameter that practically controls the conversion of volatile solids during digestion. However, higher retention times result in increased digester volumes being required, which ultimately increases the cost of the operation. Consequently, in order to make the operation of the digester more efficient and reduce the overall cost of an anaerobic digestion plant, the retention time must be reduced. Nevertheless, when applying such a strategy, care must be taken to avoid washing out

phenomena from the digester, by having a retention time of at least 10–15 days (Abbasi et al., 2012; Chandra et al., 2012).

Two different types of retention time are mentioned in literature, the hydraulic retention time (HRT) and the solids retention time (SRT). HRT is the term most commonly used to denote the retention time parameter and refers to the time for which an organic material intended for aerobic degradation, remains inside a digester. On the other hand, when the term SRT is used, it usually refers to the microorganisms (solids) inside a digester. The relationship between these two parameters is connected to the fact that, since the microbes present in a digester can only consume a limited amount of substrate each day, an adequate quantity of microbes must be provided in order to degrade a given quantity of substrate (Abbasi et al., 2012).

2.2.2.8 Presence of potentially toxic compounds

The anaerobic digestion process can be inhibited by the presence of a number of potentially toxic compounds, both of inorganic and organic nature. Inorganic compounds include ammonia, sulfide, light metal ions (e.g. Na, K, Mg, Ca, and Al) and heavy metals, while organic compounds include chlorophenols, halogenated aliphatics, N-substituted aromatics, long chain fatty acids (LCFAs), lignins and lignin related compounds. The sensitivity of this process to such substances is mainly associated with their concentration, since although in small quantities they may not cause disturbances, when their concentrations exceed specific threshold values, they begin to exert toxic effects. Other parameters that may affect the sensitivity of an anaerobic digestion system to such substances are pH and temperature (Chandra et al., 2012; Chen et al., 2008; Chen et al., 2014).

2.2.3 Feedstock types

A wide variety of solid organic substrates can be used as feedstock for anaerobic digestion, including agricultural waste and other lignocellulosic materials, the organic fraction of municipal solid waste, food waste, manure, sewage sludge, waste oils and animal fats.

2.2.3.1 Agricultural waste and biomass

Waste and biomass materials originating from agricultural activities are a relatively broad category of materials that includes the inedible portion (complex carbohydrates, such as leaves, stalks, trimmings etc.) of crops, perennial grasses, and animal waste, as well as the edible portion (oil and simple carbohydrates), especially in the cases of dedicated energy crops. Such materials are often dumped or even burned in open environments, and although direct burning theoretically does not contribute to the greenhouse effect, since biomass is carbon neutral, such a practice is not recommendable from an environmental and ecological point of view. On the other hand, the valorization of agricultural waste and biomass aiming at the production of energy and/or value-added products is viewed as a much more sustainable choice. Indeed, due to their regularly high production rates on a global level and their low cost, agricultural waste represent quite promising feedstocks for anaerobic digestion, with high potential biogas yields. Nevertheless, the organic matter present on these materials is not always readily available for degradation, due to their lignocellulosic composition, which is responsible for their eventual recalcitrance. In those cases, pretreatment is often applied before anaerobic degradation (Appels et al., 2011; Chandra et al., 2012; Li et al., 2011).

2.2.3.2 Organic fraction of municipal solid waste

Municipal solid waste in general, are a highly variable feedstock, however this is particularly true for the organic fraction of these waste (OFMSW). Indeed, the composition of this fraction varies widely, ranging from food waste (vegetable waste or fruit peels) to yard waste (leaves or grasses). The characteristics of OFMSW are highly dependent on a number of factors, including the strategy of waste collection, the sorting method, the location from which the material originates and the time of year in which collection is performed. In fact, recycling practices and types of food waste produced, may vary among different locations, according to lifestyle and cultural differences, while they will also often undergo seasonal changes. For instance, rural areas and summer months are often associated with the presence of a higher proportion of garden waste in the OFMSW, as opposed to urbanized areas and winter months (Appels et al., 2011; Li et al., 2011; Ward et al., 2008).

The OFMSW has lately been recognized as a valuable resource, with the ability of being valorized through biological processes, such as anaerobic digestion (Khalid et al., 2011). The introduction of source separation collection of the biodegradable fraction of municipal solid waste had a major role in making anaerobic treatment of these waste possible. Without source separation, a pre-sorting step would be necessary for removing compounds not suitable for anaerobic digestion, thus significantly increasing the treatment costs (Appels et al., 2011).

2.2.3.3 Food waste

Food waste (FW), usually constituting a large portion of the OFMSW, mainly originates from households, hotels, restaurants, canteens and companies. This kind of waste, which includes significant quantities of fruit and vegetable waste, is characterized by high moisture and volatile solids contents, as well as by high biodegradability. These characteristics make FW a suitable substrate for anaerobic digestion, which in this case appears as a more effective solution compared with traditional approaches, such as landfill disposal, incineration and aerobic composting (Khalid et al., 2011; Mao et al., 2015; Ward et al., 2008).

Nevertheless, due to their high content in organic solubles, these substrates tend to be hydrolyzed very rapidly, thus often resulting in excessive VFA conversion at the early stages of digestion. Such a phenomenon may lead to acidification and ultimately methanogenesis inhibition. In order to overcome this problem, co-digestion of FW with other organic substrates is often implemented. This approach helps in limiting inhibitory nutrient imbalances, such as insufficient trace elements and excessive macro nutrients, unsuitable C/N ratios and high lipid concentrations (Li et al., 2011; Mao et al., 2015; Ward et al., 2008).

2.2.3.4 Manure

Manures are readily available materials, which are often used as feedstock for anaerobic digestion. They mainly contain animal faeces, as well as varying quantities of organic fibers, originating from the straw used as bedding material. Anaerobic digestion of manure has the advantage of preventing the uncontrolled release of methane resulting from its natural degradation during storage. The methane potential of a specific type of manure mainly depends on the species, breed and growth stage of the animals, as well as on the feed, and the amount and type of bedding material and the degree of recalcitrance of its fibers. The frequent use of manures in anaerobic digestion systems is attributed to the fact that they are an excellent source of organic material, as well as to their high nitrogen content (Appels et al.,

2011; Ward et al., 2008). It has been reported that fresh goat, chicken, dairy and swine manure contain approximately 1.01, 1.03, 0.35 and 0.24% nitrogen, respectively (Mao et al., 2015). However, high nitrogen contents may potentially lead to high ammonia quantities in the manure. In fact, ammonia concentrations in certain types of manure are found to exceed the inhibition threshold concentration. For this reason, manure is often co-digested with other waste materials that have low nitrogen contents, in order to balance the C/N ratio (Appels et al., 2011; Mao et al., 2015).

2.2.3.5 Sludge

Sludge materials generated by physical, chemical and biological processes applied during wastewater treatment are another type of material that can be used as feedstock for anaerobic digestion. In fact, the disposal of these materials has become an issue of growing importance lately, due to the increasing quantities produced and anaerobic digestion has been recognized as an economic and environmentally friendly technology for treating such materials. Moreover, through anaerobic digestion, sludge stabilization, improvement of its dewaterability and inactivation and reduction of pathogens can be achieved. Recent research has also been focusing on the acceleration of sludge digestion through the application of various pretreatments (Appels et al., 2011; Mao et al., 2015).

2.2.3.6 Waste oils and animal fats

Lipid-rich waste are produced in significant quantities by a number of activities, such as food processing industries, slaughterhouses, oil manufacturing industries, dairy industries and olive oil mills. During anaerobic digestion of such waste, several problems can be manifested due to their lipids components. These materials can cause clogging, adsorption to biomass, as well as microbial inhibition, due to their conversion to high quantities of long chain fatty acids. For this reason, lipid-rich waste are typically co-digested with other substrates (Appels et al., 2011).

2.2.3.7 Lignocellulosic materials

Lignocelluloses are abundant in nature, since they are the primary building blocks of plant cell walls. They can be found as major components of several types of materials, including municipal solid waste, food waste, agricultural waste, energy crops, logging and forestry residues, as well as paper waste. Lignocellulosic materials constitute promising feedstocks for bioenergy production, in the form of biomethane, biohydrogen, bioethanol or biobutanol, as well as for a wide variety of bio-based products/chemicals, such as organic acids, bioplastic, succinic acid, citric acid, lactic acid etc. (Chandra et al., 2012; Mao et al., 2015; Sawatdeenarunat et al., 2016; Zheng et al., 2014). Nevertheless, the type of energy conversion route that would be more suitable to a specific lignocellulosic material, is highly dependent on the inherent characteristics of that material, including composition and structural and chemical properties. Lignocelluloses are composed of three main polymers, namely cellulose, hemicellulose and lignin, while they also contain smaller amounts of other components, such as pectin, protein, extractives and inorganic materials (Agbor, et al., 2011; Chandra et al., 2012; Taherzadeh and Karimi, 2008; Zheng et al., 2014). The three basic polymers are associated with each other in a hetero-matrix to a degree and with a composition, that vary for different materials, depending on their type, species and origin. In fact, the degree of

complexity of such a structure, as well as the content for each basic polymer, are directly related to the recalcitrance of these materials to bioprocessing. More specifically, biomass recalcitrance is affected by factors such as crystallinity and degree of polymerization of cellulose, accessible surface area (or porosity), protection of cellulose by lignin, cellulose sheathing by hemicellulose and fibre strength (Agbor, et al., 2011).

- *Cellulose*

Cellulose is a linear polysaccharide polymer of glucose moieties linked via β-(1,4) glycosidic bonds. These linkages make cellobiose units, which in turn constitute cellulose chains. The long-chain cellulose polymers are linked together by hydrogen bonds and van der Waals forces, so called "elementary and micro-fibrils". The micro-fibrils are often associated in the form of bundles or macrofibrils. These fibrils are attached to each other by hemicelluloses, amorphous polymers of different sugars and other polymers, such as pectin, and bonded together by lignin. This complex structure makes cellulose resistant to biological and chemical processes. In lignocellulosic structures, cellulose can be encountered in both crystalline (organized) and amorphous (unorganized) forms. This is related to the different orientations of cellulose molecules, which lead to different levels of crystallinity, with amorphous and crystalline cellulose being associated with low and high crystallinity, respectively (Agbor, et al., 2011; Chandra et al., 2012; Hendriks and Zeeman, 2009; Taherzadeh and Karimi, 2008; Zheng et al., 2014).

- *Hemicellulose*

Hemicellulose is the second most abundant polymer in nature and in contrast with cellulose, has a lower molecular weight and a random, amorphous, branched and heterogeneous structure consisting of pentoses, hexoses and acetylated sugars (Agbor, et al., 2011; Taherzadeh and Karimi, 2008). The backbone of hemicellulose is either a homo-polymer or a hetero-polymer with short branches linked by β-1,4-glucan bonds and occasionally β-1,3-glucan bonds (Chandra et al., 2012). Short and branched hemicellulose chains serve as a connection between lignin and cellulose fibers, ultimately building a cellulose-hemicellulose-lignin network characterized by extreme rigidity. Nevertheless, the same amorphous and branched properties of hemicellulose, make it highly susceptible to biological, thermal and chemical hydrolysis of their monomer compounds, in contrast to cellulose (Agbor, et al., 2011; Hendriks and Zeeman, 2009; Taherzadeh and Karimi, 2008; Zheng et al., 2014). The main component of hemicellulose in hardwood and agricultural biomass, like grasses and straw, is xylan, while in the case of softwood, is glucomannan (Hendriks and Zeeman, 2009).

- *Lignin*

Lignin is the third most abundant polymer in nature, with a very complex structure. More specifically, it is an amorphous hetero-polymer consisting of three different phenylpropane units (p-coumaryl, coniferyl and sinapyl alcohol) that are held together by different kind of linkages, ultimately forming a three-dimensional structure, which is particularly difficult to degrade. Specifically, lignin functions as a binder that connects the cellulose and hemicellulose components of lignocellulosic biomass, making it insoluble in water (Agbor, et al., 2011; Hendriks and Zeeman, 2009; Taherzadeh and Karimi, 2008). Indeed, lignin does not exist as an independent polymer, but it is associated with hemicellulose, both as physical mixtures and through covalent bonds (Monlau et al., 2013). Moreover, lignin provides

lignocellulosic materials with integrity, structural rigidity, impermeability, and resistance against microbial attack and oxidative stress (Agbor, et al., 2011; Hendriks and Zeeman, 2009; Taherzadeh and Karimi, 2008). These precise properties are those that make lignin the most recalcitrant component of lignocelluloses and consequently a major drawback to the use of such materials in anaerobic digestion processes. In fact, lignin content and distribution constitute the most common factors responsible for the recalcitrance of lignocellulosic materials. In other words, the higher the lignin proportion of a material, the higher its resistance to chemical and enzymatic degradation (Taherzadeh and Karimi, 2008).

In order to overcome the issues related to the recalcitrance of lignocellulosic materials and eventually enhance biomass digestibility, extra measures are usually adopted, which include the application of pretreatments before anaerobic digestion, or even co-digestion of lignocellulosic materials with other organic substrates. Pretreatment is typically focused on delignification, since lignin is the most recalcitrant component of these substrates. These processes may cause, among others, biomass swelling, disruption of the lignin structure and increased internal surface area. These phenomena may in turn result in alterations in the lignin structure, even without its extraction, due to changes in its chemical properties. Nevertheless, most delignification methods also result in partial hydrolysis of hemicellulose (Agbor, et al., 2011; Mao et al., 2015; Taherzadeh and Karimi, 2008).

2.2.4 Pretreatment

The application of pretreatment prior to anaerobic digestion aims at disrupting the complex structure of lignocellulosic substrates, in order to facilitate their subsequent biological degradation. In other words, pretreatments are mainly intended to break the impermeable and resistant layer of lignin, so that cellulose and hemicellulose are more easily accessible to microbes and the material has an overall enhanced digestibility. Specifically, pretreatment has the ability of reducing the cellulose and hemicellulose crystallinity and the degree of polymerization, as well as increasing the accessible surface area and porosity of the material (Behera et al., 2014; Chandra et al., 2012). Due to the variability in the structures of lignocellulosic materials, there is no unique optimum pretreatment method. On the contrary, the optimum conditions and techniques for each case, depend on the type of lignocellulosic substrate (Zheng et al., 2014).

For a pretreatment to be characterized as effective, it should meet some basic requirements, such as (Agbor, et al., 2011; Chandra et al., 2012): i) improve the formation of sugars or the ability to subsequently form sugars by hydrolysis, ii) avoid the degradation or loss of carbohydrate, iii) avoid or limit the formation of sugar and lignin degradation products that may be inhibitory to the subsequent hydrolysis and fermentation processes, iv) be cost-effective, v) be applicable and effective for a wide range and loading of lignocellulosic materials, vi) result in the separate recovery of the largest portion of lignocellulosic components in a useable form, vii) minimize the need for preparation/handling or preconditioning steps and viii) provide for low energy demand or energy recovery.

Pretreatment processes can primarily be categorized into physical, chemical and biological methods, while also combinations between these categories or between different methods within the same category can also be applied.

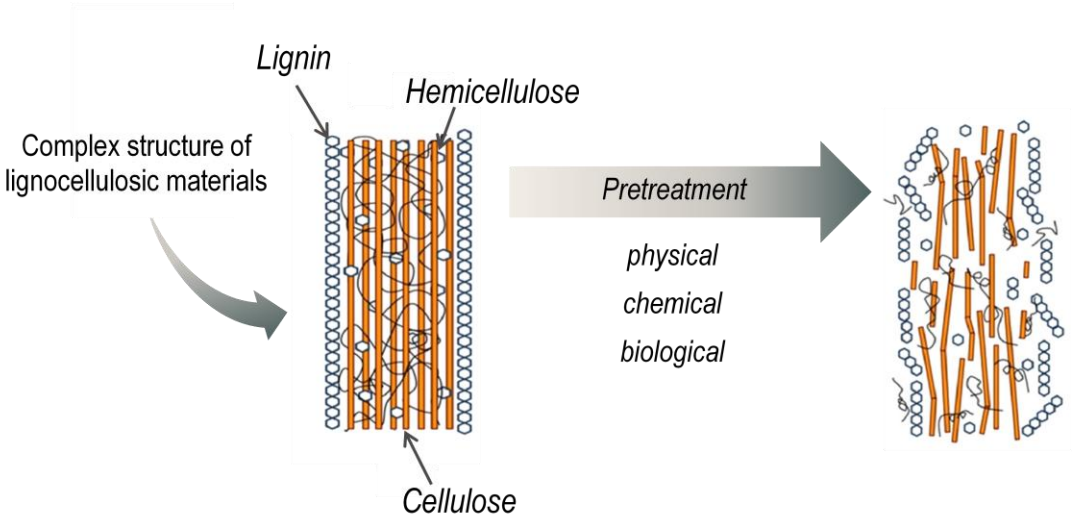


Figure 2-6: Pretreatment of lignocellulosic materials (adapted from Haghighi Mood et al., 2013)

2.2.4.1 Physical pretreatment

Physical pretreatment refers to methods that do not use chemicals or microorganisms. These include processes such as comminution (e.g. milling, chipping, grinding) and irradiation (e.g. microwave, ultrasound, gamma rays and electron beam), as well as conventional heating methods.

2.2.4.1.1 Comminution

Comminution pretreatment involves a reduction in the particle size of a substrate, through the application of methods, such as coarse size reduction, chipping, shredding, grinding and milling (ball, vibro, hammer, knife, two-roll, colloid, attrition). The selection of a specific comminution method is usually made based on the moisture content of a substrate, as well as on the desired final particle size (Agbor, et al., 2011; Zheng et al., 2014). The main action of this kind of pretreatment is concentrated on increasing the available specific surface area of the substrate, thus reducing the degree of cellulose crystallinity, and decreasing the degree of cellulose polymerization. The increased surface area ensures a better contact between substrate and microbial biomass, resulting in an enhanced anaerobic digestion performance (Agbor, et al., 2011; Ariunbaatar et al., 2014). Comminution processes are typically applied before the use of other pretreatment methods, aiming at improved feedstock handling and treatment (Zheng et al., 2014).

2.2.4.1.2 Irradiation

- *Microwave*

Microwave irradiation involves heating of a material in aqueous environments through electromagnetic irradiation, including both thermal and non-thermal effects. During this process, energy is transferred directly to the material, through molecular interaction with the electromagnetic field. More specifically, microwave irradiation induces the vibration of polar

molecules and the movement of ions, which in turn lead to the generation of heat and extensive collisions. Such phenomena, result in a more rapid and uniform heating and in reduced process times, with lower energy requirements, compared with conventional heating. The ability of a material to be heated with microwave energy depends not only on the microwave field itself, but also on the material's dielectric response (Singh et al., 2016; Zheng et al., 2014).

- *Ultrasound*

Ultrasound pretreatment is based on the generation of monolithic cavitations, which lead to physical and chemical effects in liquid solutions. The physical effects are induced by the collapse of cavitational bubbles, which in turn produce an elevated alteration in the chemical nature through the formation of free radicals. Increased biodegradability of a material after ultrasonic pretreatment is attributed to the disruption of cell wall structure, the increased specific surface area and the reduced degree of polymerization, resulting from the combination of these physical and chemical effects (Zheng et al., 2014).

- *Gamma-ray and electron beam*

It has been reported that ionizing radiation such as gamma (γ), can partially disrupt lignocellulosic structures and reduce the degree of polymerization of cellulose and lignin. In fact, when such polymeric materials absorb the energy from γ-rays or electron beams, this causes the production of ions, which in turn leads to the production of active species, such as radicals. These radicals then cause the degradation on lignocellulose molecules, by inducing chemical reactions and cleavage of the chemical bonds connecting lignin, hemicellulose, and cellulose units. This ultimately results in larger surface areas and lower crystallinity (Agbor, et al., 2011; Singh et al., 2016).

2.2.4.1.3 Conventional heating

Pretreatment of lignocellulosic substrates through conventional heating is a widely applied method simply consisting in the application of heat to a material, aiming at disrupting cell structures, more specifically the hydrogen bonds that connect crystalline cellulose and lignocellulose complexes. This ultimately results in organic matter solubilization and increased degradability of the treated material (Ariunbaatar, et al., 2014; Guo et al., 2014; Hendriks and Zeeman, 2009; Montgomery and Bochmann, 2014). This process is mainly affected by duration and temperature, with the latter having a higher impact compared with the former (Appels, et al., 2010; Ariunbaatar, et al., 2014). Such pretreatments can be conducted at various temperatures, ranging from 50 to 270 °C, with the processes performed above 100 °C usually involving the use of high pressures (Appels, et al., 2010; Ariunbaatar, et al., 2014; Montgomery and Bochmann, 2014). The reactions and mechanisms being developed during these processes are the result of heat addition and are valid not only for conventional heating, but also for different heating methods. At temperatures above 160 °C, hemicellulose and lignin solubilization will typically take place, resulting in the production of compounds such as phenolic compounds, furfural and HMF, which could potentially have an inhibitory or toxic effect on microbial populations. Especially in the cases in which pretreatment conditions are too severe, soluble lignin compounds may condensate and precipitate, sometimes even with soluble hemicellulose compounds (Hendriks and Zeeman, 2009). Moreover, heating at temperatures above 170 °C, has been associated with the

development of chemical phenomena, such as the Mallaird reaction, which occurs between carbohydrates and amino acids and results in the formation of complex compounds that are not easily degradable. Nevertheless, such reactions have been noticed to also occur at lower temperatures for longer treatment processes. Thermal treatments could also cause the loss of volatile organics from a material (Ariunbaatar, et al., 2014). It is worth mentioning that anaerobic digestion of thermally pretreated materials is enhanced only until a certain temperature, which is case-specific, depending on the substrate at hand (Montgomery and Bochmann, 2014). However, in general the use of temperatures above 250 °C is not recommended, due to the unwanted pyrolysis reactions that might occur (Hendriks and Zeeman, 2009). Heat supply during pretreatment is often used when substrate sanitation is required before anaerobic digestion. Additionally, it also represents a suitable option in the cases where a supply of waste heat is present (Montgomery and Bochmann, 2014).

Pretreatment through conventional heating, when used on its own, is characterized as a purely physical method. Nevertheless, heat is often applied in other pretreatment methods as well. These include chemical, as well as mechanical pretreatments.

2.2.4.2 Chemical pretreatment

Chemical pretreatment has been proven to be a quite promising pretreatment method for materials intended to be used as substrates in anaerobic digestion (Behera et al., 2014). Such processes have been shown to exert significant effects on the structure of lignocellulosic materials, by altering their physical and chemical characteristics (Agbor et al., 2011; Zheng et al., 2014). Chemical pretreatments may vary in the quantity of chemicals and water being required, as well as in the temperatures at which the processes are conducted. Depending on the respective amounts of these streams, recycling of chemicals and disposal of waste solutions might be needed, potentially affecting the investment and treatment costs. An important factor to consider when applying chemical pretreatments is the severity of the process, since in some cases, severe processes may result in the generation of inhibitory compounds, such as hydroxymethyl furfural (HMF) and furfural, as well as in the presence of chemical residues on the pretreated materials. Such phenomena could potentially influence the downstream anaerobic digestion process (Behera et al., 2014; Zheng et al., 2014). The type of chemical pretreatment method being used is often determinant for its effectiveness in degrading lignocellulosic structures, considering that different chemicals tend to act on different parts of the substrate (Song et al., 2014). Nevertheless, such effectiveness is also highly dependent on the type of substrate being treated, since different lignocellulosic materials are characterized by highly variable structures (Kang et al., 2013; Sambusiti et al., 2013; Zheng et al., 2014). The most commonly used chemical pretreatments involve the use of alkaline and acid reagents, oxidizing agents, inorganic salts, organic solvents and ionic liquids.

2.2.4.2.1 Alkaline reagents

Alkaline pretreatment is one of the most studied among chemical methods and involves the use of various reagents, such as sodium hydroxide, calcium hydroxide (lime), potassium hydroxide, magnesium hydroxide, hydrazine, anhydrous ammonia and ammonium hydroxide (Agbor et al., 2011; Behera et al., 2014; Mao et al., 2015; Zheng et al., 2014).

During alkaline pretreatment solvation and saphonication reactions take place, resulting in swelling of biomass and leading to increased internal surface area, decreased degree of polymerization and cellulose crystallinity, disruption of lignin structure and breakage of linkages between lignin and other carbohydrate fractions. As a result, the carbohydrates in the hetero-matrix of lignocellulosic materials are made more accessible to enzymes and bacteria, with the reactivity of the remaining polysaccharides increasing with lignin removal. Moreover, apart from lignin, acetyl and other uronic acid substitutions on hemicellulose are also removed through these processes. Alkaline pretreatment can also cause solubilization, redistribution and condensation of lignin and modifications in the crystalline state of cellulose, with these effects being able to lower or counteract the positive effects of lignin removal and cellulose swelling (Agbor et al., 2011; Hendriks and Zeeman, 2009).

The effectiveness of alkaline pretreatment is often associated with the lignin content of the materials being treated (Zheng et al., 2014). In fact, in general, pretreatment methods using alkaline reagents are more effective on those types of materials that contain a relative smaller amount of lignin. On the contrary, when materials containing a higher amount of lignin are treated with such reagents, the process becomes less effective, thus requiring much more severe conditions in order to obtain the desired results (Agbor et al., 2011; Galbe and Zacchi, 2012). Another interesting feature of alkaline pretreatment is the fact that a portion of the alkaline reagent is often consumed by the biomass itself (Agbor et al., 2011; Ariunbaatar et al., 2014).

Very often during anaerobic digestion, a pH adjustment is required, which is usually made by increasing the alkalinity. In those cases, the prior use of alkaline pretreatment seems advantageous (Ariunbaatar et al., 2014). On the other hand, a significant disadvantage of alkaline pretreatment is the generation of irrecoverable salts, with the eventuality of these salts being incorporated into the biomass during the pretreatment process. Therefore treating these large amounts of salts has become an issue of concern (Behera et al., 2014).

2.2.4.2.2 Acid reagents

The effectiveness of acid pretreatment depends on the type of acid being used, the acid concentration, the solid to liquid ratio and the process temperature. Both inorganic and organic acids can be used for such processes, including sulfuric acid, nitric acid, hydrochloric acid and phosphoric acid, acetic acid, citric acid, oxalic acid, peracetic acid, maleic acid and fumaric acid. Among them, sulfuric acid is the one that has been researched the most, due to its high catabolic activity. (Amnuaycheewa et al., 2016; Behera et al., 2014; Zheng et al., 2014).

The main reaction that occurs during acid pretreatment is the hydrolysis of hemicellulose to its monomeric units (monosaccharides such as xylose, mannose, acetic acid, galactose, glucose, etc.), which involves the disruption of covalent bonds, hydrogen bonds, and Van der Waals forces. These phenomena result in the solubilization of hemicellulose and in the improvement of the accessibility of cellulose (Agbor et al., 2011; Behera et al., 2014; Hendriks and Zeeman, 2009; Song et al., 2014). Acid pretreatment has also been found able of disrupting lignin to a high degree, but it is not so effective in dissolving it (Zheng et al., 2014). Actually, it has been reported that during this kind of pretreatment lignin may condensate and precipitate (Ariunbaatar et al., 2014).

Acid pretreatment can be conducted by using either concentrated (30-70%) or dilute (0.1 to 2%) acids, at low and high temperatures. Concentrated acids can be highly effective in solubilizing lignocelluloses, however they may also result as extremely toxic and corrosive.

Moreover, their use often requires expensive materials, such as specialized non-metallic materials or alloys for reactor construction, while in order to make the pretreatment economically feasible, measures must be taken to recover the residual acid. Additionally, chemical pretreatment with concentrated acids also generates various potentially toxic inhibitory compounds, such as carboxylic acids (e.g. formic and acetic acids), furfural, hydroxymethyl furfural (HMF) and phenolic compounds (Agbor et al., 2011; Ariunbaatar et al., 2014; Behera et al., 2014; Galbe and Zacchi, 2012; Hendriks and Zeeman, 2009; Zheng et al., 2014). Among these compounds, formic and acetic acids can be directly converted to biogas, while in the cases of furfural, HMF and phenolic compounds, their inhibitory effect is mainly associated with their concentration and in the case of phenols also with their physicochemical properties. Interestingly, although inhibitory compounds are mainly produced when using concentrated acids, this phenomenon can also be manifested with diluted acids (Zheng et al., 2014). Other possible disadvantages of such pretreatments include the loss of fermentable sugars due to the increased degradation of complex substrates, the high cost of acids and the eventual additional alkali requirements for neutralizing the hydrolysate before anaerobic digestion (Agbor et al., 2011; Ariunbaatar et al., 2014). For all these reasons, concentrated acid pretreatment is generally considered not attractive, while the use of dilute acid pretreatment is preferred, often in combination with thermal methods (Ariunbaatar et al., 2014; Zheng et al., 2014).

2.2.4.2.3 Oxidizing agents

Oxidizing agents such as, ozone, peroxides (e.g. hydrogen peroxide, peracetic acid), oxygen or air can also be used for pretreatment of lignocellulosic materials. The main effect of oxidative pretreatments consists in delignification, due to the high reactivity of oxidising agents with the aromatic ring (Harmsen et al., 2010; Zheng et al., 2014). During such processes, reactions such as electrophilic substitution, displacement of side chains, cleavage of alkyl aryl ether linkages and oxidative cleavage of aromatic nuclei can take place (Hendriks and Zeeman, 2009). In addition to affecting lignin, oxidative pretreatments also affect hemicellulose (Harmsen et al., 2010).

- *Ozone*

Ozonolysis of lignocellulosic materials results in the enhanced degradability of biomass, mainly by means of lignin degradation, with a slight alteration of hemicellulose and a minor effect on cellulose. The main parameters affecting the effectiveness of this process are moisture content, particle size and ozone concentration in the gas flow.

During ozonolysis, ozone molecules disintegrate into hydroxyl radicals (OH˙). This results in two types of reaction with organic substrates, namely direct and indirect. In the former case, the oxidation reaction depends on the structure of the reactant, while in the latter case oxidation is performed by the hydroxyl radicals. The type of reactions occurring during this pretreatment is highly dependent on the pH of the solution. Ultimately, this process results in the enhanced biodegradability and accessibility of the treated materials.

Ozonolysis pretreatment is typically carried out at ambient temperature and pressure and does not generate any inhibitory compounds, or leave any chemical residues, while it also has a disinfecting effect on pathogens. On the other hand, it usually is quite expensive, as a result of the large amounts of ozone needed (Ariunbaatar et al., 2014; Behera et al., 2014; Zheng et al., 2014).

- *Peroxides*

Peroxides oxidants include hydrogen peroxide, peracetic acid, dimethyldioxirane, and peroxymonosulphate. Among them, hydrogen peroxide is the most studied and used for pretreatment of lignocellulosic materials, most likely due to its strong oxidation ability, which results in a significantly enhanced reaction efficiency, compared with other agents (Mao et al., 2015; Zheng et al., 2014).

The oxidizing effect of peroxides is similar to that of ozonolysis, since these agents are also transformed into hydroxyl radicals ($OH^{\bullet}$), which actually are more powerful than the peroxides themselves. Pretreatment with hydrogen peroxide is a non-selective process, thus being able to affect all components of lignocellulosic materials. As a result, not only partial disruption of lignin and hemicellulose, but also release of a fraction of cellulose can occur, ultimately leading to the enhanced degradability of the treated substrates.

Nevertheless, inhibitory compounds can be generated during this process, due to the formation of soluble aromatic compounds through lignin oxidation. Moreover, high hydrogen peroxide concentrations may also be a cause of inhibition to the anaerobic digestion process, since excessive amounts of hydroxyl ions have toxic effects on methanogens.

In certain cases, the use of hydrogen peroxide alone may not be so effective, therefore a combination with other pretreatment methods, such as acid/alkali hydrolysis and microwave irradiation, is often employed. Such strategies are followed especially when treating complex and highly non-biodegradable substrates (Zheng et al., 2014).

- *Wet oxidation*

Wet oxidation pretreatment involves the use of water and an oxidizing agent (e.g. air or oxygen) at high temperatures, ranging from 125 to 300 °C, and high pressures, usually between 0.5 and 20 MPa. Treatment time can vary from a couple of minutes to hours. The critical parameters of the wet oxidation process are temperature, reaction time, oxygen pressure and water content.

When wet oxidation is used on lignocellulosic substrates, it causes a large fraction of their lignin content to be solubilized and oxidized. This pretreatment is mostly adopted for materials with a low lignin content.

Increasing concentrations of oxygen during wet oxidation result in faster reaction rates and increased production of free radicals. However, using pure oxygen leads to high operating costs. For this reason, air is usually used as an oxidizing agent. Another way of eliminating or minimizing energy inputs during wet oxidation is taking advantage of the exothermic nature of this process, by using the heat produced from the occurring reactions for keeping the temperature at a desired level after the initiation of the pretreatment (Galbe and Zacchi, 2012; Zheng et al., 2014).

2.2.4.2.4 Inorganic salts

Inorganic salts, such as NaCl, KCl, $CaCl_2$, $MgCl_2$, and $FeCl_3$, have also been employed in pretreatment processes of lignocellulosic materials. Indeed, their use has resulted quite effective for hemicellulose degradation (Liu et al., 2009) and removal of the loosely bound portion of lignin (Banerjee et al., 2016). Moreover, the use of inorganic salts for pretreatment of lignocellulosic materials, unlike the use of many acids, does not encompass problematic issues, such as corrosion or eventual neutralization of the hydrolysate, thus being easily recyclable and constituting an economically viable option (Liu et al., 2009).

2.2.4.2.5 Organic solvents

Orgavosolv pretreatment involves the use of organic solvents such as methanol, ethanol, acetone, ethylene glycol, triethylene glycol, glycerol, aqueous phenol, aqueous n-butanol and tetrahydrofurfuryl alcohol, or their aqueous solutions, as well as the eventual use of inorganic (hydlochloric and sulfuric acid) or organic acids (oxalic, salicylic and acetylsalicylic acid), which function as catalysts. These kind of processes are usually conducted at temperatures ranging from 100 to 250 °C (Agbor et al., 2011; Behera et al., 2014; Galbe and Zacchi, 2012).

Organosolv pretreatment is the only pretreatment method that is highly effective on high-lignin lignocellulosic materials. In fact, it results in the extensive removal of lignin and in an almost complete solubilization of hemicellulose. This is achieved by hydrolyzing the internal lignin bonds, as well as the bonds between lignin and hemicellulose and the glycosidic bonds present in hemicellulose and partially in cellulose. Clearly, the degree to which all these reactions are performed depends on the specific conditions under which the process is conducted. For example, the kinetics of delignification, vary depending on the type of solvent being used (Agbor et al., 2011).

An important issue to consider when applying such a pretreatment, is the possible options for solvent recovery. The procedures adopted for this scope usually include draining, evaporation, condensation and recycling of the solvents, with all of them aiming at reducing the operational costs. It is critical from an environmental and economic standpoint to optimize all these operations, in order to make the whole process environmentally feasible and cost-effective. Moreover, the separation of the pretreated material from the solvent is also very important, since depending on the type of solvent used, potential inhibitory effects to downstream processes, such anaerobic digestion, might be observed (Agbor et al., 2011; Behera et al., 2014; Galbe and Zacchi, 2012). Nevertheless, it has been reported that, if using low molecular weight alcohols and organic acids, inhibition would be less of an issue, since these compounds are easily-degradable and can usually be utilized by the microorganisms in anaerobic digestion systems (Kabir et al., 2014). In addition, once lignin solubilization is achieved, the dissolved lignin could potentially be recovered, in order to eventually be used for various purposes (e.g. chemicals, fuels) (Galbe and Zacchi, 2012).

2.2.4.2.6 Ionic liquids

Ionic liquids are a new class of solvents, which consist entirely of ions (cations and anions), exist in liquid form at room temperature and are characterized by low melting points (<100 °C), high polarities, high thermal and chemical stability, low toxicity, low hydrophobicity, low viscosity, negligible vapour pressure, high reaction rates, low volatility with potentially minimal environmental impact, non-flammability and good solvating properties (Agbor et al., 2011; Behera et al., 2014; Galbe and Zacchi, 2012; Zheng et al., 2014).

The mechanism responsible for cellulose dissolution with ionic liquids initially involves the formation of electron donor–electron acceptor complexes by the oxygen and hydrogen atoms of cellulose hydroxyl groups, which then interact with ionic liquids. The interaction between cellulose's hydroxyl groups and ionic liquids is followed by the breakage of hydrogen bonds between molecular chains of cellulose, which ultimately results in cellulose dissolution. Solubilized cellulose can be recovered by precipitation with ethanol, methanol, acetone or water and it is usually characterized by the same degree of polymerization and polydispersity as the initial cellulose, as well as by significantly different macro- and micro-structures, decreased crystallinity and increased porosity.

Ionic liquids have gained significant interest due to the fact that they can be effectively used for cellulose dissolution, even at mild conditions (90-130 °C and ambient pressure), with low energy inputs. Moreover, these processes encompass the possibility of recovering nearly 100% of the used ionic liquid to its initial purity, while leaving minimal residues (Zheng et al., 2014).

2.2.4.3 Biological pretreatment

Biological pretreatment can be performed by employing fungi, microbial consortia or enzymes (Galbe and Zacchi, 2012; Zheng et al., 2014). Its potential advantages over physical and chemical pretreatments include substrate and reaction specificity, low energy requirements, no generation of toxic compounds, and high yield of desired products (Behera et al., 2014). On the other hand, one important disadvantage that prevents the use of such pretreatments on industrial scale is its low rate, compared with most other pretreatment methods (Agbor et al., 2011; Galbe and Zacchi, 2012). Moreover, biological pretreatments involving microorganisms usually have strict requirements related to the composition, the activity and the purity of strains and the sealing of reactors, thus resulting in high investment costs, while the cost of enzymes is also high (Mao et al., 2015).

2.2.4.3.1 Fungi

Fungal pretreatment is conducted by fungi, capable of producing enzymes that can degrade lignin, hemicellulose, and polyphenols. Several fungi classes, including brown-rot, white-rot, soft-rot and basidiomycete fungi, have been used for pretreatment of lignocellulosic materials before anaerobic digestion, with white-rot being the most effective. Brown-rot fungi primarily attack cellulose, whereas white-rot and soft-rot fungi attack both lignin and cellulose through the action of lignin-degrading enzymes such as lignin peroxidases, polyphenol oxidases, manganese-dependent peroxidases, and laccases. Research is mainly focused on fungi that primarily degrade lignin fractions, since lignin constitutes the barrier to cellulose hydrolysis (Agbor et al., 2011; Galbe and Zacchi, 2012; Mao et al., 2015; Zheng et al., 2014).

2.2.4.3.2 Microbial consortia

Microbial consortium pretreatment is conducted by microbes, which are screened from natural environments and use lignocellulosic biomass as substrate. Such consortia contain yeast and cellulolytic bacteria, heat-treated sludge, *Clostridium thermocellum*, and a mixture of fungi and composting microbes. Microbial consortium pretreatment is usually focused on degrading cellulose and hemicellulose, as opposed to most fungal pretreatments, which mainly attack lignin (Mao et al., 2015; Zheng et al., 2014).

2.2.4.3.3 Enzymes

Hydrolysis of cellulose and hemicellulose is believed to be the rate-limiting step during the anaerobic digestion of lignocellulosic biomass. Enzymes are known to play an important role in hydrolysis of lignocelluloses. Specifically, enzymes with hydrolytic activity, take part in biochemical catalytic reactions, acting as a microbial supplement. The enzymes most commonly used for such a purpose include cellulase and hemicellulase. Nevertheless, the effect of enzymes in enhancing biogas production is often not satisfactory compared with

their cost, resulting in limited applications of such pretreatments (Mao et al., 2015; Zheng et al., 2014).

2.2.5 Anaerobic Co-digestion

Anaerobic co-digestion consists in the anaerobic digestion of a mixture of two or more organic substrates. This method has been proposed as an alternative strategy to anaerobic digestion of single substrates (mono-digestion), which can often present some drawbacks, depending on substrate properties. These drawbacks are usually related to organic load and nutrient balance issues. The addition of a co-substrate is a feasible option, capable of solving such problems and of improving the economic viability of anaerobic digestion systems (Mata-Alvarez et al., 2014).

In fact, anaerobic co-digestion is characterized by numerous benefits, which include dilution of eventual potentially toxic compounds, adjustment of the moisture content and pH, supply of the necessary buffer capacity to the mixture, increased load of biodegradable organic matter, improved nutrient balance, widening of the range of microorganisms taking part in the process, synergistic effect of microorganisms, better process stability, handling and biogas production, improved methane yields and production of a digested product of good quality. In addition, co-digestion of multiple substrates can also result in an excess of nutrients, leading to biostimulation and ultimately higher biodegradation rates (Anjum et al., 2016; Esposito et al., 2012; Khalid et al., 2011; Ward et al., 2008).

The positive effect of mixing different substrates on the overall stability of co-digestion systems mainly consists in the improvement of the C/N ratio of the feeding material. In fact, by appropriately combining different substrates with complementary characteristics, several problems, associated with lack or excess of carbon and nitrogen, high ammonia concentrations or accumulation of intermediate volatile compounds, can eventually be overcome. Therefore, it is of critical importance that a proper selection of co-substrates is made for each specific case, since no univocal conditions can be defined. Furthermore, inappropriate substrate combinations could lead to significant reductions of biogas quantities being produced, ultimately compromising the process efficiency (Anjum et al., 2016; Esposito et al., 2012; Khalid et al., 2011; Mata-Alvarez et al., 2014; Ward et al., 2008).

2.2.6 Biogas production in the Mediterranean area

The term “biogas” refers to the gas produced from organic matter under anaerobic conditions, however it not limited to the amount obtained from biogas production plants (anaerobic digesters) treating substrates such as manure, agricultural residues, energy crops, waste from households and food processing industry etc. Biogas sources also include landfills, as well as urban wastewater and industrial effluent treatment plants (Cioabla, et al., 2012; European Biogas Association, 2011).

As can be seen in Table 2-9 (Eurostat, 2016; UNSD, 2016; American Biogass Council, 2016), only half of the countries surrounding the Mediterranean Sea provide data regarding biogas production in 2013. According to these data, in Greece, Spain and Turkey biogas is primarily obtained through landfills, while in Croatia, Cyprus, France, Italy, Malta and Slovenia anaerobic fermentation is the major source of biogas. Specific data regarding the biogas sources in Israel were not available. Among these countries, Italy has the highest biogas production, reaching an energy equivalent of approximately 76 thousand Terajoules,

while Spain and France follow, accounting for almost 20.1 and 18.3 thousand Terajoules, respectively. Greece holds the fifth place in total biogas production with an energy equivalent of 3.7 thousand Terajoules of which 76% comes from landfills and only 5% from anaerobic fermentation. The total amount of biogas energy being produced in the Mediterranean area is less than half of the total amount that corresponds to the USA or to Germany, which is the European country with the highest biogas production (49.3% of EU-28 production).

Table 2-9: Biogas production from different sources in Mediterranean countries, expressed as energy equivalents

Country	Biogas production expressed as energy equivalents (Terajoules)				
	Landfill	Sewage sludge	Anaerobic fermentation	Thermal processes	Total
Albania	0	0	0	0	0
Algeria	-	-	-	-	-
Bosnia and Herzegovina	0	0	0	0	0
Croatia	17	98	578	0	693
Cyprus	0	19	447	0	466
Egypt	-	-	-	-	-
France	7,565	1,816	8,898	0	18,284
Greece	2,826	676	202	0	3,704
Israel	-	-	-	-	852
Italy	16,880	2,035	56,779	319	76,013
Lebanon	-	-	-	-	-
Libya	-	-	-	-	-
Malta	0	0	59	0	59
Montenegro	0	0	0	0	-
Morocco	-	-	-	-	-
Slovenia	297	117	1,040	0	1,454
Spain	8,103	6,787	4,232	950	20,072
Syrian Arab Republic	-	-	-	-	-
Tunisia	-	-	-	-	-
Turkey	7,992	0	519	0	8,511
Mediterranean area (partial total)	43,680	11,548	72,754	1,269	130,108
Germany	4,634	18,340	264,871	0	287,845
European Union (EU-28)	117,909	57,267	406,444	2,566	584,186
USA	-	-	-	-	265,794

As a result of the implementation of the European Renewable Energy Directive (2009/28/EC), which states that 20% of the final energy consumption has to be provided by renewable sources by 2020, most member states of the European Union have developed national renewable energy action plans, which include the operation of biogas production plants. Among the most widespread biogas installations are those processing agricultural substrates. Europe and North America account for thousands of operating agricultural biogas plants, while millions of small-scale digesters exist in China and the Indian sub-continent (Cioabla, et al., 2012; European Biogas Association, 2011). Table 2-10 presents data referring

to the number of biogas plants in Mediterranean countries (American Biogass Council, 2016; European Biogas Association, 2016)

Table 2-10: Number of biogas plants in Mediterranean countries

Country	Number of biogas plants
Albania	-
Algeria	-
Bosnia and Herzegovina	-
Croatia	16
Cyprus	14
Egypt	-
France	736
Greece	18
Israel	-
Italy	1491
Lebanon	-
Libya	-
Malta	-
Montenegro	-
Morocco	-
Slovenia	26
Spain	39
Syrian Arab Republic	-
Tunisia	-
Turkey	-
Mediterranean (partial total)	2340
Germany	10786
European Union (EU-28)	16611
USA	13008

Being rich in methane, biogas can be used in the majority of the applications intended for natural gas. It can be directly used for heat or combined heat and power (CHP) production, or even be upgraded to biomethane with natural gas quality and finally be injected into the gas grid. In addition, biogas can be used as an alternative renewable fuel for transport (European Biogas Association, 2011).

Table 2-11 presents data referring to the use of biogas for heat and electricity production in the European region, in both heat or electricity dedicated plants and CHP plants (Eurostat, 2016).

In Europe, 23 countries use biogas in CHP plants, 25 countries in heat only plants and 31 countries in electricity only plants. Among the Mediterranean countries situated in Europe, Italy is the country with the highest heat and electricity production obtained from biogas, with France following.

Table 2-11: Use of biogas for heat and electricity production in the European region

Countries	Gross heat production (Terajoules)				Gross electricity generation (Terajoules)			
	Main activity CHP plants	Main activity heat only plants	Autoproducer CHP plants	Autoproducer heat only plants	Main activity CHP plants	Main activity electricity only plants	Autoproducer CHP plants	Autoproducer electricity only plants
Belgium	218	0	0	0	310	263	2,088	130
Bulgaria	9	0	0	0	7	0	50	0
Czech Republic	112	0	375	0	169	115	7,891	83
Denmark	1,267	61	31	10	943	0	436	4
Germany	2,953	1,924	0	0	72,976	31,676	587	4
Estonia	65	0	0	0	72	0	0	0
Ireland	0	0	0	0	0	569	104	0
Greece	1	0	0	0	594	137	43	4
Spain	0	0	0	0	0	1,469	680	1,354
France	483	0	119	102	630	364	2,005	2,423
Croatia	114	0	0	0	162	68	47	0
Italy	8,162	11	244	0	13,921	12,229	526	137
Cyprus	42	0	0	0	43	0	133	0
Latvia	511	0	83	0	659	0	374	0
Lithuania	57	0	38	0	83	0	130	0
Luxembourg	0	0	47	0	0	0	202	0
Hungary	57	1	33	0	126	191	504	140
Malta	0	0	1	0	0	0	22	0
Netherlands	157	0	0	0	187	72	3,143	126
Austria	163	79	23	0	83	1,980	130	79
Poland	0	6	365	6	0	0	2,484	0
Portugal	0	0	0	0	0	0	36	860
Romania	8	0	137	14	18	0	68	94
Slovenia	367	0	0	0	385	0	108	14

Slovakia	47	0	70	0	119	25	227	396
Finland	157	197	62	117	104	446	263	295
Sweden	255	303	0	0	72	0	0	0
United Kingdom	0	0	0	0	0	0	2,203	19,116
Iceland	0	0	0	0	0	0	0	0
Norway	5	41	0	0	7	40	0	0
Montenegro	0	0	0	0	0	0	0	0
Former Yugoslav Republic of Macedonia	0	0	0	0	0	0	0	0
Albania	0	0	0	0	0	0	0	0
Serbia	0	0	0	0	0	0	72	0
Turkey	1,499	0	0	0	788	2,088	54	108
Bosnia and Herzegovina	0	0	0	0	0	0	0	0
Moldova	0	0	0	0	0	0	4	4
Ukraine	0	0	0	0	0	0	0	0
European Union (EU-28)	15,205	2,582	1,628	249	91,663	49,604	24,484	25,258

2.3 References

Abbasi, T., Tauseef, S. M., Abbasi, S. A. (2012). Anaerobic digestion for global warming control and energy generation—An overview. *Renewable and Sustainable Energy Reviews*, *16*(5), 3228–3242.

Agbor, V. B., Cicek, N., Sparling, R., Berlin, A., Levin, D. B. (2011). Biomass pretreatment: Fundamentals toward application. *Biotechnology Advances*, *29*(6), 675–685.

Albanna, M. (2013). Anaerobic digestion of the organic fraction of municipal solid waste. In: Malik, A., Grohmann, E., Alves, M. (Eds.), Management of Microbial Resources in the Environment. Springer, Dordrecht, Heidelberg, New York, London, p. 313-340.

Alburquerque, J.., Gonzálvez, J., García, D., Cegarra, J. (2004). Agrochemical characterisation of "alperujo", a solid by-product of the two-phase centrifugation method for olive oil extraction. *Bioresouce. Technology*, *91*, 195–200.

Alibardi, L., Cossu, R. (2015). Composition variability of the organic fraction of municipal solid waste and effects on hydrogen and methane production potentials. *Waste Management*, *36*, 147–155.

American Biogas Council (https://www.americanbiogascouncil.org/) (accessed on 2016)

Amnuaycheewa, P., Hengaroonprasan, R., Rattanaporn, K., Kirdponpattara, S., Cheenkachorn, K., Sriariyanun, M. (2016). Enhancing enzymatic hydrolysis and biogas production from rice straw by pretreatment with organic acids. *Industrial Crops and Products*, *87*, 247–254.

Anjum, M., Khalid, A., Mahmood, T., Aziz, I. (2016). Anaerobic co-digestion of catering waste with partially pretreated lignocellulosic crop residues. *Journal of Cleaner Production*, *117*, 56–63.

Appels, L., Degrève, J., Van der Bruggen, B., Van Impe, J., Dewil, R. (2010). Influence of low temperature thermal pre-treatment on sludge solubilisation, heavy metal release and anaerobic digestion. *Bioresource Technology*, *101*, 5743–5748.

Appels, L., Lauwers, J., Degrève, J., Helsen, L., Lievens, B., Willems, K., Van Impe, J., Dewil, R. (2011). Anaerobic digestion in global bio-energy production: Potential and research challenges. *Renewable and Sustainable Energy Reviews*, *15*(9), 4295–4301.

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., Lens, P.N.L. (2014). Pretreatment methods to enhance anaerobic digestion of organic solid waste. *Applied Energy*, *123*, 143–156.

Arvanitoyannis, I.S., Ladas, D., Mavromatis, A. (2006). Wine waste treatment methodology. *Internationa Journal of Food Science and Technology*, *41*, 1117–1151.

Azbar, N., Bayram, A., Filibeli, A., Muezzinoglu, A., Sengul, F., Ozer, A. (2004). A Review of Waste Management Options in Olive Oil Production. *Critical Reviews in Environmental Science and Technology*, *34*, 209–247.

Banerjee, D., Mukherjee, S., Pal, S., Khowala, S. (2016). Enhanced saccharification efficiency of lignocellulosic biomass of mustard stalk and straw by salt pretreatment. *Industrial Crops and Products, 80*, 42–49

Battista, F., Fino, D., Ruggeri, B. (2014). Polyphenols Concentration ' s Effect on the Biogas Production by Wastes Derived from Olive Oil Production. *Chemical Engineering Transactions, 38*, 373–378.

Behera, S., Arora, R., Nandhagopal, N., Kumar, S. (2014). Importance of chemical pretreatment for bioconversion of lignocellulosic biomass. *Renewable and Sustainable Energy Reviews, 36*, 91–106.

Borja, R., Rincón, B., Raposo, F. (2006). Anaerobic biodegradation of two-phase olive mill solid wastes and liquid effluents: kinetic studies and process performance. *Journal of Chemical Technology & Biotechnology, 81*, 1450–1462.

Boukroufa, M., Boutekedjiret, C., Petigny, L., Rakotomanomana, N., Chemat, F. (2015). Bio-refinery of orange peels waste: A new concept based on integrated green and solvent free extraction processes using ultrasound and microwave techniques to obtain essential oil, polyphenols and pectin. *Ultrasonics Sonochemistry, 24*, 72–79.

Bustamante, M.A., Moral, R., Paredes, C., Pérez-Espinosa, A., Moreno-Caselles, J., Pérez-Murcia, M.D. (2008). Agrochemical characterisation of the solid by-products and residues from the winery and distillery industry. *Waste Management, 28*(2), 372–380.

Caceres, C.X., Caceres, R.E., Hein, D., Molina, M.G., Pia, J.M. (2012). Biogas production from grape pomace: Thermodynamic model of the process and dynamic model of the power generation system. *International Journal of Hydrogen Energy, 37*, 10111–10117.

Campuzano, R., González-Martínez, S. (2016). Characteristics of the organic fraction of municipal solid waste and methane production: A review. *Waste Management, 54*, 3–12.

Carlini, M., Castellucci, S., Moneti, M. (2015). Anaerobic Co-digestion of Olive-mill Solid Waste with Cattle Manure and Cattle Slurry: Analysis of Bio-methane Potential. *Energy Procedia, 81*, 354–367.

Cesaro, A., Belgiorno, V. (2014). Pretreatment methods to improve anaerobic biodegradability of organic municipal solid waste fractions. *Chemical Engineering Journal, 240*, 24–37.

Chandra, R., Takeuchi, H., Hasegawa, T. (2012). Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. *Renewable and Sustainable Energy Reviews*, *16*(3), 1462–1476.

Chen, Y., Cheng, J.J., Creamer, K.S. (2008). Inhibition of anaerobic digestion process: A review. *Bioresource Technology 99*, 4044–4064.

Chen, J.L., Ortiz, R., Steele, T.W.J., Stuckey, D.C. (2014). Toxicants inhibiting anaerobic digestion: a review. *Biotechnology Advances, 32*, 1523–34.

Cioabla, A.E., Ionel, I., Dumitrel, G.A., Negrea, P., Pode, V. (2012). Biogas Production through Anaerobic Digestion of some Agro-Industrial Residues. *Chemistry Magazine*, *63*(6), 629-632.

Cotton Australia (cottonaustralia.com.au) (accessed on 2016)

Da Ros, C., Cavinato, C., Pavan, P., Bolzonella, D. (2016). Mesophilic and thermophilic anaerobic co-digestion of winery wastewater sludge and wine lees: An integrated approach for sustainable wine production. *Journal of Environmental Management.*

Department of Biotechnology, Ministry of Science & Technology, Ministry of Environment and Forests, Govt. of India (2011). Biology of *Gossypium spp.* (Cotton).

Díaz, A.B., Alvarado, O., de Ory, I., Caro, I., Blandino, A. (2013). Valorization of grape pomace and orange peels: Improved production of hydrolytic enzymes for the clarification of orange juice. *Food and Bioproducts Processing, 91*, 580–586.

DIRECTIVE 2008/98/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 19 November 2008 on waste and repealing certain Directives [2008] OJ L312/3-30.

DIRECTIVE 2009/28/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/E [2009] OJ L140/16-62.

El Achkar, J.H., Lendormi, T., Hobaika, Z., Salameh, D., Louka, N., Maroun, R.G., Lanoisellé, J.-L. (2016). Anaerobic digestion of grape pomace: Biochemical characterization of the fractions and methane production in batch and continuous digesters. *Waste Management, 50*, 275–282.

Esposito, G., Frunzo, L., Giordano, A., Liotta, F., Panico, A., Pirozzi, F. (2012). Anaerobic co-digestion of organic wastes. *Reviews in Environmental Science and Bio/Technology*, *11*(4), 325–341.

European Biogas Association (2011). "Biogas – simply the best". Available at: european-biogas.eu

European Biogas Association (http://european-biogas.eu/) (accessed on 2016)

Eurostat (http://ec.europa.eu/eurostat) (accessed on 2016)

Fabbri, A., Bonifazi, G., Serranti, S. (2015). Micro-scale energy valorization of grape marcs in winery production plants. *Waste Management, 36*, 156–165.

FAOSTAT (http://www.fao.org/faostat/en) (accessed on 2015 & 2016)

Galbe, M., Zacchi, G. (2012). Pretreatment: The key to efficient utilization of lignocellulosic materials. *Biomass and Bioenergy*, *46*, 70–78.

Gidarakos, E., Havas, G., Ntzamilis, P. (2006). Municipal solid waste composition determination supporting the integrated solid waste management system in the island of Crete. *Waste Management, 26*(6), 668–679. 8

Grainger, K., Tattersall, H. (2007). Wine Production: Vine To Bottle, Wine Production: Vine To Bottle. Blackwell Publishing.

Guo, J., Wang, W., Liu, X., Lian, S., Zheng, L. (2014). Effects of thermal pre-treatment on anaerobic co-digestion of municipal biowastes at high organic loading rate. *Chemosphere*, *101*, 66–70.

Haghighi Mood, S., Hossein Golfeshan, A., Tabatabaei, M., Salehi Jouzani, G., Najafi, G.H., Gholami, M., Ardjmand, M. (2013). Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. *Renewable and Sustainable Energy Reviews*, *27*, 77–93.

Hamawand, I., Sandell, G., Pittaway, P., Chakrabarty, S., Yusaf, T., Chen, G., Seneweera, S., Al-Lwayzy, S., Bennett, J., Hopf, J. (2016). Bioenergy from Cotton Industry Wastes: A review and potential. *Renewable and Sustainable Energy Reviews, 66*, 435–448.

Harmsen, P., Huijgen, W., Bermudez, L., Bakker, R. (2010). Literature review of physical and chemical pretreatment processes for lignocellulosic biomass. Energy Research Centre of the Netherlands, 10-13.

Hendriks, A.T.W.M., Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology, 100*, 10–18.

Hoornweg, D., Bhada-Tata, P. (2012). What a Waste: A Global Review of Solid Waste Management. World Bank, Washington DC, USA.

Kabir, M.M., Rajendran, K., Taherzadeh, M.J., Sárvári Horváth, I. (2014). Experimental and economical evaluation of bioconversion of forest residues to biogas using organosolv pretreatment. *Bioresource Technology, 178*, 201–208.

Kalderis, D., Diamadopoulos, E. (2010). Valorization of Solid Waste Residues from Olive Oil Mills: A Review. *Terrestrial and Aquatic Environmental Toxicology, 4*(Special Issue 1), 7–20.

Kang, K.E., Park, D.-H., Jeong, G.-T. (2013). Effects of inorganic salts on pretreatment of Miscanthus straw. *Bioresource Technology, 132*, 160–165.

Karantonis, H.C., Tsantila, N., Stamatakis, G., Samiotaki, M., Panayotou, G., Antonopoulou, S., Demopoulos, C.A. (2008). Bioactive polar lipids in olive oil, pomace and waste byproducts. *Journal of Food Biochemistry, 32*, 443–459.

Kayhanian, M. (1999). Ammonia Inhibition in High-Solids Biogasification: An Overview and Practical Solutions. *Environmental Technology*, *20*(4), 355–365.

Khalid, A., Arshad, M., Anjum, M., Mahmood, T., Dawson, L. (2011). The anaerobic digestion of solid organic waste. *Waste Management, 31*(8), 1737–1744.

Koppar, A., Pullammanappallil, P. (2013). Anaerobic digestion of peel waste and wastewater for on site energy generation in a citrus processing facility. *Energy, 60*, 62–68.

Li, Y., Park, S. Y., Zhu, J. (2011). Solid-state anaerobic digestion for methane production from organic waste. *Renewable and Sustainable Energy Reviews, 15*(1), 821–826.

Liu, L., Sun, J., Cai, C., Wang, S., Pei, H., Zhang, J. (2009). Corn stover pretreatment by inorganic salts and its effects on hemicellulose and cellulose degradation. *Bioresource Technology*, *100*(23), 5865–5871.

Macias-Corral, M., Samani, Z., Hanson, A., Smith, G., Funk, P., Yu, H., Longworth, J. (2008). Anaerobic digestion of municipal solid waste and agricultural waste and the effect of co-digestion with dairy cow manure. *Bioresource Technology*, *99*(17), 8288–8293.

Mao, C., Feng, Y., Wang, X., Ren, G. (2015). Review on research achievements of biogas from anaerobic digestion. *Renewable and Sustainable Energy Reviews*, *45*, 540–555.

Marculescu, C., Ciuta, S. (2013). Wine industry waste thermal processing for derived fuel properties improvement. *Renewable Energy*, *57*, 645–652.

Martín, M. A., Siles, J. A., Chica, A. F., Martín, A. (2010). Biomethanization of orange peel waste. *Bioresource Technology*, *101*(23), 8993–8999.

Mata-Alvarez, J., Dosta, J., Romero-Güiza, M. S., Fonoll, X., Peces, M., Astals, S. (2014). A critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renewable and Sustainable Energy Reviews*, *36*, 412–427.

McGraw-Hill Education. (http://www.mhhe.com/biosci/pae/botany/botany_map/articles/article_30.html) (accessed on 2016)

Monlau, F., Barakat, A., Trably, E., Dumas, C., Steyer, J.-P., Carrère, H. (2013). Lignocellulosic Materials Into Biohydrogen and Biomethane: Impact of Structural Features and Pretreatment. *Critical Reviews in Environmental Science and Technology*, *43*(3), 260–322.

Montgomery, L., Bochmann, G. (2014). Pretreatment of feedstock for enhanced biogas production. IEA Bioenergy 1–20.

National Cotton Council of America. (http://www.cotton.org/) (accessed on 2016)

Negro, V., Ruggeri, B., Mancini, G., Fino, D. (2016). Recovery of D-limonene through moderate temperature extraction and pyrolytic products from orange peels. *Journal of Chemical Technology & Biotechnology*.

Nogales, R., Cifuentes, C., Benitez, E. (2005). Vermicomposting of Winery Waste: A Laboratory Study. *Journal of Environmental Science and Health , Part B : Pesticides , Food Contaminants , and Agricultural Wastes*, *40*(4), 659–673.

Okino Delgado, C.H., Fleuri, L.F. (2016). Orange and mango by-products: Agro-industrial waste as source of bioactive compounds and botanical versus commercial description—A review. *Food Reviews International*, *32*(1), 1–14.

Oliveira, M., Duarte, E. (2016). Integrated approach to winery waste: waste generation and data consolidation. *Frontiers of Environmental Science & Engineering*, *10*(1), 168–176.

Pellera, F.-M., Pasparakis, E., Gidarakos, E. (2016). Consecutive anaerobic-aerobic treatment of the organic fraction of municipal solid waste and lignocellulosic materials in laboratory-scale landfill-bioreactors. *Waste Management*, *56*, 181–189.

Petrakis, C. (2006). Olive oil extraction. Olive oil: Chemistry and technology, 191-224.

Placido, J., Capareda, S. (2013). Evaluation of ligninolytic enzymes, ultrasonication and liquid hot water as pretreatments for bioethanol production from cotton gin trash, in: *ASABE Annual International Meeting*. pp. 203–208.

Rezzadori, K., Benedetti, S., Amante, E.R. (2012). Proposals for the residues recovery: Orange waste as raw material for new products. *Food and Bioproducts Processing*, *90*(4), 606–614.

Roig, A., Cayuela, M.L., Sánchez-Monedero, M.A. (2006). An overview on olive mill wastes and their valorisation methods. *Waste Management*, *26*(9), 960–969.

Ruiz, B., Flotats, X. (2014). Citrus essential oils and their influence on the anaerobic digestion process: an overview. *Waste Management*, *34*(11), 2063–79.

Ruiz, B., Flotats, X. (2016). Effect of limonene on batch anaerobic digestion of citrus peel waste. *Biochemical Engineering Journal*, *109*, 9–18.

Sambusiti, C., Monlau, F., Ficara, E., Carrère, H., Malpei, F. (2013). A comparison of different pre-treatments to increase methane production from two agricultural substrates. *Applied Energy*, *104*, 62–70.

Sánchez Moral, P., Ruiz Méndez, M.V. (2006). Production of pomace olive oil. *Grasas Y Aceites*, *57*(1), 47–55.

Sawatdeenarunat, C., Nguyen, D., Surendra, K.C., Shrestha, S., Rajendran, K., Oechsner, H., Xie, L., Khanal, S.K. (2016). Anaerobic biorefinery: Current status, challenges, and opportunities. *Bioresource Technology*, *215*, 304–313.

Siles, J.A., Vargas, F., Gutiérrez, M.C., Chica, A.F., Martín, M.A. (2016). Integral valorisation of waste orange peel using combustion, biomethanisation and co-composting technologies. *Bioresource Technology*, *211*, 173–182.

Singh, R., Krishna, B. B., Kumar, J., Bhaskar, T. (2016). Opportunities for utilization of non-conventional energy sources for biomass pretreatment. *Bioresource Technology*, *199*, 398–407.

Sinha, N., Sidhu, J., Barta, J., Wu, J., Cano, M. P. (Eds.) (2012). Handbook of fruits and fruit processing. John Wiley & Sons.

Song, Z., Yang, G., Liu, X., Yan, Z., Yuan, Y., Liao, Y. (2014). Comparison of seven chemical pretreatments of corn straw for improving methane yield by anaerobic digestion. *PLoS ONE*, *9*(4), 1–8.

Sweep-net (2014a). Country Report on Solid Waste Management in Algeria.

Sweep-net (2014b). Country Report on Solid Waste Management in Egypt.

Sweep-net (2014c). Country Report on Solid Waste Management in Lebanon.

Sweep-net (2014d). Country Report on Solid Waste Management in Morocco.

Sweep-net (2014e). Country Report on Solid Waste Management in Tunisia.

Taherzadeh, M.J., Karimi, K. (2008). *Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review*. *International Journal of Molecular Sciences* (Vol. 9).

United Nations Statistics Division (UNSD) (http://unstats.un.org/) (accessed on 2016)

Ward, A.J., Hobbs, P.J., Holliman, P.J., Jones, D.L. (2008). Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technology*, *99*(17), 7928–40.

Waste Atlas (http://www.atlas.d-waste.com/) (accessed on 2016)

Weiland, P. (2010). Biogas production: current state and perspectives. *Applied Microbiology and Biotechnology*, *85*, 849.860.

Wikandari, R., Nguyen, H., Millati, R., Niklasson, C., Taherzadeh, M.J.M. (2015). Improvement of biogas production from orange peel waste by leaching of limonene. *BioMed Research International*, *2015*, 1–6.

Wilkins, M.R., Suryawati, L., Maness, N.O., Chrz, D. (2007). Ethanol production by Saccharomyces cerevisiae and Kluyveromyces marxianus in the presence of orange-peel oil. *World Journal of Microbiology and Biotechnology*, *23*(8), 1161–1168.

Zheng, Y., Zhao, J., Xu, F., Li, Y. (2014). Pretreatment of lignocellulosic biomass for enhanced biogas production. *Progress in Energy and Combustion Science*, *42*, 35–53.

CHAPTER 3

Effect of substrate to inoculum ratio and inoculum type on the biochemical methane potential of solid agroindustrial waste

This chapter focuses on evaluating the influence of different substrate to inoculum ratios (SIR) and inoculum types on the methane potential of four solid agroindustrial waste, namely winery waste (WW), cotton gin waste (CGW), olive pomace (OP) and juice industry waste (JW). To this purpose, Biochemical Methane Potential (BMP) assays were conducted, in which four SIR, i.e. 0.25, 0.5, 1 and 2 (on a volatile solids (VS) basis) were tested and three different inocula, namely anaerobic sludge, landfill leachate and thickened anaerobic sludge, were compared. All four materials were proved viable substrates for anaerobic digestion. Furthermore, anaerobic sludge was found the most adequate inoculum among tested samples, and due to its high availability it may be considered a manageable choice in real-scale applications. Contrarily, using landfill leachate and thickened anaerobic sludge for the same purpose showed lower efficiencies. The optimum SIR for determining the methane potential of the studied substrates were of 0.5 for WW and JW, yielding 446.23 and 445.97 $mLCH_{4,\ STP}/gVS_{substrate}$, respectively, and of 0.25 for CGW and OP, yielding 267.96 and 258.65 $mLCH_{4,\ STP}/gVS_{substrate}$, respectively. Higher SIR delayed methane production, indicating process inhibition. Experimental methane potentials were lower than theoretical, suggesting that eventual pretreatments prior to anaerobic digestion might be worth investigating. The association of different SIR with 2- and 3-parameter kinetic models manifested the complexity of the anaerobic digestion of the studied substrates. Moreover, a different modeling approach, assuming the occurrence of multiple-stages, appeared to be more suitable for describing the behavior of the experimental data.

3.1 Introduction

The use of alternative energy sources instead of traditional has been increasingly gaining interest in the last decades and has become a major research topic, which is under continuous development. The significant amounts of greenhouse gases that are released worldwide, coupled to the imminent depletion of fossil fuels, demand energy generation to be possible by applying different methods and technologies that are sustainable, more convenient form an economical standpoint and use renewable materials as feedstock (Eskicioglu and Ghorbani, 2011; Nzila et al., 2010).

Anaerobic digestion involves the use of a consortium of microorganisms for the degradation and stabilization of complex organic substrates, such as manure, wastewater, sludge and solid waste, with the main product being biogas, an energy-rich gas mainly composed of methane and carbon dioxide (Raposo et al., 2011a). Recently, this technology has been studied aiming at non conventional energy production (Sawatdeenarunat et al., 2015). The use of solid organic substrates in particular, has been attracting a lot of attention, with agricultural and agroindustrial biomass and waste materials being the main candidates for such use (Feng et al., 2013).

Agricultural and agroindustrial activities are among the most widespread around the world and especially in the Mediterranean region, they make a major contribution to national economies (Fountoulakis et al., 2008). Among the most profitable sectors of this area are the wine and olive oil production industries, while the citrus fruits (mainly orange) and cotton processing activities are also of high importance. In fact, according to the Statistical Database of the Food and Agriculture Organization of the United Nations (FAOSTAT) (2016), data referring to twenty Mediterranean countries report that in 2013, approximately 29.6 million tons of grapes, 19.2 million tons of olives, 13.5 million tons of oranges and 3.9 million tons of seed cotton were totally produced. The increasing development of activities related to the processing of these commodities has, over the past decades, resulted in large amounts of solid waste being generated on a regular basis. Depending on the activity, such wastes can vary in type and composition. In particular, the solid residue obtained after grape processing for wine production is known as grape marc or grape pomace and is composed of skins, seeds, pulp and stalks. This material has been found to contain considerable quantities of phenolic compounds (El Achkar et al., 2016; Nogales et al., 2006; Pala et al., 2014). The olive oil production process generates a type of solid residue that consists of pieces of olive skin, pulp and kernels, as well as some oil. This specific material, also known as olive pomace, contains a variety of compounds (e.g. phenolic compounds, fatty acids etc.) and is characteristic of the three-phase olive oil extraction system (Karantonis et al., 2008; Carlini et al., 2014). Similarly, cotton processing waste is usually comprised of stalks, leaves and cottonseed hulls (Isci and Demirer, 2007). Moreover, the waste obtained from orange processing, mostly for juice production, represents approximately 50-60% of the initially processed fruit and is composed of peels, seeds and segment membranes (Forgács et al., 2012; Martín et al., 2010; Wilkins et al., 2007).

The accumulation of agroindustrial waste due to high production rates, may often cause several management and disposal problems. In fact, there are many cases in which agroindustrial waste have no further use and are ultimately inappropriately discarded, or deposited in landfills, thus eventually leading to environmental degradation. Therefore, anaerobic digestion of such materials seems a much suitable option for combined waste management and alternative energy production. Additionally to their abundance and low cost, agroindustrial waste, have also the advantage that they do not compete with food or feed

production, something that makes them viable substitutes for energy crops (Dinuccio et al., 2010; Fountoulakis et al., 2008; Isci and Demirer, 2007; Nigam et al., 2009; Sawatdeenarunat et al., 2015).

Nevertheless, in order to consider a specific substrate as a possible feedstock material for an anaerobic digestion plant, an important parameter that needs to be determined is its methane potential, i.e. the maximum methane quantity that is potentially produced during anaerobic digestion (Alzate et al., 2012). The most common method used to determine methane potential is conducting Biochemical Methane Potential (BMP) assays. This protocol has not been standardized yet, in order to fit every possible substrate, therefore numerous variations can be found in literature, which concern several parameters related not only to the substrate, but also to the inoculum and to the experimental conditions under which the assays are conducted. Among these parameters, two of the most important, are the substrate to inoculum ratio (SIR) and the inoculum source/type (Raposo et al., 2011a). The SIR is determinant for the correct operation of the anaerobic digestion process (Pozdniakova et al., 2012) and its selection is related to the substrate properties. Several studies (Alzate et al., 2012; Eskicioglu and Ghorbani, 2011; Feng et al., 2013; González-Fernández and García-Encina, 2009; Kawai et al., 2014; Kim et al., 2012; Lim and Fox, 2013; Raposo et al., 2009; Zhou et al., 2011) had as their main focus the optimization of this parameter and their results were in fact dependent on the substrate in use. Inoculum type is another basic parameter in anaerobic digestion. The inoculum provides the system with the initial microbial population, which will then participate to the reactions constituting the organic matter degradation processes. It also contains several macronutrients which can positively affect enzyme activity and biogas production (Gu et al., 2014). A wide variety of samples has been used for such purpose in other researches, including sludge from anaerobic digesters treating municipal or agroindustrial wastewater, animal manures, landfill leachate, etc. (Córdoba et al., 2016; Gu et al., 2014; Pozdniakova et al., 2012).

The purpose of this study was to evaluate the influence of parameters such as substrate to inoculum ratio (SIR) and inoculum type, on the methane potential of four solid agroindustrial waste, namely winery waste, cotton gin waste, olive pomace and juice industry waste. In order to optimize these parameters, Biochemical Methane Potential (BMP) assays were performed, for all materials. Four SIR values were tested (0.25, 0.5, 1 and 2, on a VS basis), while three different inocula were compared, specifically anaerobic sludge, landfill leachate and thickened anaerobic sludge. The materials examined in the present study are among the most produced agroindustrial waste in the Mediterranean region, thus, the investigation of possible management alternatives, eventually leading to energy production, is of great importance. To date, a few studies can be found regarding the methane potential of these kinds of substrates, especially when referring to the investigation of different substrate to inoculum ratios. The approach adopted in the present research has not been enough studied, particularly when considering that no specific researches examining the use of different inocula have been conducted in relation to such materials. Anaerobic sludge from wastewater treatment plants and leachate from landfills can be easily found in an urban environment, as well as in the vicinity of industrial areas and are characterized by high availability throughout the year. Therefore, the possible use of such materials as inocula for anaerobic digestion is of high relevance and is worth investigating. Additionally, in this paper kinetic modeling of the anaerobic digestion process with the application of multiple equations was conducted. More specifically, two approaches were adopted. The first one was a single-modeling approach, in which the experimental data were fitted through non-linear regression to five separate kinetic models, i.e. the first-order exponential, two-phase exponential, logistic, transference

(reaction-curve) and modified Gompertz models. In the second approach, a multiple-stage modeling of the data was conducted, by combining the first-order exponential model and the logistic model. The application of such an approach to BMP data referring to different substrate to inoculum ratio and inoculum type, while concerning the specific substrates and conditions examined in this research, has not been studied before.

3.2 Materials and methods

3.2.1 Substrates and inocula

Four different substrates were used in this study, more precisely, winery waste (WW), comprising of grape skins, seeds and stalks, cotton gin waste (CGW) comprising of cotton fiber, stalks, burs and leaves, olive mill solid waste, specifically olive pomace (OP), as well as juice industry waste (JW), comprising of orange peels. As soon as the samples were collected, they were stored at -20 °C in zip-lock bags, for preservation purposes. Prior to digestion WW and JW were comminuted without drying using a food processor, CGW was dried at 60 °C and then comminuted to a particle size less than 500 μm, using a universal cutting mill, while OP was kept as received.

The use of three types of inocula, which differed in either source and/or composition, was evaluated. The first sample consisted of anaerobic sludge (AS) originating from a mesophilic anaerobic digester of the Municipal Wastewater Treatment Facility of Chania, Crete. The second type of inoculum was a landfill leachate (LL) sample which was taken from the Sanitary Landfill situated in the Akrotiri area of Chania. Finally, the third inoculum was a sample of anaerobic sludge which was gravitationally thickened (TAS) in the laboratory prior to its use. Using the latter sample aims at investigating the suitability of a partially dewatered anaerobic sludge sample as inoculum. TAS was obtained by leaving AS to settle for 24 h and afterwards decanting the supernatant.

3.2.2 Experimental setup and procedure

Two series of experiments were conducted, of which the first one aimed at determining the optimum substrate to inoculum ratio (SIR) for each substrate. More specifically, four SIR values on a volatile solids (VS) basis were tested, namely 0.25, 0.5, 1 and 2 $gVS_{substrate}/gVS_{inoculum}$. In order to obtain these ratios, the quantity of inoculum in each assay was maintained constant at 15 gVS/L, while the quantity of substrate varied according to the respective SIR value. In these experiments only AS was used as inoculum. In the second series of experiments the effect of inoculum type on the methane production process was investigated. This was done by comparing the performance of LL and TAS as inocula, with that of AS at the optimum SIR, as it resulted for each substrate, from the first series of experiments.

The apparatus used for BMP assays consisted of 250 mL reactors (conical flasks) covered with rubber stoppers, in which two PVC (Polyvinyl chloride) tubes were inserted. These tubes allowed methane measurement, as well as N_2 flushing in the flasks, in order to ensure an inert atmosphere. The working volume of the reactors was set to 100 mL. In both series of experiments, blank assays containing only inoculum (SIR=0) were also performed, since none of the inocula was degassed prior to BMP assays.

BMP assays were initiated by first introducing the inoculum and the substrates in the flasks in appropriate amounts and by subsequently adding deionized water to the mixture, if needed, in order to bring the total volume to approximately 100 mL. It is noted that deionized water was used instead of tap water, in order to avoid the eventual presence of traces of unknown substances, which might have altered the results. Following this step, the pH of the mixture was adjusted at 7.8 ± 0.05, by adding small amounts of NaOH (1 M). Introducing the substrates in the reactors caused the initial pH of the mixture to decrease, especially at higher SIR. Therefore, the adjusting procedure served both to create a more favorable environment for the startup of the anaerobic digestion process, and as a means of assuring as much as possible comparable conditions between different SIR of the same substrate. After pH adjusting, the flasks were covered with the rubber stoppers and finally, they were flushed with N_2 for 5 min. The digestion was conducted in an incubator set at 35 °C. The mesophilic temperature range is the most commonly used in literature for conducting anaerobic digestion experiments and it refers to values between 20 and 45 °C (Raposo et al., 2011a). The selection of the specific value of 35 °C in the present study was made based on previous researches (Alzate et al., 2012; Barrantes Leiva et al., 2014; Córdoba et al., 2016; Donoso–Bravo et al., 2010; Eskicioglu and Ghorbani, 2011; Fernández-Cegrí et al., 2012; Fountoulakis et al., 2008; González-Fernández and García-Encina, 2009; Gunaseelan, 2004; Isci and Demirer, 2007; Liao et al., 2014; Lim and Fox, 2013; Martín et al., 2010; Raposo et al., 2009; Raposo et al., 2011b; Rincón et al., 2013; Wang et al., 2012). It is noted that all assays conducted in this study were duplicated.

Methane production was measured daily for the first seven days and for the rest of the incubation period, every two days. BMP assays were terminated when methane quantity was undetectable or less than 5% of the total amount produced. Therefore, the total duration of the assays varied depending on each sample and ranged between 41 and 68 days.

3.2.3 Analytical methods

Total Solids (TS) and Volatile Solids (VS) contents were determined according to American Public Health Association (APHA) method 2540G. A portable pH-meter (PH25, Crison) was used for pH determination, and specifically for the pH of the solid substrates, the measurement was performed in a deionized water suspension with a solid/liquid ratio of 1/10 g/mL. Elemental analysis (C, H, N, S) was conducted using an EA300 Euro Vector elemental analyzer. Oxygen content was determined by difference, considering the VS content of each substrate. Fiber analysis, i.e. the determination of NDF (Neutral Detergent Fiber), ADF (Acid Detergent Fiber) and ADL (Acid Detergent Lignin), was based on the method described by Fernández-Cegrí et al. (2012). Total Alkalinity (TA) at the end of digestion was determined according to APHA method 2320B. Finally, methane production was determined by means of volume displacement using an 11.2% KOH solution, as it was done in previous studies (Altaş, 2009; Nain and Jawed, 2006). More specifically, each BMP reactor was connected to an inverted bottle containing the alkaline solution. Subsequently, biogas was released to flow inside the bottle, in order to remove CO_2 and H_2S by absorption and leave only CH_4. The volume of CH_4 being transferred to the bottle caused the displacement of an equal amount of KOH solution, which was then quantified using a graduated cylinder.

3.3 Data analysis

3.3.1 Theoretical methane potential

The Theoretical Methane Potential (TMP) of the four substrates at Standard Temperature and Pressure (STP) conditions was estimated through their elemental composition and the stoichiometry of the degradation reaction (Equation 1), using Buswell's formula (Equation 2) (Kim et al., 2012; Lim and Fox, 2013):

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right)H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right)CO_2 + dNH_3 + eH_2S \quad (1)$$

$$TMP\left[\text{mL CH}_{4,\text{STP}}/\text{g VS}\right] = 22.4 \cdot \left[\frac{\left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)}{12a + b + 16c + 14d + 32e}\right] \cdot 1000 \quad (2)$$

3.3.2 Theoretical oxygen demand

The Theoretical Oxygen Demand (TOD) of the four substrates was estimated through their elemental composition and the stoichiometry of the oxidation reaction (Equation 3), using Equation 4 (Raposo et al., 2011b):

$$C_aH_bO_cN_dS_e + \left(a + \frac{b}{4} - \frac{c}{2} - \frac{3d}{4}\right)O_2 \rightarrow aCO_2 + \left(\frac{b}{2} - \frac{3d}{2}\right)H_2O + dNH_3 \quad (3)$$

$$TOD\left[\text{mg O}_2/\text{g VS}\right] = \frac{\left(2a + \frac{b}{2} - c - \frac{3d}{2}\right) \cdot 16}{12a + b + 16c + 14d} \cdot 1000 \quad (4)$$

3.3.3 Model fitting

Methane production was modeled by fitting the data with five kinetic models through non-linear regression, using the Solver tool of Microsoft Office Excel. The goodness of fit was evaluated by taking into consideration both the Residual Sum of Squares (RSS) and the R-square (R^2) values.

The first-order exponential, two-phase exponential, logistic, transference (reaction-curve) and modified Gompertz models were used (Donoso–Bravo et al., 2010; Luna-delRisco et al., 2011), which are described by Equations 5 – 9, respectively.

$$B = B_{\max} \cdot \left[1 - \exp\left(-kt\right)\right] \quad (5)$$

$$B = B_1 \cdot \left[1 - \exp\left(-k_1 t\right)\right] + B_2 \cdot \left[1 - \exp\left(-k_2 t\right)\right] \quad (6)$$

$$B = \frac{P}{1 + \exp\left[4R_m \cdot (\lambda - t)/P + 2\right]} \quad (7)$$

$$B = P \cdot \left\{1 - \exp\left[-\frac{R_m \cdot (t - \lambda)}{P}\right]\right\} \quad (8)$$

$$B = P \cdot \exp\left\{-\exp\left[\frac{R_m \cdot e}{P}(\lambda - t) + 1\right]\right\} \quad (9)$$

where, t is time (d), B is the cumulative methane production at time t (mL CH_4), B_{max} is the maximum methane production (mL CH_4), P is the methane production potential (mL CH_4), R_m is the maximum methane production rate (mL CH_4/d), k , k_1 and k_2 are the rate constants (d^{-1}) and λ is the lag phase (d).

In an attempt to better describe the experimental data, an approach which combined the application of the first-order exponential model and the logistic model, was adopted. This procedure was followed in order to evaluate whether the biodegradation of the studied substrates might be more accurately modeled under the assumption of multiple stages taking place. The first-order exponential model was applied for the very first days of incubation, while the logistic model was used for the remaining period, eventually for more than one interval. A slightly modified version of Equation 7 was used in this case (Rincón et al., 2013) (Eq. 10)

$$B = B_0 + \frac{P}{1 + \exp\left[4R_m \cdot (\lambda - t)/P + 2\right]} \quad (10)$$

where, B_0 represents the cumulative methane production at the startup of the stage in question (mL CH_4).

3.3.4 Specific Methane Yield

The specific methane yield (SMY) resulting after the end of digestion was obtained by subtracting the ultimate cumulative methane production of the blank assay (mL CH_4) from the ultimate cumulative methane production of each assay containing the tested substrates, and by subsequently dividing it by the initially added amounts of VS of the substrates. These values were then converted to STP conditions.

3.4 Results and discussion

3.4.1 Characterization

The characteristics of the four substrates and of the inocula are presented in Table 3-1. Inocula AS and LL show similar low TS and VS contents. TAS, on the other hand is characterized by higher values, however VS/TS approaches the value of AS. These results are expected, since TAS is obtained through thickening of AS, consequently it would have an increased TS percentage, but a comparable VS content. Moreover, all three samples are in the

alkaline region, with LL having the highest value. WW and JW are relatively low in TS compared with CGW and OP, while the VS/TS ratio is observed above 0.90 in the cases of WW, OP and JW. The pH of all substrates is found in the acidic region, with WW and JW having the lowest values. CGW and OP are characterized by the lowest and highest C and H contents, respectively, while WW and JW show a similar composition. Nevertheless, the C/N of JW is significantly higher than those of the other substrates, which are all within the recommended range (20/1 – 30/1) for anaerobic digestion (Li et al., 2011). OP and CGW are the substrates with the highest and the lowest TMP, respectively. NDF, ADF and ADL contents of CGW and OP are found at similar levels with minor differences, while WW and JW present significantly lower values. The results of Table 3-1 are comparable to most of those found in several earlier publications (Adl et al., 2012; Carlini et al., 2014; Dinuccio et al., 2010; Fabbri et al., 2015; Gunaseelan, 2004; Isci and Demirer, 2007; Kaparaju and Rintala, 2006; Nzila et al., 2010; Rincón et al., 2013; Ruggeri et al., 2015; Subagyono et al., 2015), although the values for specific characteristics within these results may differ.

Table 3-1: Characteristics of inocula and substrates

Properties	Inocula			Substrates			
	AS	LL	TAS	WW	CGW	OP	JW
TS (%) (wb**)	2.7	2.6	4.3	28.1	70.6	53.5	16.2
VS (%)(wb**)	1.7	1.4	2.8	25.8	52.9	52.3	15.7
VS/TS	0.62	0.53	0.64	0.92	0.75	0.98	0.97
pH	7.8	8.2	7.4	3.7	6.9	6.1	4.6
Elemental composition (db***)							
C (%)	33.7*	-	-	45.9	32.8	54.2	45.3
H (%)	0.2*	-	-	5.95	4.40	7.53	6.29
N (%)	4.0*	-	-	1.80	1.40	2.09	0.90
S (%)	< DL*	-	-	< DL	< DL	< DL	< DL
O (%)	24.1*	-	-	38.3	36.4	34.5	44.3
C/N	8.4*	-	-	25.5	23.4	25.9	50.3
Empirical formula	-	-	-	$C_{29.8}H_{46.4}O_{18.7}N$	$C_{27.4}H_{44.2}O_{22.8}N$	$C_{30.2}H_{50.4}O_{14.4}N$	$C_{58.8}H_{97.8}O_{43.0}N$
TMP ($mLCH_{4,\ STP}/gVS$)	-	-	-	489.26	392.09	593.14	453.50
TOD (mgO_2/gVS)	-	-	-	1398	1120	1695	1296
Fiber composition (%) (wb**)							
NDF	-	-	-	12.1	43.9	34.5	3.2
ADF	-	-	-	11.7	42.6	26.9	2.3
ADL	-	-	-	11.0	31.5	24.7	0.3

DL: Detection Limit , *analysis performed on sample solids , **wb: wet basis , ***db: dry basis

3.4.2 Effect of substrate to inoculum ratio

3.4.2.1 Methane production

Fig. 3-1 shows the daily and cumulative methane production profiles (in terms of volume, (mL)) for each substrate at different SIR values (0.25, 0.5, 1, 2), during the BMP assays of the first series of experiments, which were conducted using only AS as inoculum. Throughout the digestion period a similar general trend is noticed for all substrates. More specifically, methane production profiles are generally characterized by an initial lag phase, a subsequent more rapid increasing phase and finally a stabilization phase. The duration of the lag phase is different for each substrate, as well as for each SIR value. A resemblance between the data for CGW (Fig.3-1 (c) and (d)) and OP (Fig. 3-1 (e) and (f)), as well as between the data for WW (Fig. 3-1 (a) and (b)) and JW (Fig. 3-1 (g) and (h)), is noticed. In the first case, as the SIR is raised from 0.25 to 2 an increase in the quantity of methane produced is observed. This can be attributed to the presence of more available substrate in the reactors (Raposo et al., 2011b). In the second case however, methane production corresponding to the samples with SIR=0.5 prevails. In fact, the order followed by methane production values has the general trend: SIR 0.5 > SIR 0.25 > SIR 1 > SIR 2. Alzate et al. (2012) and Zhou et al. (2011) have observed a similar trend of methane yields as a function of substrate loading, with their maximum values being attained for substrate to inoculum ratios of 0.5 and 0.6, respectively. It is possible that, in the present study, the quantity of biodegradable material and of available nutrients corresponding to a SIR=0.25 for WW and JW, was not sufficient for the microbial biomass to induce enzymes and thus to complete methanogenesis (Lim and Fox, 2013; Pozdniakova et al., 2012). In addition, the curves referring to SIR=1 follow an odd behavior compared to the other samples, for both WW and JW. More specifically, the methane production peak value for these specific samples is observed at days 29 and 35, respectively, while for the remaining assays the peak value is reached no later than the 15th day of incubation. This caused the cumulative methane production of these samples to increase and reach the levels of the assays with SIR=0.25 and SIR=0.5, for WW and JW, respectively, with the latter ultimately exceeding it. This behavior may be explained by the eventual delayed consumption of previously produced Volatile Fatty Acids (VFA) by methanogens (Alkan-Ozkaynak and Karthikeyan, 2011). Probably for the same reason, higher amounts of substrate led to a slightly prolonged lag phase in the majority of cases, but this phenomenon is more pronounced for WW and to some extent for JW. The eventual VFA accumulation would have led to acidification inside the reactors. More specifically, the latter phenomenon occurs when the ratio between the rate of the acidogenic process and the rate of the methanogenic process is out of balance, i.e. when the acidogenesis rate exceeds the methanogenesis rate. In that case, VFA accumulation is verified with a consequent drop in pH, which in turn causes inhibition to methanogens (Esposito et al., 2012; Vavilin and Angelidaki, 2005). Nevertheless, the acidification phenomenon manifested in the present study would have probably been a case of reversible acidification, since methane production recovered after a certain period of time for all relevant samples, implying eventual VFA consumption (González-Fernández and García-Encina, 2009; Kawai et al., 2014). The enhanced manifestation of this phenomenon for WW and JW could be attributed to their properties. Indeed, Wang et al. (2012) have mentioned that substrates with a low nitrogen content and a high C/N ratio, such as WW and JW in this study, are often characterized by a low pH and poor buffering capacity, which may lead to VFA accumulation during the digestion process.

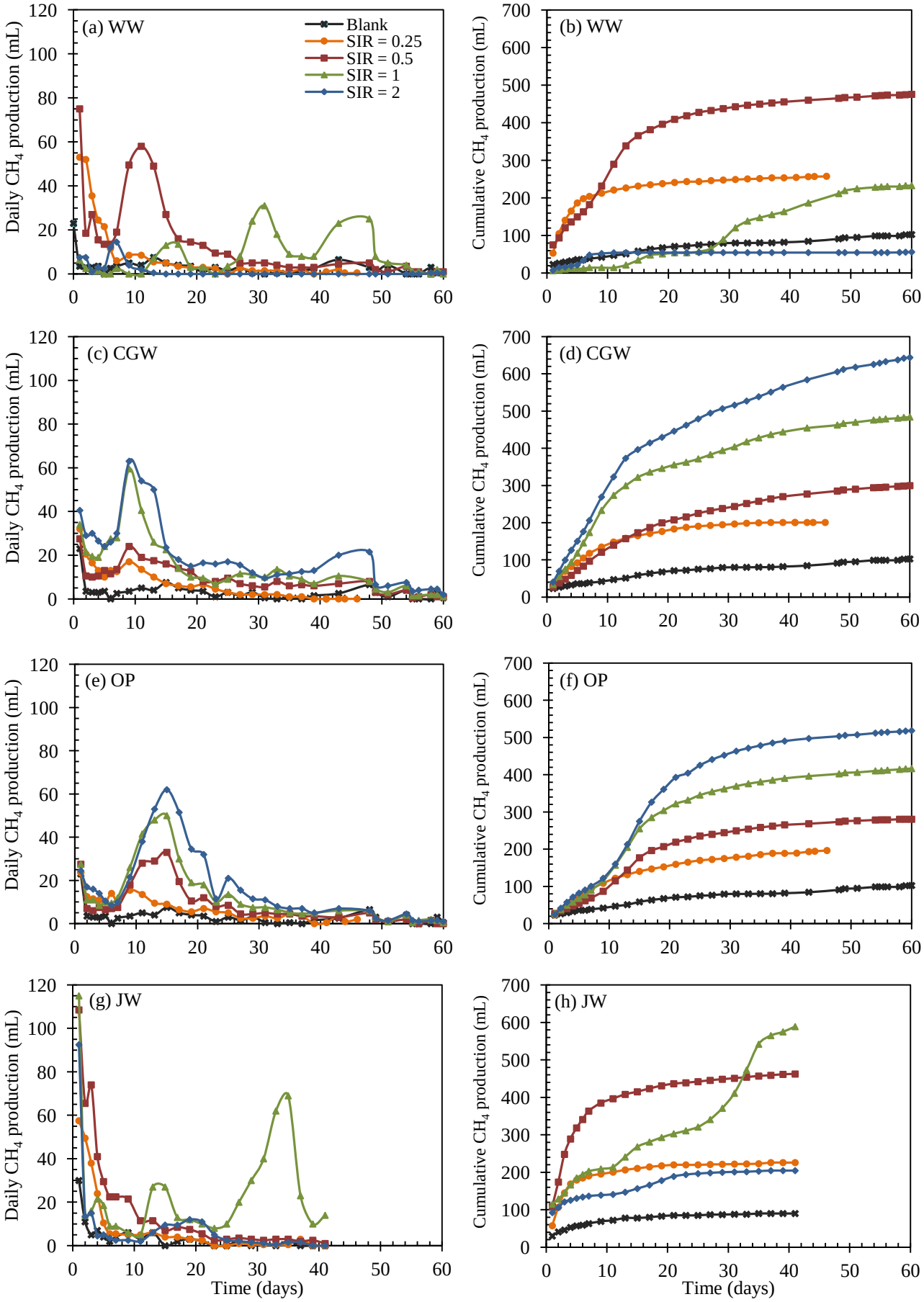


Figure 3-1: Daily and cumulative CH_4 production profiles as a function of time, for trials with different SIR [SIR=0.25(●), SIR=0.5 (■), SIR=1(▲), SIR=2 (♦)], for WW (a, b), CGW (c, d), OP (e, f) and JW (g, h)

3.4.2.2 Specific methane yields

The data reported in Table 3-2 ($mLCH_4/gVS_{substrate}$) are consistent with the results depicted in Fig. 3-1. The highest SMY are obtained for WW and JW both at SIR=0.5, with the values in the table, corresponding to 115.36 and 70.22 $mLCH_4/gRaw_{substrate}$, respectively, if expressed in relation to the raw mass of each substrate instead of the VS mass. CGW and OP yielded values having a decreasing trend as a function of increasing SIR, with those corresponding to CGW being slightly higher. The maximum values obtained for these two substrates correspond, in terms of raw mass, to 141.88 and 135.20 $mLCH_4/gRaw_{substrate}$, respectively. The overall lowest methane yield was provided by WW at SIR=2, followed by JW at the same SIR. Methane production (Fig. 3-1) and SMY values (Table 3-2) combined with the significantly more acidic pH, around 5.1 (Table 3-4), corresponding to these two assays, indicates that complete degradation of the organic matter present in the reactors was not achieved in these cases (Alkan-Ozkaynak and Karthikeyan, 2011). In the case of JW the respective TA value for SIR=2 may be an additional indication of this phenomenon. As far as WW is concerned, the negative SMY can be explained by the fact that the methane volume produced from this particular assay was less than the volume obtained from the blank assay, clearly showing that microbial inhibition may have occurred in this case. It is also noticed that for all substrates, maximum SMY are lower than their respective TMP (Table 3-2).

Decreased SMY at higher SIR may be attributed to system overloading. Higher substrate amounts in the reactor imply increased availability of easily hydrolysable material (Alzate et al., 2012), which in turn leads to VFA accumulation, decreased pH values, as shown in Table 3-4 in the present study, and ultimately anaerobic digestion inhibition (Alkan-Ozkaynak and Karthikeyan, 2011; Eskicioglu and Ghorbani, 2011; Feng et al., 2013; Lim and Fox, 2013; Pozdniakova et al., 2012; Raposo et al., 2009).

The SMY that were obtained in the present study are comparable to those reported by other authors, who studied similar substrates. Gunaseelan (2004) reported methane yields of 283 and 455 $mLCH_4/gVS_{added}$, for grape pressings and orange peel, respectively, both corresponding to a SIR of 0.5. Grape marcs yielded 116 NL CH_4/kg VS (SIR=0.5) in the study performed by Dinuccio et al. (2010), while in another study carried out by Fabbri et al. (2015) two similar samples yielded 273.08 and 156.85 $NmLCH_4/gVS$ (both at SIR=0.5), respectively. Adl et al. (2012) reported a methane yield for ground cotton stalks equal to 52.8 $mLCH_4/gVS$ at a feed to inoculum ratio of 0.24, whereas when Nzila et al. (2010) evaluated the energy potential of different materials, the result for cotton residues was 365 m^3CH_4/tVS at an inoculum to substrate ratio of 1.5 (i.e. SIR=0.67). Finally, Rincón et al. (2013), who studied thermal pretreatment of two-phase olive mill solid waste, reported a methane yield of 373 $mLCH_4/gVS$ for the untreated sample, using a SIR of 0.5.

3.4.2.3 Achievement of t_{80}

The time period required in order to achieve at least 80% (t_{80}) of the total CH_4 production was determined from the methane production data and is shown in Table 3-3. t_{80} is clearly affected by the variation in the SIR and it generally tends to increase with higher amounts of substrate in the reactors. A similar observation was made by Feng et al. (2013). More specifically, twice the time is required for CGW to attain above 80% of the total methane production, at an SIR=0.5 (31 days) compared with the time required at SIR=0.25 (15 days). For the remaining assays (SIR=1 and SIR=2), t_{80} seems to stabilize, since no substantial differences are

observed. In the case of OP the variation in SIR does not appear to significantly affect t_{80}. WW and JW show similar patterns, i.e. increasing t_{80} for SIR between 0.25 and 1, and a much earlier achievement of the target percentage at SIR=2, nevertheless with a lower CH_4 quantity. At this point however, it should be mentioned that SIR and consequently the amount of substrate for each assay is different. Therefore, methane production values presented in Table 3-3 are not directly comparable with each other, since this fact has not been taken into consideration for the data reported in this case.

Table 3-2: SMY of substrates, comparison between different SIR values and different inocula

Varying parameter	Specific methane yield (SMY) (mL $CH_{4,\ STP}$ / g $VS_{substrate}$)			
	WW	CGW	OP	JW
SIR				
0.25	404.70	267.96	258.65	325.62
0.5	446.23	235.71	213.09	445.97
1	90.12	228.23	188.03	298.79
2	-13.92	161.96	124.55	34.28
Inoculum type	SIR=0.5	SIR=0.25	SIR=0.25	SIR=0.5
AS	446.23	267.96	258.65	445.97
LL	407.06	59.78	119.76	274.19
TAS	403.56	212.88	224.84	242.50

3.4.3 Effect of inoculum type on methane potential

3.4.3.1 Methane production

After determining the optimum SIR for each substrate, on the basis of the aforementioned results, a new series of BMP assays was performed using LL and TAS as inocula. The data obtained from this series of experiments were compared to the respective data obtained with AS. Fig. 3-2 shows the daily (D) and cumulative (C) methane production profiles for each of the substrates (S), as well as for the blank assays (B).

In the case where TAS was used as inoculum, methane production patterns resemble those obtained using AS, as expected, since the two samples originate from the same source. On the other hand, using LL, in all cases led to a not only lower but also shorter methane production. To be more specific, methane production was noticed since the beginning of the assays; however by the end of the first week of incubation it had already reached a plateau. After a gap period, during which the amount of methane being produced was minimal or not sufficient in order to be measured, hence the plateau, methane production started to rise again maintaining an increasing trend until the end of the assays. This gap period, in which no or little methane was produced, is a common characteristic for all substrates, nevertheless its duration varied for each substrate. The fact that in the case of CGW the period without gas production lasted until the end of incubation, does not preclude the eventuality of methane production rising again at a later time, if the digestion had not been interrupted.

The LL sample used in this study is characterized by a pH of 8.2, which classifies it as mature, according to the information reported by Renou et al. (2008) regarding the characteristics of landfill leachate depending on age. This classification of LL would imply the prevalence of refractory compounds such as humic and fulvic acids. The presence of these

substances usually leads to low biological activity and thus tends to limit the effectiveness of biological processes (Christensen et al., 2001; Renou et al., 2008; Öman and Junestedt, 2008). In the present study, this would mean inhibition of anaerobic digestion, manifested by the lower methane production observed in Fig. 3-2. Another factor that might have acted as an inhibitor when LL was used as inoculum, is the eventual presence of high ammonia nitrogen concentrations, either already existing in the leachate or developed afterwards during digestion (Liao et al., 2014). Ammonia is known to have a certain buffering effect in anaerobic digestion systems. In contrast to what was observed in this study, other authors (Barrantes Leiva et al., 2014; Liao et al., 2014) have stated that this buffering capacity may contribute in maintaining conditions favorable for methanogenesis by counteracting the effect of acid accumulation. Thus, the results of the present study could be more related to the ratio between the amounts of leachate and the amounts of substrates present in the reactors. More specifically, it is possible that the quantity of leachate was too high compared to the quantity of substrate and as a result the buffering effect prevailed, bringing pH and alkalinity to higher levels. This eventuality could be supported by the pH and TA values measured at the end of incubation (Table 3-4). In fact, in a study conducted by Gu et al. (Gu et al., 2014), a final pH of 8.8 was thought to be connected to the reduced biogas production rate.

3.4.3.2 Specific methane yields

As far as the SMY obtained using different inocula are concerned, it is noticed that LL and TAS are inferior to AS in all cases. More specifically, the use of LL yielded values that are about 9, 78, 54 and 38% lower than those obtained when AS was used, for WW, CGW, OP and JW, respectively. Similarly, the SMY of TAS assays were approximately 10, 21, 13 and 46% lower than those of AS assays, for WW, CGW, OP and JW, respectively.

It is possible that both LL and TAS did not contain the same microbial or/and nutrient load as AS, both in quantity and in quality. In the former case, although anaerobic processes take place inside a landfill, LL would probably not contain a high amount of nor anaerobic microorganisms or nutrients, since their majority would have been retained within the waste mass, in order for biodegradation to be carried out. In addition, the toxic and inhibiting substances that usually end up in the leachate, due to the heterogeneity of municipal solid waste deposited in the landfill, would have prevented the action of anaerobic bacteria. A longer digestion period would be eventually needed to overcome any of these issues and to reach the same levels of methane production that were achieved with the use of AS as inoculum. In the case of TAS, the thickening process applied in order to obtain this sample, may have removed part of the microorganisms and/or nutrients present in the supernatant phase, while retaining most of them within the settled matter.

3.4.3.3 Achievement of t_{80}

By observing the data referring to different inoculum types in Table 3-3, it can be noticed that AS and TAS assays show a similar behavior regarding both time (t_{80}) and methane produced, for three out of the four substrates, namely WW, CGW and OP. In the case of JW, the time required to achieve approximately 82% of the total CH_4 production with TAS is significantly higher than t_{80} with AS, while the respective attained gas volume is lower. Inoculation with LL, on the other hand, results in lower methane production achieved at longer time periods for WW, OP and JW, while for CGW t_{80} is much lower (4 days).

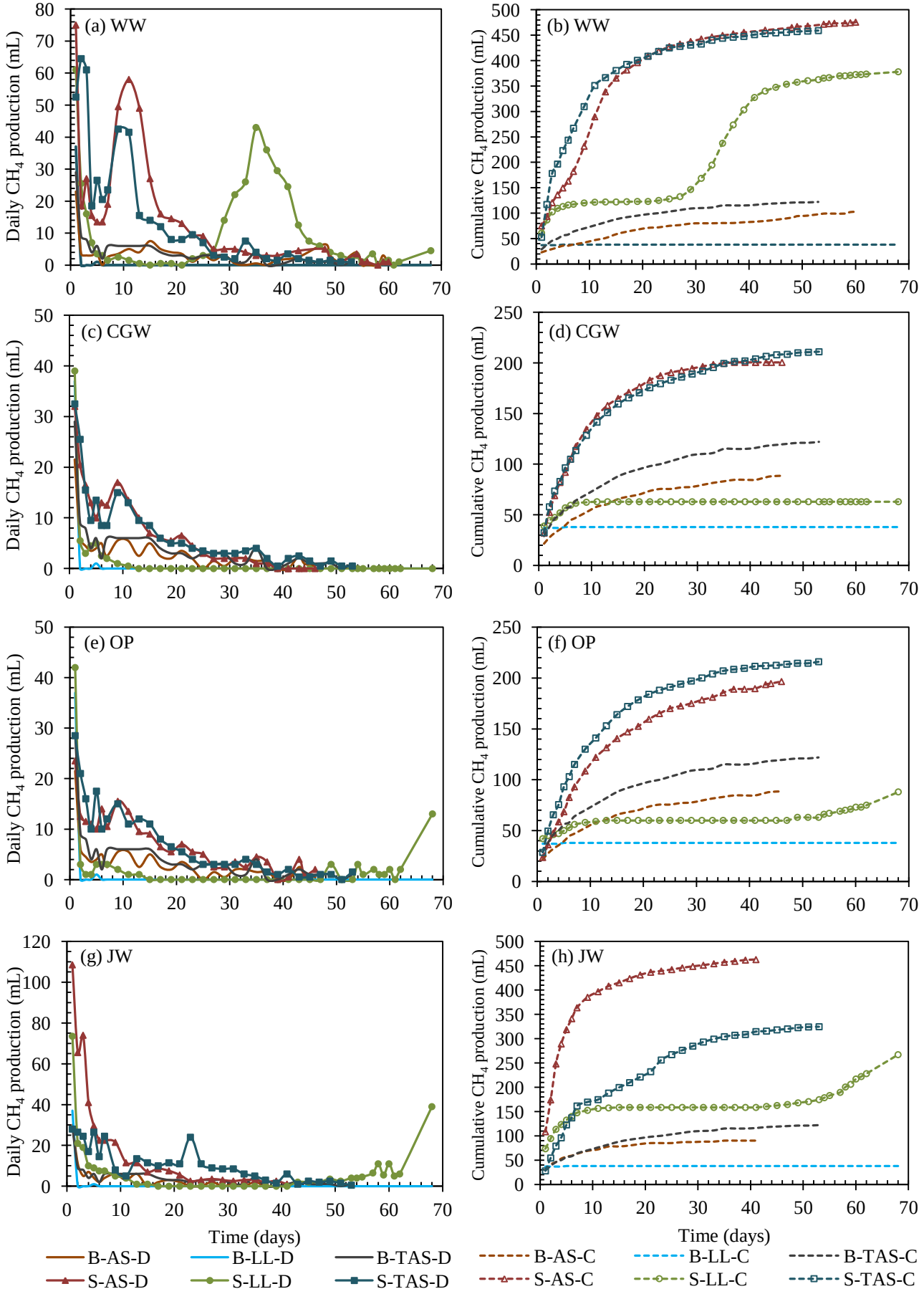


Figure 3-2: Daily and cumulative CH_4 production profiles as a function of time, for trials with different inocula [AS (–, --, ▲, Δ), LL (–, --, ●, ○), TAS (–, --, ■, □)], for WW (a, b), CGW (c, d), OP (e, f) and JW (g, h)

Table 3-3: Time period for achievement of methane production >80% of total amount (t_{80}), comparison between different SIR values and different inocula

Varying parameter	Substrates											
	WW			CGW			OP			JW		
	Time (day)	CH_4 produced (mL)	Percentage of total production (%)	Time (day)	CH_4 produced (mL)	Percentage of total production (%)	Time (day)	CH_4 produced (mL)	Percentage of total production (%)	Time (day)	CH_4 produced (mL)	Percentage of total production (%)
SIR												
0.25	9	212.5	82.5	15	165.0	82.3	21	159.5	81.2	6	185.0	81.9
0.5	17	381.5	80.2	31	243.5	81.3	23	227.0	80.9	9	385.0	83.2
1	43	186.5	80.2	29	394.0	81.4	25	345.5	83.0	33	473.0	80.3
2	7	48.0	85.7	31	516.0	80.1	25	425.5	82.1	17	166.0	81.2
Inoculum type												
AS	17	381.5	80.2	15	165.0	82.3	21	159.5	81.2	9	385.0	83.2
LL	39	303.0	80.2	4	52.0	82.5	59	71.0	80.7	60	217.0	81.3
TAS	15	380.5	82.9	19	170.5	80.8	19	178.5	82.6	25	267.0	82.3

Table 3-4: pH and Total Alkalinity values at the end of BMP assays

Varying parameter	Blanks		Substrates							
			WW		CGW		OP		JW	
	pH	Total Alkalinity (mg $CaCO_3$/L)	pH	Total Alkalinity (mg $CaCO_3$/L)	pH	Total Alkalinity (mg $CaCO_3$/L)	pH	Total Alkalinity (mg $CaCO_3$/L)	pH	Total Alkalinity (mg $CaCO_3$/L)
SIR	7.66	2950								
0.25			7.59	3750	7.51	3000	7.43	2900	7.40	3352
0.5			7.44	3550	7.48	3450	7.52	3200	7.34	3639
1			7.50	3750	7.35	3700	7.43	3200	7.36	3448
2			5.12	3700	7.37	4000	7.40	3350	5.11	2586
Inoculum type										
AS	7.66	2950	7.44	3550	7.51	3000	7.43	2900	7.34	3639
LL	8.32	12694	8.58	14966	8.99	8397	8.58	14818	8.15	13385
TAS	7.34	1867	7.25	2371	7.26	2068	7.35	1917	7.16	2169

3.4.4 Model fitting

3.4.4.1 Single-modeling approach

The experimental data obtained from the two series of experiments were fitted to five single kinetic models, which can be divided in two groups, a) the first comprises of two 2-parameter models, i.e. the first-order exponential and the two-phase exponential, and b) the second includes three 3-parameter models, namely the logistic, the transference (reaction-curve) and the modified Gompertz. In the following paragraphs, for each assay, the best-fitting models from each group will be provided, with those mentioned first being the overall best.

The modeling data for the first series of experiments are presented in Table 3-5. In the case of WW, the data for the assay with SIR=0.25 fit best the two-phase exponential and the transference models, while the data referring to SIR 0.5, 1 and 2 agree more with the logistic model and mostly the two-phase exponential model. For CGW, all samples are best fitted by the two-phase exponential and the transference models. The kinetics for OP for SIR above 0.5, are best described by the modified Gompertz model and both 2-parameter models, while for SIR=0.25 the two-phase exponential and the transference models are proved better. As far as JW is concerned, for SIR 0.25, 0.5 and 2, the two-phase exponential and the transference models fit best the data, while for SIR=1 the logistic and two-phase exponential models are better.

From a general overview of these results, it is observed that methane production for assays with a low SIR (0.25) is best fitted by 2-parameter models, while generally for higher SIR, i.e. 1 and 2, the experimental data are better described by 3-parameter models. Assays with SIR=0.5 have no particular affinity, since in most cases the differences between 2-parameter and 3-parameter models are very small. All the above are true for WW, OP and JW, while for CGW, primarily 2-parameter models seem to agree more with the data, especially on the basis of RSS values.

Table 3-6 provides the parameters obtained through the modeling process for the data of the second series of experiments. A general affinity towards the two-phase exponential model and the transference model can be noticed for the majority of the assays. There are only three exceptions showing a slightly different behavior, for WW, OP and JW, respectively, all of them containing LL as inoculum. More specifically, the data for the WW-LL assay are primarily fitted by the logistic model, with both 2-parameter models giving similar results, while for the OP-LL and JW-LL assays, although the most suitable model is the 2-parameter two-phase exponential, among 3-parameter models they differ, showing better results with the application of the logistic and modified Gompertz models, respectively. The affinity of the logistic model to anaerobic digestion assays using LL has also been reported elsewhere (Donoso–Bravo et al., 2010).

Table 3-5: Modeling parameters for single-modeling approach, trials with different SIR

Model	Parameters	WW				CGW				OP				JW			
		SIR = 0.25	SIR = 0.5	SIR = 1	SIR = 2	SIR = 0.25	SIR = 0.5	SIR = 1	SIR = 2	SIR = 0.25	SIR = 0.5	SIR = 1	SIR = 2	SIR = 0.25	SIR = 0.5	SIR = 1	SIR = 2
1st Order Exponential	B_{max}	247.2	477.3	10486	55.60	200.0	309.8	486.0	651.0	194.3	298.4	447.7	577.1	218.9	444.7	834.6	188.6
	k	0.2597	0.0857	0.0004	0.1717	0.1248	0.0529	0.0642	0.0559	0.0872	0.0546	0.0510	0.0450	0.3289	0.2463	0.0255	0.2424
	RSS	1341	6112	13382	484	307	401	4832	6581	304	4604	14583	30143	874	3136	83265	10816
	R^2	0.9798	0.9895	0.9475	0.9268	0.9957	0.9984	0.9927	0.9944	0.9961	0.9829	0.9770	0.9713	0.9780	0.9850	0.8317	0.6456
Two-phase Exponential	B_1	214.3	68.72	21928	17.38	185.4	196.9	398.6	475.4	107.3	247.2	328.9	419.3	189.9	373.2	101.6	99.88
	B_2	65.86	408.5	1032.2	38.21	17.30	148.7	1074	4663	131.2	51.20	118.8	157.8	42.57	120.3	60127	131.7
	k_1	0.3320	0.0857	0.0002	0.1717	0.1080	0.0730	0.0803	0.0789	0.0204	0.0546	0.0510	0.0450	0.4166	0.3228	112.6	1.981
	k_2	0.0237	0.0857	0.0002	0.1717	1.430	0.0208	0.0015	0.0006	0.1275	0.0546	0.0510	0.0450	0.0478	0.0338	0.0002	0.0451
	RSS	174	6112	13236	484	36	245	3869	3651	77	4604	14583	30143	271	221	25584	758
	R^2	0.9974	0.9895	0.9771	0.9268	0.9995	0.9990	0.9942	0.9969	0.9990	0.9829	0.9770	0.9713	0.9932	0.9989	0.9483	0.9752
Logistic	P	242.9	458.6	245.4	54.84	192.9	284.9	452.1	598.3	182.2	272.3	400.8	504.4	215.1	435.9	13293	182.3
	R_m	40.3	25.5	7.18	7.41	15.82	10.5	20.4	23.7	10.88	11.7	18.5	22.4	45.1	68.3	121	31.2
	λ	0.00	0.00	15.4	1.18	0.00	0.00	0.00	0.00	0.00	1.13	2.43	3.36	0.00	0.00	69.2	0.00
	RSS	4461	6039	1442	141	2879	6050	25697	44393	3010	1622	4176	3936	2769	13477	11347	16865
	R^2	0.9328	0.9896	0.9943	0.9787	0.9597	0.9765	0.9612	0.9620	0.9612	0.9940	0.9934	0.9962	0.9304	0.9355	0.9771	0.4474
Transference	P	247.2	477.3	635401	55.36	200.3	310.0	485.1	651.0	194.3	293.4	434.6	554.9	218.9	444.8	819.8	188.6
	R_m	64.19	40.9	4.47	11.3	24.73	16.4	31.5	36.4	16.95	17.7	26.2	30.2	72.0	110	21.5	45.7
	λ	0.00	0.00	4.68	0.78	0.00	0.00	0.09	0.00	0.00	0.87	1.44	1.69	0.00	0.00	0.00	0.00
	RSS	1341	6112	8591	403	235	353	4808	6581	304	3916	10195	21358	874	3136	83276	10816
	R^2	0.9798	0.9895	0.9663	0.9390	0.9967	0.9986	0.9927	0.9944	0.9961	0.9854	0.9839	0.9796	0.9780	0.9850	0.8317	0.6456
Modified Gompertz	P	243.8	462.4	285.4	55.01	194.1	288.7	458.3	606.9	184.0	277.7	408.9	515.5	216.0	437.6	107954	182.7
	R_m	42.61	27.1	6.23	7.21	16.77	11.2	21.5	25.1	11.61	11.7	18.1	21.8	47.4	72.4	244	33.7
	λ	0.00	0.00	12.6	0.81	0.00	0.00	0.00	0.00	0.00	0.48	1.62	2.33	0.00	0.00	146	0.00
	RSS	3406	6167	2717	222	2235	4022	16311	29086	2089	995	2296	3659	2064	10407	11784	15828
	R^2	0.9487	0.9894	0.9893	0.9664	0.9687	0.9844	0.9753	0.9751	0.9730	0.9963	0.9964	0.9965	0.9481	0.9502	0.9762	0.4814

Table 3-6: Modeling parameters for single-modeling approach, trials with different inocula

Model	Parameters	Blanks			WW		CGW		OP		JW	
		AS	LL	TAS	LL	TAS	LL	TAS	LL	TAS	LL	TAS
1st Order	B_{max}	97.22	37.92	115.9	835.8	447.8	62.68	202.8	10487	211.5	164.2	326.7
Exponential	k	0.0635	3.665	0.1093	0.0101	0.1337	0.6221	0.1135	0.0001	0.1050	4019	0.0720
	RSS	1387	3	1313	70431	2609	174	1695	27571	618	45084	5046
	R^2	0.9318	0.2425	0.9434	0.8675	0.9931	0.8506	0.9780	-8.812	0.9933	0.0000	0.9787
Two-phase	B_1	90.87	37.73	30.72	0.00	157.0	61.43	65.98	50.67	177.3	129.5	208.4
Exponential	B_2	20.73	431.1	94.64	835.8	312.6	425.1	150.3	783.6	548.4	5254.9	5243.6
	k_1	0.0337	3.894	1.365	0.0452	0.0512	0.6658	0.4151	1.463	0.1343	0.7047	0.1295
	k_2	3.313	0.0000	0.0595	0.0101	0.1894	0.0001	0.0618	0.0004	0.0015	0.0002	0.0005
	RSS	212	2	19	70431	890	163	76	664	194	9599	3100
	R^2	0.9896	0.3881	0.9992	0.8675	0.9976	0.8601	0.9990	0.7639	0.9979	0.7871	0.9869
Logistic	P	92.26	315.9	112.0	476.5	434.9	472.9	195.3	1556.2	203.3	173.3	310.2
	R_m	3.72	0.02	7.77	6.89	38.2	0.317	14.7	2.39	14.0	33.4	14.0
	λ	0.00	0.00	0.00	0.00	0.00	0.00	0.00	230	0.00	0.00	0.00
	RSS	2261	3	3079	35309	17527	820	6323	730	4551	25925	16726
	R^2	0.8887	0.2466	0.8672	0.9336	0.9534	0.2952	0.9181	0.7401	0.9505	0.4250	0.9295
Transference	P	97.27	37.92	115.9	820.2	447.8	62.68	202.8	63.10	211.5	175.0	326.7
	R_m	6.17	139	12.7	8.52	59.9	39.1	23.0	32.4	22.2	54.9	23.5
	λ	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	RSS	1387	3	1313	70436	2609	174	1695	1857	618	23001	5046
	R^2	0.9318	0.2425	0.9434	0.8675	0.9931	0.8506	0.9780	0.3391	0.9933	0.4898	0.9787
Modified	P	92.77	570.7	112.3	1061	437.1	853.4	196.3	3298	204.5	22767	310.3
Gompertz	R_m	4.04	0.01	8.49	6.52	40.4	0.280	15.7	1.78	15.0	14.7	15.5
	λ	0.00	0.00	0.00	0.00	0.00	0.00	0.00	295	0.00	373	0.00
	RSS	2357	3	2883	41814	13450	818	5389	735	3564	12751	14812
	R^2	0.8840	0.2468	0.8757	0.9214	0.9642	0.2977	0.9302	0.7383	0.9612	0.7172	0.9376

3.4.4.2 Multiple-stage-modeling approach

The data presented in Tables 3-5 and 3-6 show that the single-model approach managed to describe quite well the kinetics of the majority of BMP assays. Nevertheless, in certain cases model fitting was either poor (R-square < 0.90) or not accurate enough (0.90 < R-square < 0.99). Interestingly, it is also observed that in the cases where the transference model prevails over the other 3-parameter models, its RSS and R-square values are equal to the respective values obtained for the first-order exponential model. Moreover, it is noticed that the experimental data have a recurrent affinity for the two-phase exponential model. The combination of both these facts suggests that in those cases the kinetics may be fitted by more than one model at the same time, thus the digestion process would probably comprise of multiple stages. In an attempt to verify this theory, a modeling approach considering the occurrence of multiple stages was applied. In the single-modeling approach each kinetic model was applied to the whole digestion period. On the other hand, in the multiple-stage approach the digestion period was separated in different time intervals and the kinetic models were applied to each interval separately. The two kinetic models selected for the latter approach were the first-order exponential and the logistic (sigmoidal) models. This selection was based on the supposition that usually at the initial stage of digestion the more readily degradable materials are consumed, thus causing the methane production curve to follow a first-order pattern. As the digestion process progresses, more recalcitrant materials are made available, leading to delayed methane production, which is often manifested by a sigmoidal shape of the data curve. The association of the first-order model to easily degradable materials and of sigmoidal-type models, such as the logistic, to more recalcitrant materials has also been made in previous studies (Rincón et al., 2013; Vavilin et al., 2008).

Fig. 3-3 and Fig. 3-4 provide the results of the multiple-stage-modeling for the data of the first and second series of experiments, respectively (detailed results are provided in Tables A-1 and A-2, of Appendix A). This modeling procedure yielded a different number of stages for each sample, depending on the form of the experimental data as a function of time, ranging from at least two, to up to seven stages. Parameters determining the goodness of fit for each assay generally show a better description of the experimental data by the multiple-stage-modeling approach. In fact, RSS and R-square values are respectively lower and higher, compared to those obtained for the single-modeling approach, with emphasis towards the RSS values. There may be only two possible outliers, namely CGW-SIR=0.25 and OP-SIR=0.25, for which the combined (sum) RSS of the multiple-stage-modeling approach is found higher, i.e. 149 and 87 (Table A-1), rather than 36 and 77 (in the single-modeling approach) (Table 3-5). This prevalence of the multiple-stage-modeling is particularly pronounced in all cases where LL was the inoculum of choice. The level of agreement of this fitting approach can also be more clearly verified from the graphical representation of the data (Fig. 3-3, 3-4 and A-1).

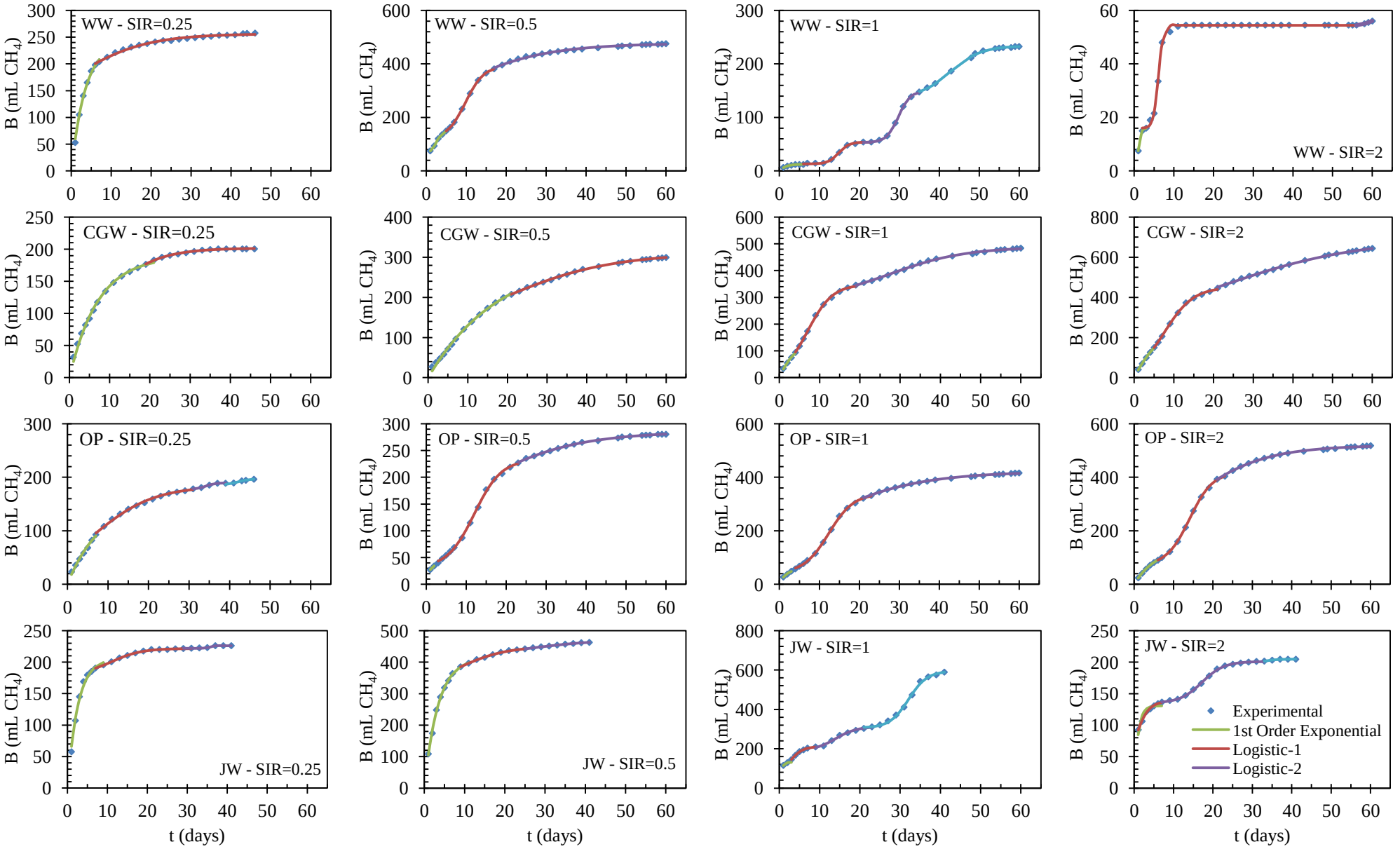


Figure 3-3: Fitting for combination of 1st Order Exponential and Logistic models (cumulative methane production [*B*] as a function of time [*t*]), trials with different SIR

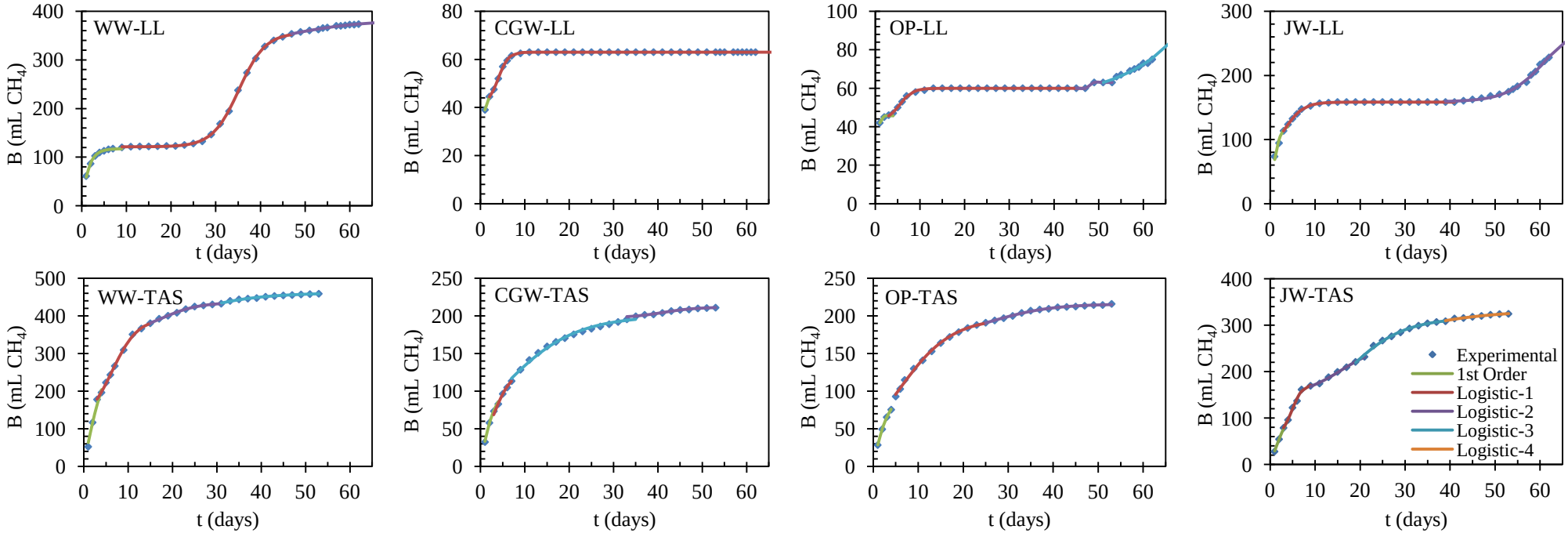


Figure 3-4: Fitting for combination of 1st Order Exponential and Logistic models (cumulative methane production [*B*] as a function of time [*t*]), trials with different inocula

3.5 Conclusions

This study investigated the effect of different substrate to inoculum ratios (SIR) and inoculum types on the biochemical methane potential of four solid agroindustrial waste, namely winery waste (WW), cotton gin waste (CGW), olive pomace (OP) and juice industry waste (JW). Experimental data confirmed the viability of the tested substrates for anaerobic digestion, as well as the suitability of the tested experimental conditions, for serving the purpose of the study. It was demonstrated that lower SIR (0.25 and 0.5) are more adequate for determining the methane potential of the examined agroindustrial waste. Specifically, a SIR equal to 0.5 resulted in methane yields of 446.23 $mLCH_4$, $_{STP}/gVS_{substrate}$ for WW and of 445.97 $mLCH_4$, $_{STP}/gVS_{substrate}$ for JW, while a SIR equal to 0.25 yielded 267.96 $mLCH_4$, $_{STP}/gVS_{substrate}$ for CGW and 258.65 $mLCH_4$, $_{STP}/gVS_{substrate}$ for OP. On the other hand, results obtained for higher SIR indicated that representative measurements are not possible if using higher substrate amounts, since anaerobic digestion inhibition is manifested, due to system overloading phenomena. Methane potentials obtained experimentally were lower than theoretical methane potentials, with this being particularly true for CGW and OP. This suggests that eventual pretreatment before anaerobic digestion should be the subject of future investigations, in order to not only attempt to enhance substrate digestibility, but also verify the effect of different methods on the composition and characteristics of each substrate. Assays with different inoculum types showed the prevalence of anaerobic sludge (AS) over landfill leachate (LL) and thickened anaerobic sludge (TAS) for such purpose. Lower methane production in the cases where the two latter materials were used as inocula, suggest that they eventually were inferior to AS regarding microbial and nutrient load possibly in both quality and quantity. Considering the aforementioned facts, as well as the high availability of AS, the use of such a material as inoculum for anaerobic digestion represents a good option for large-scale applications when dealing with similar substrates. Nevertheless, a material such as TAS, or with similar characteristics, may be a satisfactory alternative to AS. On the other hand, a more in depth investigation, eventually for a more extended time period, should be considered in the case of LL. Kinetic modeling results suggest that the SIR plays an important role in determining an eventual affinity of the data to specific kinetic models. In fact, at low SIR, substrate degradation develops more easily due to lower organic matter loading, hence the association with 2-parameter models, which usually describe more simple processes. However, at higher SIR, degradation processes become more complex due to higher organic load, thus resulting in the association with 3-parameter models. Moreover, the results obtained from the multiple-stage-modeling approach suggest that when dealing with substrates such as those investigated in the present study, the evaluation of the anaerobic digestion kinetics should be carefully conducted, by taking into consideration the complexity of the processes taking place.

3.6 References

Adl, M., Sheng, K., Gharibi, A. (2012). Technical assessment of bioenergy recovery from cotton stalks through anaerobic digestion process and the effects of inexpensive pre-treatments. *Applied Energy*, *93*, 251–260.

Alkan-Ozkaynak, A., Karthikeyan, K.G. (2011). Anaerobic digestion of thin stillage for energy recovery and water reuse in corn-ethanol plants. *Bioresource Technology*, *102*(21), 9891–9896.

Altaş, L. (2009). Inhibitory effect of heavy metals on methane-producing anaerobic granular sludge. *Journal of Hazardous Materials*, *162*(2–3), 1551–1556.

Alzate, M.E., Muñoz, R., Rogalla, F., Fdz-Polanco, F., Pérez-Elvira, S.I. (2012). Biochemical methane potential of microalgae: Influence of substrate to inoculum ratio, biomass concentration and pretreatment. *Bioresource Technology*, *123*, 488–494.

Barrantes Leiva, M., Koupaie, E.H., Eskicioglu, C. (2014). Anaerobic co-digestion of wine/fruit-juice production waste with landfill leachate diluted municipal sludge cake under semi-continuous flow operation. *Waste Management*, *34*(10), 1860–1870.

Carlini, M., Castellucci, S., Moneti, M. (2015). Anaerobic Co-digestion of Olive-mill Solid Waste with Cattle Manure and Cattle Slurry: Analysis of Bio-methane Potential. *Energy Procedia*, *81*, 354–367.

Christensen, T. H., Kjeldsen, P., Bjerg, P. L., Jensen, D. L., Christensen, J. B., Baun, A., Albrechtsen, H.-J., Heron, G. (2001). Biogeochemistry of land leachate plumes. *Applied Geochemistry*, *16*, 659–718.

Córdoba, V., Fernández, M., Santalla, E. (2016). The effect of different inoculums on anaerobic digestion of swine wastewater. *Journal of Environmental Chemical Engineering*, *4*(1), 115–122.

Dinuccio, E., Balsari, P., Gioelli, F., Menardo, S. (2010). Evaluation of the biogas productivity potential of some Italian agro-industrial biomasses. *Bioresource Technology*, *101*(10), 3780–3783.

Donoso-Bravo, A., Pérez-Elvira, S.I., Fdz-Polanco, F. (2010). Application of simplified models for anaerobic biodegradability tests. Evaluation of pre-treatment processes. *Chemical Engineering Journal*, *160*(2), 607–614.

El Achkar, J.H., Lendormi, T., Hobaika, Z., Salameh, D., Louka, N., Maroun, R.G., Lanoisellé, J.-L. (2016). Anaerobic digestion of grape pomace: Biochemical characterization of the fractions and methane production in batch and continuous digesters. *Waste Management*, *50*, 275–282.

Eskicioglu, C., Ghorbani, M. (2011). Effect of inoculum/substrate ratio on mesophilic anaerobic digestion of bioethanol plant whole stillage in batch mode. *Process Biochemistry*, *46*(8), 1682–1687.

Esposito, G., Frunzo, L., Giordano, A., Liotta, F., Panico, A., Pirozzi, F. (2012). Anaerobic co-digestion of organic wastes. *Reviews in Environmental Science and Bio/Technology*, *11*(4), 325–341.

Fabbri, A., Bonifazi, G., Serranti, S. (2015). Micro-scale energy valorization of grape marcs in winery production plants. *Waste Management*, *36*, 156–165.

FAOSTAT (http://faostat3.fao.org) (accessed on 2016)

Feng, L., Li, Y., Chen, C., Liu, X., Xiao, X., Ma, X., Zhang, R., He, Y., Liu, G. (2013). Biochemical methane potential (BMP) of vinegar residue and the influence of feed to inoculum ratios on biogas production. *BioResources*, *8*(2), 2487–2498.

Fernández-Cegrí, V., De la Rubia, M.A., Raposo, F., Borja, R. (2012). Effect of hydrothermal pretreatment of sunflower oil cake on biomethane potential focusing on fibre composition. *Bioresource Technology*, *123*, 424–429.

Forgács, G., Pourbafrani, M., Niklasson, C., Taherzadeh, M.J., Hováth, I.S. (2012). Methane production from citrus wastes: process development and cost estimation. *Journal of Chemical Technology & Biotechnology*, *87*(2), 250–255.

Fountoulakis, M.S., Drakopoulou, S., Terzakis, S., Georgaki, E., Manios, T. (2008). Potential for methane production from typical Mediterranean agro-industrial by-products. *Biomass and Bioenergy*, *32*(2), 155–161.

González-Fernández, C., García-Encina, P.A. (2009). Impact of substrate to inoculum ratio in anaerobic digestion of swine slurry. *Biomass and Bioenergy*, *33*(8), 1065–1069.

Gu, Y., Chen, X., Liu, Z., Zhou, X., Zhang, Y. (2014). Effect of inoculum sources on the anaerobic digestion of rice straw. *Bioresource Technology*, *158*, 149–155.

Gunaseelan, V.N. (2004). Biochemical methane potential of fruits and vegetable solid waste feedstocks. *Biomass and Bioenergy*, *26*(4), 389–399.

Isci, A., Demirer, G.N. (2007). Biogas production potential from cotton wastes. *Renewable Energy*, *32*(5), 750–757.

Kaparaju, P.L.N., Rintala, J.A. (2006). Thermophilic Anaerobic Digestion of Industrial Orange Waste. *Environmental Technology*, *27*(6), 623–633.

Karantonis, H.C., Tsantila, N., Stamatakis, G., Samiotaki, M., Panayotou, G., Antonopoulou, S., Demopoulos, C.A. (2008). Bioactive polar lipids in olive oil, pomace and waste byproducts. *Journal of Food Biochemistry*, *32*, 443–459.

Kawai, M., Nagao, N., Tajima, N., Niwa, C., Matsuyama, T., Toda, T. (2014). The effect of the labile organic fraction in food waste and the substrate/inoculum ratio on anaerobic digestion for a reliable methane yield. *Bioresource Technology*, *157*, 174–180.

Kim, S.H., Kim, C.-H., Jin, G.C., Yoon, Y.M. (2012) Effects of substrate to inoculum ratio on biochemical methane potential assay of livestock waste biomass. Proceedings Venice 2012, *Fourth International Symposium on Energy from Biomass and Waste*, San Servolo, Venice, Italy; 12-15 November 2012, CISA publisher, Italy.

Li, Y., Park, S.Y., Zhu, J. (2011). Solid-state anaerobic digestion for methane production from organic waste. *Renewable and Sustainable Energy Reviews*, *15*(1), 821–826.

Liao, X., Zhu, S., Zhong, D., Zhu, J., Liao, L. (2014). Anaerobic co-digestion of food waste and landfill leachate in single-phase batch reactors. *Waste Management*, *34*(11), 2278–2284.

Lim, S. J., Fox, P. (2013). Biochemical methane potential (BMP) test for thickened sludge using anaerobic granular sludge at different inoculum/substrate ratios. *Biotechnology and Bioprocess Engineering*, *18*(2), 306–312.

Luna-delRisco, M., Normak, A., Orupõld, K. (2011). Biochemical methane potential of different organic wastes and energy crops from Estonia. *Agronomy Research*, *9*(1–2), 331–342.

Martín, M.A., Siles, J.A., Chica, A.F., Martín, A. (2010). Biomethanization of orange peel waste. *Bioresource Technology*, *101*(23), 8993–8999.

Nain, M. Z., Jawed, M. (2006). Performance of anaerobic reactors at low organic load subjected to sudden change in feed substrate types. *Journal of Chemical Technology & Biotechnology*, *81*, 958–965.

Nigam,P.S.N. Gupta, N., Anthwal, A. (2009). Pre-treatment of agro-industrial residues, P.S.N. Nigam, A. Pandey (Eds.), Biotechnology for agro-industrial residues utilisation, Springer Science and Business Media BV, Dordrecht, 13–32.

Nogales, R., Cifuentes, C., Benitez, E. (2005). Vermicomposting of Winery Waste: A Laboratory Study. *Journal of Environmental Science and Health , Part B : Pesticides , Food Contaminants , and Agricultural Wastes*, *40*(4), 659–673.

Nzila, C., Dewulf, J., Spanjers, H., Kiriamiti, H., van Langenhove, H. (2010). Biowaste energy potential in Kenya. *Renewable Energy*, *35*(12), 2698–2704.

Öman, C.B., Junestedt, C. (2008). Chemical characterization of landfill leachates - 400 parameters and compounds. *Waste Management*, *28*(10), 1876–1891.

Pala, M., Kantarli, I.C., Buyukisik, H.B., Yanik, J. (2014). Hydrothermal carbonization and torrefaction of grape pomace: A comparative evaluation. *Bioresource Technology*, *161*, 255–262.

Pozdniakova, T. A., Costa, J.C., Santos, R. J., Alves, M.M., Boaventura, R.A.R. (2012). Anaerobic biodegradability of Category 2 animal by-products: Methane potential and inoculum source. *Bioresource Technology*, *124*, 276–282.

Raposo, F., Borja, R., Martín, M.A., Martín, A., de la Rubia, M.A., Rincón, B. (2009). Influence of inoculum-substrate ratio on the anaerobic digestion of sunflower oil cake in batch mode: Process stability and kinetic evaluation. *Chemical Engineering Journal*, *149*(1–3), 70–77.

Raposo, F., De la Rubia, M.A., Fernández-Cegrí, V., Borja, R. (2011a). Anaerobic digestion of solid organic substrates in batch mode: An overview relating to methane yields and experimental procedures. *Renewable and Sustainable Energy Reviews*, *16*(1), 861–877.

Raposo, F., Fernández-Cegrí, V., De la Rubia, M.A., Borja, R., Béline, F., Cavinato, C., Demirer, G., Fernández, B., Fernández-Polanco, M., Frigon, J.C., Ganesh, R., Kaparaju, P., Koubova, J., Méndez, R., Menin, G., Peene, A., Scherer, P., Torrijos, M.. Uellendahl, H.. Wierinck, I., de Wilde, V. (2011b). Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an

international interlaboratory study. *Journal of Chemical Technology & Biotechnology, 86*, 1088-1098.

Renou, S., Givaudan, J. G., Poulain, S., Dirassouyan, F., Moulin, P. (2008). Landfill leachate treatment: Review and opportunity. *Journal of Hazardous Materials*, *150*(3), 468–493.

Rincón, B., Bujalance, L., Fermoso, F.G., Martín, A., Borja, R. (2013). Biochemical methane potential of two-phase olive mill solid waste: Influence of thermal pretreatment on the process kinetics. *Bioresource Technology*, *140*, 249–255.

Ruggeri, B., Battista, F., Bernardi, M., Fino, D., Mancini, G. (2015). The selection of pretreatment options for anaerobic digestion (AD): A case study in olive oil waste production. *Chemical Engineering Journal*, *259*, 630–639.

Sawatdeenarunat, C., Surendra, K.C., Takara, D., Oechsner, H., Khanal, S.K. (2015). Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. *Bioresource Technology*, *178*, 178–186.

Subagyono, D.J.N., Marshall, M., Jackson, W.R., Chaffee, A.L. (2015). Pressurized thermal and hydrothermal decomposition of algae, wood chip residue, and grape marc: A comparative study. *Biomass and Bioenergy*, *76*, 141–157.

Vavilin, V.A., Angelidaki, I. (2005). Anaerobic degradation of solid material: importance of initiation centers for methanogenesis, mixing intensity, and 2D distributed model. *Biotechnology and Bioengineering*, *89*(1), 113–122.

Vavilin, V.A., Fernandez, B., Palatsi, J., Flotats, X. (2008). Hydrolysis kinetics in anaerobic degradation of particulate organic material: an overview. *Waste Management*, *28*(6), 939–951.

Wang, X., Yang, G., Feng, Y., Ren, G., Han, X. (2012). Optimizing feeding composition and carbon-nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. *Bioresource Technology*, *120*, 78–83.

Wilkins, M.R., Suryawati, L., Maness, N.O., Chrz, D. (2007). Ethanol production by Saccharomyces cerevisiae and Kluyveromyces marxianus in the presence of orange-peel oil. *World Journal of Microbiology and Biotechnology*, *23*(8), 1161–1168.

Zhou, Y., Zhang, Z., Nakamoto, T., Li, Y., Yang, Y., Utsumi, M., Sugiura, N. (2011). Influence of substrate-to-inoculum ratio on the batch anaerobic digestion of bean curd refuse-okara under mesophilic conditions. *Biomass and Bioenergy*, *35*(7), 3251–3256.

CHAPTER 4

Microwave pretreatment of solid agroindustrial waste

This chapter focuses on the use of microwave heating as a pretreatment for lignocellulosic agroindustrial waste prior to anaerobic digestion for methane production. Initially, an optimization procedure was carried out, in order to determine the most suitable conditions for microwave pretreatment of the investigated agroindustrial waste. To this purpose, the variation in operational parameters, such as heating rate, holding time, solid to liquid ratio and temperature was examined. Ultimately, the scope of this chapter was to evaluate the effect of microwave pretreatment on the solubilization and degradability of the four substrates. Results showed that pretreatment temperature is the most important operational parameter for microwave pretreatment, while different effects of this process on the solubilization and methane potential of the studied substrates were presented. The variations were attributed to the specific characteristics of each substrate combined with the pretreatment conditions. In the cases of winery waste and juice industry waste, microwave pretreatment resulted in relatively high solubilization levels for both materials. Nevertheless, the portion retained on the solid fraction after pretreatment was larger and less biodegradable for winery waste, while for juice industry waste it was smaller but with a higher biodegradability degree. As far as cotton gin waste and olive pomace are concerned, microwave pretreatment seems not to cause high organic matter solubilization, while it most likely induced structural changes in the materials matrices. This resulted in methane production levels that indicate the presence of recalcitrant or/and inhibitory compounds on the pretreated samples. Moreover, the increased moisture and hydrogen contents for pretreated samples suggested that additional changes were made to the substrates. Ultimately, although microwave pretreatment did not improve methane production, results indicated that at temperatures between 125 and 150 °C, such a process could eventually provide samples which are more suitable for methane production. Moreover, an improved energy balance could be obtained by combining these temperatures with lower exposure times and higher solid to liquid ratios.

4.1 Introduction

Anaerobic digestion (AD) is a process which involves the action of microbes aiming at the degradation of complex organic substrates and their ultimate conversion into biogas and digestate. It occurs in the absence of oxygen and comprises of four steps, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Due to its low impact on the environment, AD has, over the past decades, become a well-established waste stabilization technology, while it has also been considered as an efficient energy production method (Ariunbaatar et al., 2014, Jackowiak et al., 2011a). In fact, biogas, which is mainly composed of CH_4 and CO_2, is a viable energy source with several possible applications, among which are combined heat and power plants (Marin et al., 2010; Pecorini et al., 2016).

Research regarding the use of lignocellulosic materials as substrates for anaerobic digestion has been increasing in the last years with agricultural and agroindustrial waste representing a viable and low cost option, due to their high production rates and availability (Pellera and Gidarakos, 2016). In fact, a wide variety of such materials has been studied lately, including sunflower oil cake (Fernández-Cegrí et al., 2012), rice straw, pennisetum (Huang et al., 2012), olive mill solid waste (Rincón et al., 2013), wheat straw (Jackowiak et al., 2011a; Sapci et al., 2013), oat straw, barley straw (Sapci, 2013) and switchgrass (Jackowiak et al., 2011b). Among the most profitable agroindustrial sectors in Mediterranean countries are the wine production and olive oil production industries, as well as the cotton and citrus fruits processing activities. All these sectors are associated with significant amounts of waste materials being produced annually (Pellera and Gidarakos, 2016). In fact, it has been reported that wine production solid residues account for more than 20% of the quantity of grapes being processed (Marculescu and Ciuta, 2013; Oliveira and Duarte, 2016), while olive mill solid waste in three-phase systems represent approximately 30% of the initial olive quantity (La Cara et al., 2012; Rincón et al., 2012). Furthermore, it is estimated that around 50-60% of the total quantity of oranges processed for juice or marmalade production is discarded as waste (Negro et al., 2016; Ruiz and Flotats, 2014; Wilkins et al., 2007). Lastly, according to Placido and Capareda (2013) the amount of cotton gin waste being produced per ton of cotton is estimated around 0.31-0.42 tons. Residues such as those mentioned above may potentially be managed using anaerobic digestion. Nevertheless, these materials, being mainly composed of cellulose, hemicellulose and lignin, are characterized by a complex structure and recalcitrance to biodegradation, the degree of which can vary depending on the substrate. Indeed, the lignin content of a substrate is often determining, since this particular component acts as a barrier, preventing degradation and limiting the effectiveness of biological processes, such as AD. For this reason, pretreatments are often applied to lignocellulosic materials, as a means of disrupting their structure and eventually enhancing their digestibility (Fernández-Cegrí et al., 2012; Jackowiak et al., 2011a). Over the years, a wide variety of processes has been studied, regarding their possible application as pretreatments, aiming at more efficient methane production. These processes can mainly be categorized as physical (e.g. milling, extrusion, grinding, freezing, radiation), chemical (e.g. acid, alkaline, oxidative, organosolv, ionic liquid), physico-chemical (e.g. steam explosion, ammonia fiber explosion, liquid hot water, wet oxidation, CO_2 explosion) and biological, although combinations have also been tested (Ariunbaatar et al., 2014; Haghighi Mood et al., 2013; Hendriks and Zeeman, 2009). In order to be considered effective and viable, a pretreatment should not only be able to cause the disruption of a substrate matrix, as well as additional structural changes (e.g. increasing the porosity and surface area), but also have a moderate energy demand and limited cost (Haghighi Mood et al., 2013; Li et al., 2016).

Microwave (MW) radiation has gained considerable attention as a promising pretreatment method for improving the degradability of biomass materials. It consists of electromagnetic irradiation with a frequency between 300 MHz and 300 GHz and a corresponding wavelength ranging from 0.001 to 1 m (Huang et al., 2012; Marin et al., 2010). MW pretreatment includes a two-fold action on materials, i.e. an athermal and a thermal one (De Souza, 2015; Houtmeyers et al., 2014). During this process the transfer of heat is performed through the development of two mechanisms, namely the vibration of dipolar molecules and the migration of ions (Huang et al., 2012; Singh et al., 2016). The major difference between conventional and MW heating is the fact that while the former occurs via superficial heat transfer, the latter involves the direct interaction of the material's molecules with the electromagnetic field. This, results in a more rapid, uniform and selective heating, achieved within a reduced process time and with lower energy requirements, compared with conventional heating (Haghighi Mood et al., 2013; Jackowiak et al., 2011b; Li et al., 2016; Sapci, 2013). MW pretreatment has been proved highly effective in breaking organic molecules and disrupting complex structures, leading to the release of extracellular and intracellular material, which in turn increases the accessibility and bioavailability of a substrate (Pecorini et al., 2016; Sapci, 2013). Nevertheless, the actual effect that MW irradiation exerts on different types of substrates is still not fully clear (Marin et al., 2010) and it varies depending not only on the specific substrate being treated, but also on the temperature, the power and the duration of the process applied (Pecorini et al., 2016; Sapci, 2013). Therefore, further research concerning the application of MW pretreatment to different types of substrates is of great importance, in order to better understand the mechanisms behind this technology and the manner in which its specific effects can vary among different materials.

The purpose of this study is to determine the effect of microwave pretreatment on four lignocellulosic materials originating from some of the most widespread agroindustrial activities in the Mediterranean region, namely winery waste (WW), cotton gin waste (CGW), olive pomace (OP) and juice industry waste (JW). More specifically, this paper focuses on the impact of microwave pretreatment on their solubilization and degradability. Currently, there is lack of research concerning the application of such a treatment on these substrates, therefore the present study makes a contribution to this regard. Microwave heating was performed using a laboratory scale microwave reaction system, with the experiments being carried out at five different temperatures, namely 75, 125, 150, 175 and 200 °C, and by examining varying solid to liquid ratios, heating rates and holding times. The liquid fractions obtained after pretreatment were analyzed for soluble chemical oxygen demand (sCOD) and total phenols (TPH) concentrations, in order to assess the effect of pretreatment on the substrates solubilization. The effect on degradability was evaluated by determining the methane potential of the solid fractions through Biochemical Methane Potential (BMP) assays.

4.2 Materials and methods

4.2.1 Substrates and inoculum

Four agroindustrial waste typical of the Mediterranean area were used in the present study. More specifically, winery waste (WW), composed of grape skins, seeds and stalks, cotton gin waste (CGW) comprising of cotton fiber, stalks, burs and leaves, olive pomace (OP), which is

the solid waste obtained in three-phase olive mills and juice industry waste (JW) comprised of orange peels. All samples were taken from the respective agroindustrial facilities in which they are produced, i.e. a winery, a ginnery, a three-phase olive mill and a juice industry. Sample handling was not the same for the four substrates, due to their different characteristics. For WW and JW, the materials were initially separated in batches, placed in zip-lock bags and stored at -20 °C. One day before each use, appropriate amounts were transferred to 4 °C and on the day of the experiment they were comminuted without drying using a food processor. On the other hand, CGW was immediately dried at 60 °C and then comminuted to a particle size less than 500 μm, using a universal cutting mill, while OP was immediately stored at -20 °C without size reduction. The full characterization of the four substrates has been performed in a previous study (Pellera and Gidarakos, 2016) (Chapter 3).

The inoculum used in this study consisted of mesophilic anaerobic sludge with total solids (TS) and volatile solids (VS) contents of 2.56% and 1.71%, respectively (VS/TS=0.67), and it was obtained from an anaerobic digester situated in the Municipal Wastewater Treatment Facility of Chania, Crete.

4.2.2 Microwave pretreatment

Microwave (MW) pretreatment of the substrates was performed using a Mars 6 (CEM) microwave reaction system. Initially, an optimization procedure was performed, using two of the four investigated substrates, i.e. WW and CGW (Pellera and Gidarakos, 2014). Through this procedure, the variation of four parameters was investigated, namely a) solid to liquid ratio (S/L) (50, 75 and 100 g/L), b) heating rate (HR) (2.5, 5 and 10 °C/min), c) holding time (HT) (5, 10, 15 and 30 min) and d) temperature (T) (75, 125, 150, 175 and 200 °C). These experimental conditions are given in summary in Table 4-1. The selection of optimum conditions was made aiming towards a procedure with less energy and time consumption. The main experiments were performed under the selected optimum conditions, in terms of S/L, HR and HT, and at five different temperatures, namely 75, 125, 150, 175 and 200 °C. It is noted that the units of S/L refer to the form in which each material was stored, i.e. raw for WW, JW and OP and dried at 60 °C for CGW.

MW pretreatment was performed by initially inserting appropriate substrate amounts in Teflon vessels (100 mL capacity), to which 50 mL of deionized water were then added. After sealing the vessels, the latter were placed inside the microwave reaction system, for the heating to take place. At the end of MW pretreatment, the slurries were transferred in centrifuge tubes, and their final pH was measured. They were subsequently centrifuged at 3,900 rpm for 15 min and the solid and liquid fractions were collected separately. The liquid fractions were filtered through a 0.45 μm pore size membrane filter, in order to determine sCOD (soluble Chemical Oxygen Demand) and TPH (Total Phenols) concentrations, while the solid fractions were first subjected to vacuum filtration in order to remove excess water and then stored at -20 °C until further use. Additionally, a portion of each solid material was dried at 60 °C for further analyses, which included the determination of TS and VS contents, as well as elemental (CHNS) composition. All experiments were performed in triplicate.

Table 4-1: MW pretreatment experimental conditions

Experiments	S/L (g/L)	HR (°C/min)	HT (min)	T (°C)
Effect of S/L	50, 75, 100	5	15	150
Effect of HR	50	2.5, 5, 10	15	150
Effect of HT	50	10	5, 10, 15, 30	75, 150, 200
Effect of T	50	10	5	75, 125, 150, 175, 200

4.2.3 Biochemical methane potential assays

The experimental apparatus for BMP (biochemical methane potential) assays consisted of 250 mL conical flasks covered with rubber stoppers. Three PVC (Polyvinyl chloride) tubes were inserted in the stoppers, which allowed N_2 flushing in the flasks, daily methane measurement and weekly sampling for pH measurement and TPH concentrations determination.

The working volume for the BMP assays was set to 100 mL, the inoculum quantity was the same for all assays, i.e. 15 gVS/L, while the substrate to inoculum ratio (SIR) on a VS basis ($gVS_{substrate}/gVS_{inoculum}$) was 0.5 for WW and JW and 0.25 for CGW and OP. These values were chosen on the basis of the results of a previous study (Pellera and Gidarakos, 2016) (Chapter 3). Blank assays (SIR=0), containing only the inoculum were also performed, in order to determine the residual methane potential of the inoculum and to then be able to calculate the net methane potential of each substrate. It is noted that preliminary BMP assays were also performed for the pretreated substrates obtained after the optimization procedure, using the same inoculum quantities and SIR, but with a working volume of 50 mL. The results of these assays can be seen in Appendix B (Fig. B-2).

BMP assays were carried out by firstly introducing the inoculum and substrates in the flasks, in appropriate amounts and by subsequently adding deionized water to the mixture, if needed, in order to bring the total volume to approximately 100 mL. After adjusting the pH of the mixture at 7.8±0.05, by adding small amounts of NaOH (1 M), the flasks were covered with the rubber stoppers and finally flushed with N_2 for 2 min. The reactors were finally placed in an incubator set at 35 °C. Methane production was measured daily for the first seven days of incubation and subsequently every two days. BMP assays were terminated when methane production was undetectable or less than 5% of the total amount. All the assays were performed in duplicate.

4.2.4 Analytical methods

TS and VS contents were determined according to APHA (American Public Health Association) method 2540G. Elemental analysis (C, H, N, S) of raw (untreated) and pretreated samples was performed using an EA300 Euro Vector elemental analyzer, via flash combustion at 1,020 °C. The oxygen content was determined by difference, considering the VS content of each sample. pH was determined using a portable pH-meter. The sCOD concentrations in the liquid fractions obtained after pretreatment were determined through APHA method 5220C, while TPH concentrations were determined according to Folin-Ciocalteu's method, on the basis of the procedure described by Singleton et al. (1999).

Briefly, 40 μL of sample were placed in glass cuvettes, to which 3.16 mL of deionized water and 200 μL of Folin-Ciocalteu reagent were then added. After mixing using a vortex mixer, the cuvettes were left for 4 min and subsequently 600 μL of sodium carbonate solution (20%) were added to the mixture. Finally, the solutions were once again mixed and left for 2 h at 20 °C. The absorbance of each solution was determined at 765 nm. The final TPH concentrations are expressed in gallic acid equivalents (GAE). Methane production was determined by means of volume displacement using an 11.2% KOH solution, as it was done in previous studies (Altaş, 2009; Nain and Jawed, 2006). More specifically, each BMP reactor was connected to an inverted bottle containing the alkaline solution. Subsequently, biogas was released to flow inside the bottle, in order to remove CO_2 and H_2S by absorption and leave only CH_4. The volume of CH_4 being transferred to the bottle caused the displacement of an equal amount of KOH solution, which was then quantified using a graduated cylinder.

4.3 Data analysis

4.3.1 Pretreatment mass yields

The yield of the MW pretreatment process in terms of mass recovery was estimated by calculating three different mass yield values for each pretreated sample, namely Y_{Wet}, Y_{TS} and Y_{VS}. More specifically, the calculations were made by using Equation 1 and considering the initial mass of substrate (m_i) and the mass of sample obtained after pretreatment at each different temperature (m_p), with the values being expressed in three different ways, i.e. a) $gWet_{pretreated\ sample}/gWet_{raw\ sample}$ for Y_{Wet}, b) $gTS_{pretreated\ sample}/gTS_{raw\ sample}$ for Y_{TS} and c) $gVS_{pretreated\ sample}/gVS_{raw\ sample}$ for Y_{VS}.

$$Y = \frac{m_p}{m_i} \tag{1}$$

4.3.2 Theoretical methane potential

The Theoretical Methane Potential (TMP) of the MW-pretreated samples at Standard Temperature and Pressure (STP) conditions was estimated through their elemental composition and the stoichiometry of the degradation reaction (Equation 2), using Equation 3 (Raposo et al., 2011):

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right)H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right)CO_2 + dNH_3 + eH_2S \tag{2}$$

$$TMP\left[\text{mL CH}_{4,\text{STP}}/\text{g VS}\right] = 22.4 \cdot \left[\frac{\left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)}{12a + b + 16c + 14d + 32e}\right] \cdot 1000 \tag{3}$$

4.3.3 Theoretical oxygen demand

The Theoretical Oxygen Demand (TOD) (mg O_2/g VS) of the four substrates in their untreated (raw) form, as well as after pretreatment at different temperatures, was estimated through their elemental composition and the stoichiometry of the oxidation reaction (Equation 4), using Equation 5 (Raposo et al., 2011):

$$C_a H_b O_c N_d S_e + \left(a + \frac{b}{4} - \frac{c}{2} - \frac{3d}{4}\right) O_2 \rightarrow aCO_2 + \left(\frac{b}{2} - \frac{3d}{2}\right) H_2O + dNH_3 \quad (4)$$

$$TOD\left[\text{mg O}_2/\text{g VS}\right] = \frac{\left(2a + \frac{b}{2} - c - \frac{3d}{2}\right) \cdot 16}{12a + b + 16c + 14d} \cdot 1000 \quad (5)$$

4.3.4 Specific methane yield

The Specific Methane Yield (SMY) of each MW-pretreated sample, resulting after the end of digestion, was obtained by subtracting the ultimate cumulative methane production of the blank assay (mL CH_4) from the ultimate cumulative methane production of each assay containing the pretreated samples, and by subsequently dividing it either by the amount of VS of the pretreated samples being added to each reactor, or by the corresponding amount of VS of the raw substrates, which were calculated on the basis of the mass yields of the pretreatment process. These values were then converted to STP conditions. This way, the SMY was expressed in two different ways, namely SMY_P (mL $CH_{4,\,STP}$/g VS_P) and SMY_{Raw} (mL $CH_{4,\,STP}$/g VS_{Raw}), respectively.

4.3.5 Biodegradability Index

The Biodegradability Index (BI) (%) was calculated using the TMP (mL $CH_{4,\,STP}$/g VS) and the SMY_P (mL $CH_{4,\,STP}$/g VS_P) of each pretreated sample, as it is shown in Equation 6.

$$BI\left[\%\right] = \frac{SMY_P}{TMP} \cdot 100 \quad (6)$$

4.3.6 Solubilization

COD solubilization (%) was calculated using the TOD (mg O_2/g VS) of the untreated substrates and the sCOD (mg O_2/g VS) values determined for each substrate at the different pretreatment temperatures, according to Equation 7.

$$\text{COD solubilization}\left[\%\right] = \frac{sCOD}{TOD} \cdot 100 \quad (7)$$

4.3.7 Kinetic modeling

Methane production was modeled by fitting the data with the first-order exponential model (Fernández-Cegrí et al., 2012) (Equation 8) through non-linear regression, using the Solver tool of Microsoft Office Excel. The goodness of fit was evaluated by taking into consideration both the Residual Sum of Squares (RSS) and the R-square (R^2) values.

$$B = B_0 \cdot \left[1 - \exp\left(-kt\right)\right] \tag{8}$$

where, t is the digestion time (d), k is the rate constant (d^{-1}), B is the cumulative methane production at time t (mL CH_4) and B_0 is the maximum methane production (mL CH_4).

4.3.8 Energy calculations

The specific energy consumption, E_C (kJ/kg VS), resulting from the pretreatment process was calculated according to Equation 9 (Passos et al., 2013).

$$E_C = \frac{P_W \cdot t_E}{m} \tag{9}$$

where, P_W is the power of the microwave reaction system (600 W), t_E is the exposure time (s) and m is the mass of VS of raw substrate being subjected to pretreatment (kg VS).

The specific energy corresponding to the methane quantity produced from the pretreated samples, E_M (kJ/kg VS), was calculated according to Equation 10 (Passos et al., 2013).

$$E_M = \frac{SMY_{Raw} \cdot \xi}{1000} \tag{10}$$

where, ξ is the lower heating value of methane (35800 kJ/m^3 CH_4).

Considering that MW pretreatment temperatures are much higher than the temperature of the mesophilic anaerobic digestion, the pretreated samples would require an intermediate cooling procedure, which would result in the release of energy in the form of heat. The specific energy corresponding to the potential recovery of the latter amount of energy, E_Q (kJ/kg VS), was calculated according to Equation 11 (Ma et al., 2011).

$$E_Q = \frac{\left[m_s \cdot C_{P,s} \cdot \Delta T + m_w \cdot C_{P,w} \cdot \Delta T\right]}{m} \tag{11}$$

where, m_s is the mass (kg) of dry solids of substrate being subjected to pretreatment, m_w is the mass (kg) of water in the vessel during pretreatment (including the water contained in the substrate), $C_{P,s}$ and $C_{P,w}$ are the specific heat capacities of solids (1.95 kJ/kg °C) and water (4.18 kJ/kg °C), respectively and ΔT is the temperature difference (°C) between the temperature of the slurry at the end of pretreatment and the temperature of the mesophilic anaerobic digestion (35 °C).

The specific energy profit of the pretreatment, E_T (kJ/kg VS), was calculated considering E_M, E_Q and E_C, according to Equation 12 (Kuglarz et al., 2013).

$$E_T = E_M + E_Q - E_C \quad (12)$$

with the sum of E_M and E_Q comprising the specific energy output, E_o (kJ/kg VS), and E_C constituting the specific energy input E_i (kJ/kg VS).

In order to be able to provide an energy balance for the MW pretreatment process applied in the present study, all the above mentioned energy parameters were expressed as kJ per kg of VS of raw (untreated) substrate, aiming at a more accurate approach.

4.4 Results and discussion

4.4.1 Microwave pretreatment

4.4.1.1 Optimization procedure

Effect of solid to liquid ratio and heating rate

Fig. 4-1 presents the variations of sCOD and TPH released concentrations, as well as final pH of the slurries, as a function of S/L and HR, as they were determined after microwave pretreatment during the optimization procedure. As far as substrate solubilization is concerned, the differences between values are mostly minor, however they are enough to indicate that an increase in S/L affects solubilization negatively, while changing HR between 2.5 and 10 °C/min has no particular effect. The pH of the slurries remained the same under all conditions, namely between 3.3 and 3.6 for WW, and between 5.7 and 6.1 for CGW. Jackowiak et al. (2011a) also reported that heating rate variation did not affect wheat straw solubilization during microwave pretreatment. Considering these results, optimum values of S/L 50 g/L and HR 10 °C/min were chosen for the subsequent experiments.

Effect of holding time and temperature

The effect of HT on substrate solubilization and final pH at different pretreatment temperatures is depicted in Fig. 4-2. For both substrates, increasing T from 75 to 200 °C appears to positively influence TPH and sCOD release. The variation in HT in the range of 5–30 min caused less significant changes, maintaining pH steady, and making a slight increasing solubilization trend visible in some cases. However, a different behavior can be observed regarding sCOD release from WW (Figure 4-2a), since the concentrations corresponding to the pretreatment performed at 150 °C are the highest. As far as pH is concerned, higher T is generally combined with lower pH values in the slurries, with this effect being more evident for CGW (total range 7.1–4.7). The above mentioned results were used to determine the optimum HT value of 5 min. Since the variation in solubilization as a function of HT was not significant for either substrate, the shortest pretreatment period was selected, for time and energy saving reasons.

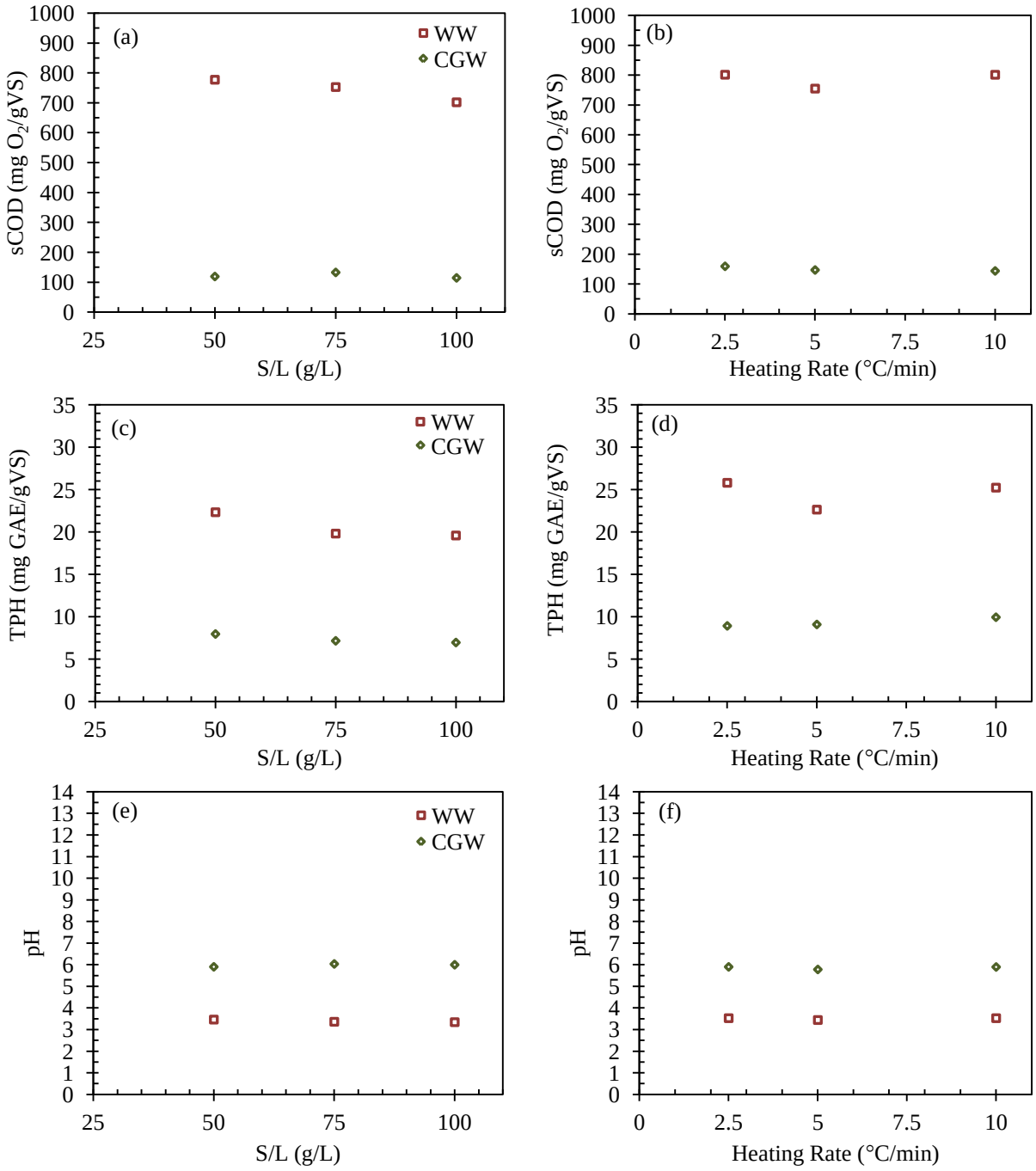


Figure 4-1: sCOD release (a, b), TPH release (c, d) and final pH (e, f) after microwave pretreatment as a function of S/L (a, c, e) and HR (b, d, f) (HT=15 min, T=150 °C)

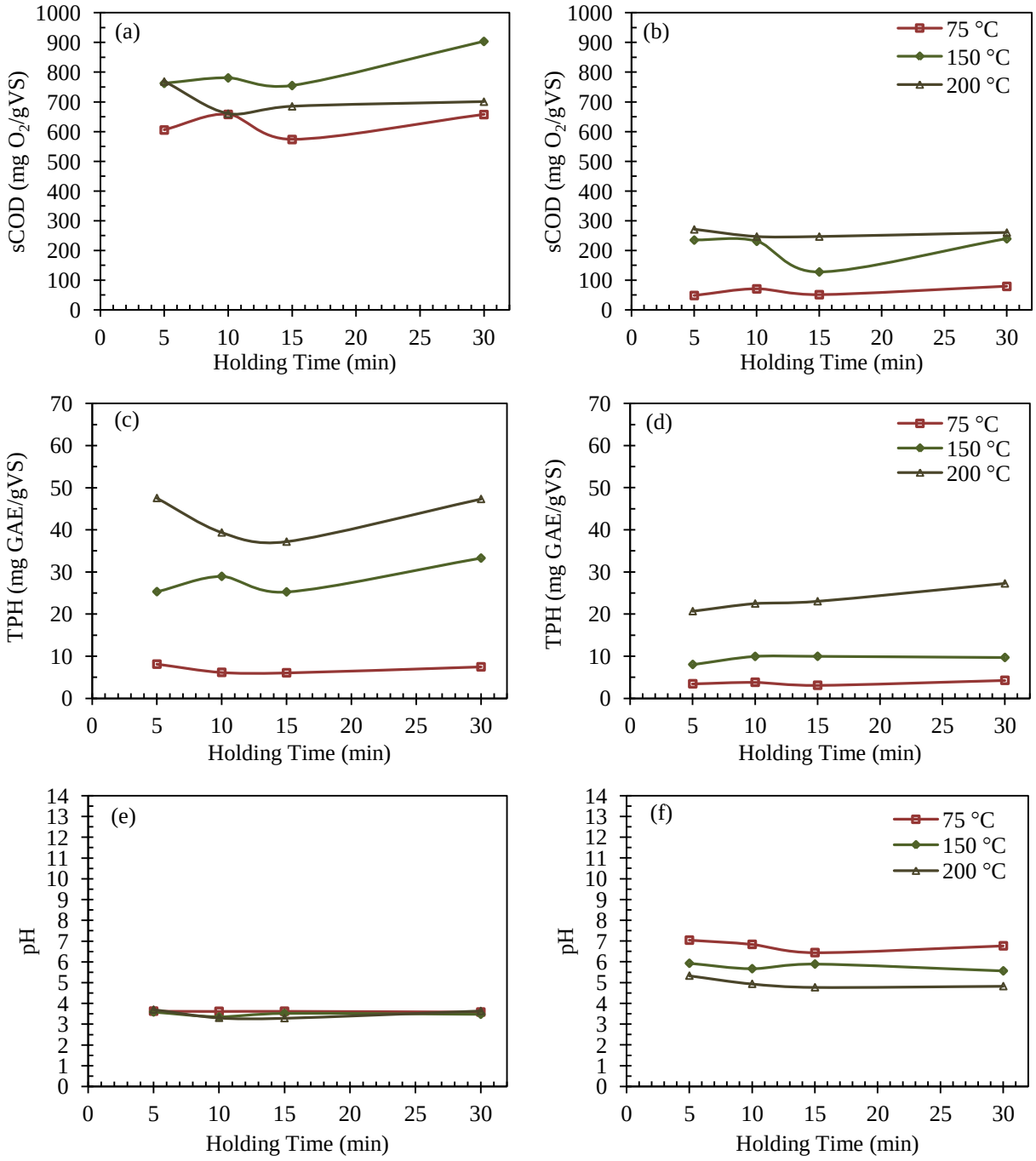


Figure 4-2: sCOD release (a, b), TPH release (c, d) and final pH (e, f) after microwave pretreatment HT and T (S/L=50 g/L, HR=10 °C/min) for WW (a, c, e) and CGW (b, d, f)

4.4.1.2 Main experiments

4.4.1.2.1 Effect on substrate composition

Table 4-1 presents the composition of MW-pretreated samples. Pretreated samples were found to have higher moisture contents in comparison with the untreated (raw) substrates. This comes into conflict with certain other studies, reporting decreased moisture contents for similarly pretreated samples as a function of temperature (Chen et al., 2012; Huang et al., 2012; Rincón et al., 2013). Indeed, it has been reported that MW irradiation often causes the release of bound water into the free liquid phase, as a result of the disruption phenomena in the structure of a given material (Beszédes et al., 2011; Bougrier et al., 2008; Marin et al., 2010; Shahriari et al., 2012; Tyagi et al., 2014). Nevertheless, Mollekopf et al. (2011) have verified that both moisture removal and an increase in water absorption capacity can occur simultaneously during MW treatment. Moreover, increased water absorption capacity in plant materials has been linked to the increase in their porosity, induced by thermal treatment (Kratchanova et al., 2004; Merino-Pérez et al., 2015). In a study conducted by Sapci et al. (2013), wheat straw was pretreated with both MW irradiation and steam-explosion. Interestingly, a decrease in moisture content was observed for MW-pretreated samples, while for steam-exploded wheat straw a significant increase was manifested. The authors attributed the latter result to the water vapor being added during steam-explosion. It is possible that in the present study, a combination of phenomena similar to those described above occurred at the same time, leading to the obtained results. An additional explanation may also be found in the nature of the procedure followed during the pretreatment process. In fact, although the solid fraction obtained after centrifugation was subjected to vacuum filtration, precisely to remove excess moisture, some quantity of the water that was initially added to the vessels, would inevitably have been retained within the mass of the samples in the form of free water, contributing to the higher moisture contents compared with the raw substrates. This is especially true in the case of CGW, which is naturally prone to water absorption. Furthermore, in most studies, the samples being irradiated are introduced in the reaction vessels alone, without any additional water quantity, contrarily to what was done in this study. This fact probably contributed to the obtained results as well.

Compared with raw substrates, VS/TS values for MW-pretreated samples appear to have been increased for WW and OP and decreased for JW, while in the case of CGW increased values are observed only for three out of five samples, specifically those corresponding to pretreatment temperatures between 75 and 150 °C. Indeed, these three values also follow an increasing trend as a function of temperature, while a decreasing trend is subsequently observed. Higher temperatures are associated with slightly higher VS/TS values for WW, while no particular effect is noticed in the case of OP, although the sample produced at 200 °C has the highest VS/TS compared with the others. Finally, for JW, decreasing values are noticed moving from 75 to 175 °C, while the sample produced at 200 °C appears to contain a similar relative amount of VS as the sample obtained at 75 °C. Both decreased (Bougrier et al., 2008; Huang et al., 2012; Liu et al., 2012; Tyagi et al., 2014) and increased (Chen et al., 2012; Jackowiak et al., 2011a; Rincón et al., 2013; Sapci et al., 2013) VS/TS values have been observed in literature, as a function of increasing intensity of wet thermal treatment, either microwave, hydrothermal, or steam-explosion. In all cases the changes were attributed to the hydrolysis of the lignocellulosic structure of each studied material into smaller molecules. Based on the fact that MW pretreatment indeed causes the disruption/decomposition of lignocelluloses (Pecorini et al., 2016; Sapci, 2013), the above

mentioned explanation suggests that the nature of each specific material in combination with the pretreatment conditions may lead to different final results. Specifically, in one case a portion of the volatiles may be released in the liquid phase and therefore removed from the solid matrix, while in another case eventual changes in the material structure may cause the rearrangement of already existing components, leading to an increase in the final VS content of the material.

Elemental analysis shows that C content of pretreated materials generally increased as pretreatment temperature was raised to higher levels, while O content decreased. Nevertheless, in the case of CGW, an exception is presented, since the highest C and O contents are observed for the samples obtained at 150 and 125 °C, respectively. As far as H content is concerned, an almost constant behavior is noticed for WW and JW, with a slight increase for JW at temperatures of 175 and 200 °C. On the other hand, for CGW and OP, a significant variation occurs when raising the temperature from 75 to 125 °C, whereas for more elevated temperatures, H contents can be considered constant. Interestingly, the above mentioned significant variation is a decrease in the case of CGW, while in the case of OP is an increase. Finally, N content profiles are generally characterized by an initial increase followed by a subsequent decrease, with the peak values being observed at 125 °C for WW and OP and at 150 °C for CGW. On the contrary, the values referring to JW appear not to have a definite trend. By comparing these results with the elemental composition of the respective raw substrates, it is observed that MW pretreatment resulted in increased C and H contents, as well as decreased O contents, for all substrates. Regarding N contents, a clear effect leading to decreased values, was noticed only for CGW and OP. Previous studies (Chen et al., 2012; Huang et al., 2012; Sapci, 2013) have reported an increase in C contents after microwave treatment, which is in agreement with the present results. On the other hand, for H and O contents a decrease was usually observed, partly disagreeing with what was obtained in this study. The explanation to these results is similar to the one given earlier regarding the increased VS/TS of pretreated samples. More specifically, the disruption of larger molecules into smaller ones, would most likely have led to an easier and more efficient combustion of the volatile matter of the substrates during elemental analysis (as well as during VS determination), resulting in higher C and H contents being determined.

It is worth mentioning that S contents are not included in elemental analysis results, since all values were below the detection limit of the instrument (0.01%).

All the above mentioned information reveal that MW pretreatment induced notable changes in the composition of the investigated substrates, thus demonstrating the effectiveness of this process in affecting the structure of such materials.

Table 4-2: Composition of MW-pretreated samples

Substrates	Pretreatment temperature (°C)	M (%) [a]	VS/TS	Elemental analysis				
				C (%) [b]	H (%) [b]	O (%) [b]	N (%) [b]	C/N
WW	- (raw)	71.9 ± 0.0	0.920 ± 0.009	45.9 ± 4.2	5.95 ± 1.17	38.3 ± 5.3	1.80 ± 0.31	25.5 ± 5.5
	75	84.3 ± 5.2	0.969 ± 0.031	56.2 ± 0.8	9.16 ± 0.18	29.6 ± 0.3	1.94 ± 0.31	29.3 ± 5.1
	125	87.6 ± 2.7	0.979 ± 0.025	57.2 ± 0.3	9.25 ± 0.06	29.0 ± 0.2	2.41 ± 0.01	23.8 ± 0.3
	150	83.2 ± 3.2	0.980 ± 0.016	57.9 ± 0.0	9.03 ± 0.06	29.2 ± 0.1	1.86 ± 0.10	31.1 ± 1.6
	175	82.7 ± 4.1	0.984 ± 0.009	61.4 ± 0.3	9.00 ± 0.11	26.2 ± 0.5	1.76 ± 0.09	34.9 ± 1.6
	200	83.3 ± 1.7	0.984 ± 0.009	65.2 ± 0.0	9.11 ± 0.02	22.9 ± 0.1	1.19 ± 0.03	54.9 ± 1.5
CGW	- (raw)	29.4 ± 2.5	0.750 ± 0.098	32.8 ± 1.2	4.40 ± 0.22	36.4 ± 1.4	1.40 ± 0.20	23.4 ± 3.8
	75	79.9 ± 0.4	0.763 ± 0.011	38.9 ± 1.5	6.89 ± 0.22	29.6 ± 1.3	0.88 ± 0.00	44.4 ± 1.6
	125	79.3 ± 1.1	0.772 ± 0.008	37.9 ± 0.7	6.32 ± 0.12	31.9 ± 0.7	1.06 ± 0.08	35.8 ± 3.2
	150	80.5 ± 1.7	0.778 ± 0.009	40.0 ± 2.9	6.46 ± 0.42	30.2 ± 3.2	1.11 ± 0.06	36.3 ± 4.6
	175	80.5 ± 0.2	0.751 ± 0.010	38.3 ± 4.4	6.40 ± 0.55	29.3 ± 5.0	1.09 ± 0.03	35.0 ± 3.2
	200	79.0 ± 0.4	0.739 ± 0.016	39.1 ± 1.0	6.54 ± 0.06	27.2 ± 1.1	1.04 ± 0.13	37.8 ± 3.6
OP	- (raw)	46.5 ± 1.9	0.977 ± 0.001	54.2 ± 3.2	7.53 ±1.14	34.5 ± 5.0	2.09 ± 0.07	25.9 ± 2.4
	75	62.6 ± 2.5	0.988 ± 0.001	57.7 ± 0.9	9.52 ± 0.20	30.3 ± 1.6	1.26 ± 0.55	50.6 ± 21.4
	125	60.0 ± 2.6	0.988 ± 0.003	60.9 ± 0.8	10.5 ± 0.1	25.5 ± 1.0	1.89 ± 0.09	32.3 ± 1.2
	150	59.6 ± 3.5	0.989 ± 0.048	61.4 ± 2.3	10.6 ± 0.5	25.4 ± 2.9	1.55 ± 0.16	39.8 ± 2.6
	175	64.3 ± 2.2	0.988 ± 0.007	62.8 ± 0.6	10.4 ± 0.2	24.6 ± 1.3	1.04 ± 0.46	67.0 ± 29.1
	200	66.9 ± 2.4	0.998 ± 0.008	64.8 ± 0.9	10.3 ± 0.1	24.0 ± 1.2	0.69 ± 0.07	94.4 ± 7.9
JW	- (raw)	83.8 ± 0.8	0.968 ± 0.000	45.3 ± 0.1	6.29 ± 0.89	44.3 ± 1.2	0.90 ± 0.15	50.3 ± 6.9
	75	94.0 ± 0.1	0.967 ± 0.012	46.2 ± 0.1	8.59 ± 0.01	41.1 ± 0.1	0.83 ± 0.01	55.4 ± 0.3
	125	95.3 ± 0.5	0.958 ± 0.013	46.7 ± 0.1	8.48 ± 0.13	39.6 ± 0.0	1.05 ± 0.02	44.3 ± 0.6
	150	95.2 ± 0.2	0.953 ± 0.019	46.5 ± 0.1	8.38 ± 0.01	39.6 ± 0.2	0.82 ± 0.04	57.0 ± 2.5
	175	93.6 ± 0.6	0.952 ± 0.035	49.8 ± 0.2	9.10 ± 0.01	35.2 ± 0.2	1.08 ± 0.03	46.0 ± 1.6
	200	92.8 ± 1.7	0.968 ± 0.009	52.4 ± 0.6	8.99 ± 0.07	34.5 ± 0.8	0.97 ± 0.10	54.1 ± 5.2

[a] wet basis, [b] dry basis, All values are expressed as average ± standard deviation

4.4.1.2.2 Mass yields

Mass yield values referring to the MW pretreatment process are presented in Table 4-2, where Y_{Wet}, Y_{TS} and Y_{VS} represent the mass yields expressed on a wet sample, a total solids and a volatile solids basis, respectively.

It can be seen that Y_{Wet} values are generally greater than unity. This is attributed to the increased moisture content of pretreated samples compared with the raw substrates. Especially in the case of CGW, the wet mass of the samples obtained after pretreatment is almost three times greater that the initial wet mass of raw CGW. As far as Y_{TS} and Y_{VS} are concerned, the two respective values referring to each separate sample are very close to each other and range from 0.65 to 0.86 for WW, from 0.60 to 0.85 for CGW and from 0.32 to 0.47 for JW. On the other hand, in the case of OP it is observed that samples obtained after pretreatment at 75, 125 and 150 °C reach values greater than unity for Y_{TS} and Y_{VS}. This fact indicates an increase in the TS and VS contents of these specific pretreated samples. It has been reported that thermal treatment of biomass can cause several simultaneous reactions, including dehydration, depolymerization, rehydration, rearrangement, condensation and carbonization (Kim et al., 2016). Generally, when applying thermal treatment processes which adopt conventional heating, these phenomena, involving lignocelluloses and mainly lignin, occur at temperatures higher than 160 °C (Hendriks and Zeeman, 2009; Singh et al., 2016; Zhao et al., 2012). However, it is possible that the different mode of action of MW heating, compared with conventional heating, allowed some of these phenomena to take place at lower temperatures, thus not requiring such severe conditions (Li et al., 2016). The eventual occurrence of such processes and the consequent structural changes would explain an increase in TS and VS contents of a substrate treated by MW irradiation, as was observed for OP at 75, 125 and 150 °C in the present study. In fact, considering the particular composition of OP, which includes several types of compounds, such as sugars, volatile acids, polyphenols, polyalcohols, proteins and pigments (Rincón et al., 2013), the eventual condensation and precipitation of soluble degradation compounds could have possibly led to the above mentioned results.

4.4.1.2.3 Effect on solubilization

The effect of MW pretreatment on material solubilization was evaluated by determining sCOD and TPH concentrations in the liquid phase obtained after the process conducted at optimum conditions, i.e. S/L=50g/L, HR=10 °C/min and HT=5 min. According to the data presented in Fig. 4-3 increasing pretreatment temperature from 75 to 200 °C appears to generally have a positive influence on sCOD release. It is noticed that the data referring to WW and JW follow similar variation patterns, as do those referring to CGW and OP. In the former case, an increasing trend is observed for temperatures between 75 and 150 °C, while for temperatures >150 °C, values tend to stabilize. On the other hand, a continuous increase is noticed for CGW and OP throughout the tested temperature range. Moreover, a more pronounced effect of pretreatment temperature, manifested by a larger difference between values, can be seen from 125 to 150 °C, for WW and JW, while a similar observation can be made for CGW and OP in the range 150-175 °C. As far as TPH release profiles (Fig. 4-4) are concerned, they are characterized by an exponential increase as a function of temperature. It has been previously reported that thermal pretreatment exerts such an effect on organic substrates, that as the process temperature is increased, higher amounts of organic matter are released in soluble form (e.g. sCOD) (Kuglarz et al., 2013; Pecorini et al., 2016). More

specifically, heat addition at temperatures between 150 and 180 °C is known to cause solubilization of lignocellulosic biomass components, firstly of hemicellulose and then of lignin, leading to increased concentrations of products, such as phenolic compounds (Hendriks and Zeeman, 2009). In two studies referring to sludge pretreatment (Eskicioglu et al., 2008; Hosseini Koupaie and Eskicioglu, 2016) the authors had also observed increased soluble COD values after MW irradiation at increasing temperatures. Similar results were obtained when wheat straw was treated at a range of 100-180 °C (Jackowiak et al., 2011a), but also when switchgrass was treated at a range of 90-150 °C (Jackowiak et al., 2011b).

It is noted, that higher solubilization levels (Table 4-3) are obtained for WW and JW, while CGW and OP present lower values. These differences among substrates are most likely due to their fiber composition. In fact, in a previous study (Pellera and Gidarakos, 2016) (Chapter 3) it was found that the NDF (Neutral Detergent Fiber) contents of untreated WW, CGW, OP and JW are 12.1, 43.9, 34.5 and 3.2%, respectively. Thus, the substrates with higher fiber contents would have been less easily hydrolysable. A similar result was observed in the study performed by Jackowiak et al. (2011a), in which wheat straw, with an NDF content as high as 77.1%, was pretreated with microwave irradiation achieving COD solubilization levels ranging from 6.9 to 12.5%. These values are comparable to those observed in the present study.

The pH of the slurries after pretreatment can be seen in Fig. 4-5, where higher temperatures are generally associated with lower pH values. This is especially true for CGW, OP and JW, while WW presents a constant behavior. The hydrolysis reactions induced by MW pretreatment caused the release of organic acids in the liquid phase, resulting in reduced pH values (Jackowiak et al., 2011a; Pecorini et al., 2016). This effect is more evident in the cases of CGW and OP, since the variation ranges being observed for these substrates moving from 75 to 200 °C, i.e. 6.72-4.99 and 5.72-3.24, respectively, are wider compared with the other two substrates.

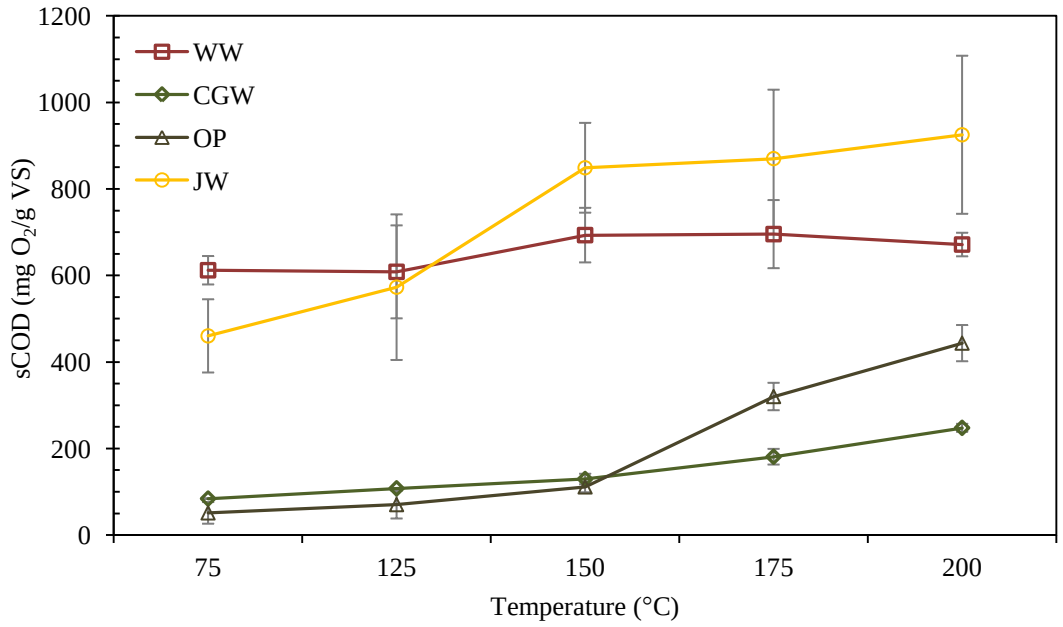


Figure 4-3: sCOD release after pretreatment as a function of temperature (S/L=50 g/L, HR=10 °C/min, HT=5 min) for WW (—□—), CGW (—◇—), OP (—△—) and JW (—○—), (error bars represent standard deviation)

Table 4-3: Mass yields for MW pretreatment at different temperatures

Substrates	Pretreatment temperature (°C)	Mass Yields		
		Y_{Wet}	Y_{TS}	Y_{VS}
WW	75	1.5	0.82	0.86
	125	1.3	0.56	0.60
	150	1.3	0.76	0.81
	175	1.1	0.66	0.71
	200	1.1	0.65	0.70
CGW	75	2.7	0.77	0.78
	125	2.8	0.82	0.85
	150	2.6	0.71	0.73
	175	2.6	0.73	0.73
	200	2.1	0.61	0.60
OP	75	1.6	1.10	1.11
	125	1.6	1.22	1.24
	150	1.5	1.12	1.13
	175	1.3	0.84	0.85
	200	1.3	0.81	0.83
JW	75	1.4	0.39	0.39
	125	1.6	0.36	0.35
	150	1.7	0.37	0.37
	175	1.6	0.47	0.47
	200	0.9	0.32	0.32

Table 4-4: COD solubilization (%) of substrates after MW pretreatment at different temperatures

Substrate	COD solubilization (%)				
	75 °C	125 °C	150 °C	175 °C	200 °C
WW	43.8 ± 2.4	43.5 ± 7.7	49.6 ± 4.5	49.7 ± 5.6	48.0 ± 1.9
CGW	7.50 ± 0.0	9.61 ± 0.0	11.5 ± 1.1	16.1 ± 1.6	22.1 ± 0.8
OP	3.01 ± 1.5	4.13 ± 1.9	6.55 ± 0.7	18.9 ± 1.9	26.2 ± 2.5
JW	35.5 ± 6.5	44.2 ± 13.0	65.5 ± 8.0	60.0 ± 10.9	71.4 ± 14.1

All values are expressed as average ± standard deviation

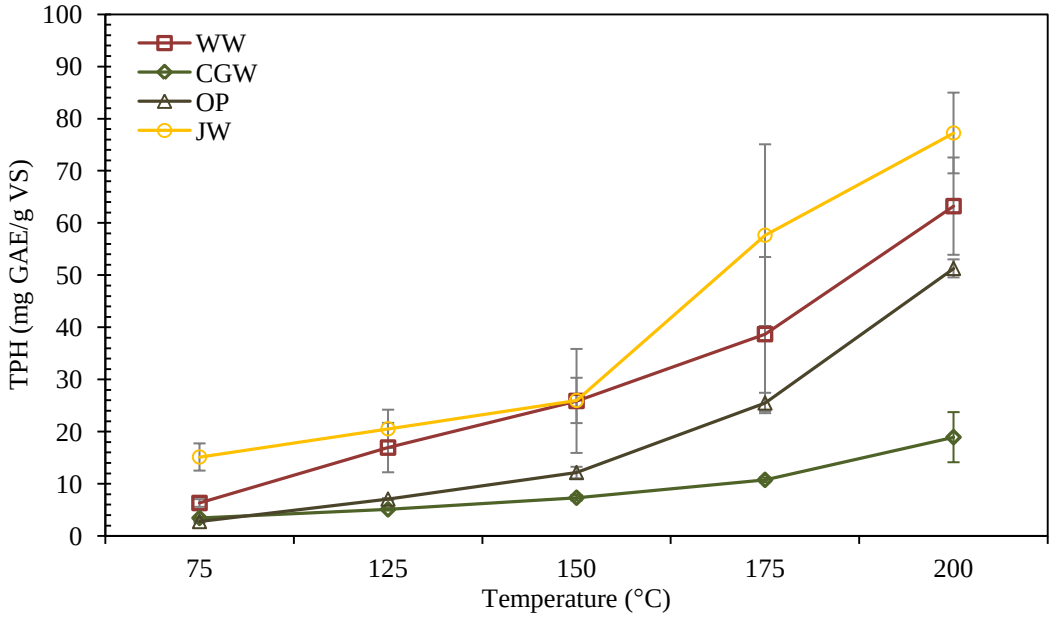


Figure 4-4: TPH release after pretreatment as a function of temperature (S/L=50 g/L, HR=10 °C/min, HT=5 min) for WW (□), CGW (◊), OP (Δ) and JW (○), (error bars represent standard deviation)

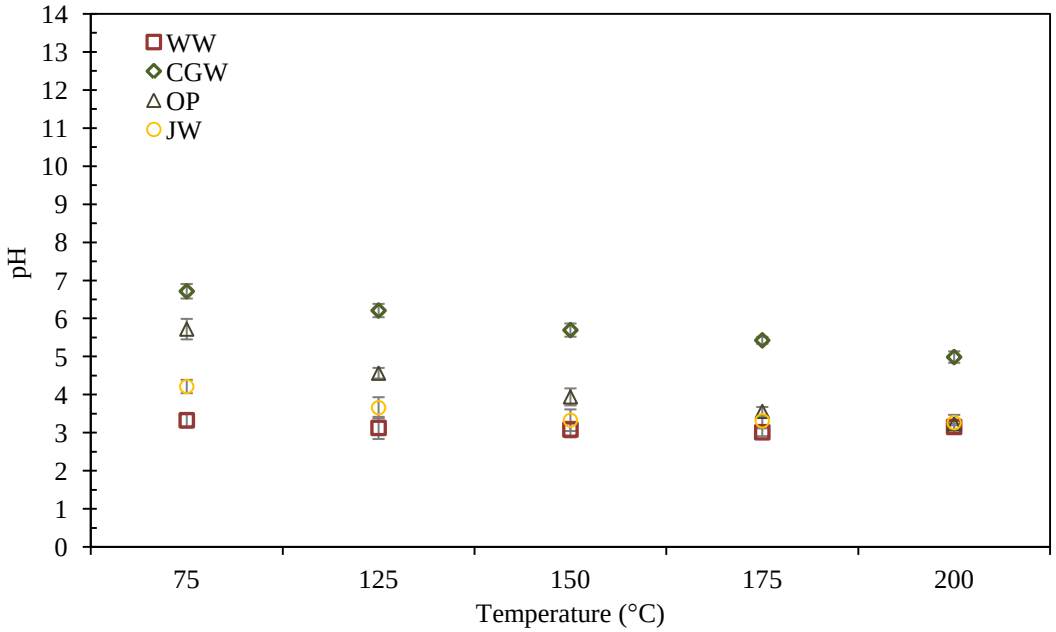


Figure 4-5: Final pH of slurries after pretreatment as a function of temperature (S/L=50 g/L, HR=10 °C/min, HT=5 min) for WW (□), CGW (◊), OP (Δ) and JW (○), (error bars represent standard deviation)

4.4.2 Biochemical methane potential assays

4.4.2.1 Methane production

The solid samples obtained after MW pretreatment at five different temperatures (75, 125, 150, 175 and 200 °C) for each substrate were used for conducting BMP assays. Cumulative methane production (mL CH_4) during digestion can be seen in Fig. 4-6. No lag phase was observed for any of the WW, CGW and OP samples, since the beginning of methane production was observed on day 1. On the other hand, two of the JW samples, specifically those obtained after pretreatment at 175 and 200 °C, showed some signs of methane production inhibition, manifested by the sigmoidal shape of their curves (Rincón et al., 2013).

The specific methane yields of MW-pretreated samples (SMY_P) are provided in Table 4-4. The same table provides TMP, TOD and BI values, specific methane yields expressed on the basis of the corresponding amount of raw substrate (SMY_{Raw}), as well as COD-equivalents for the respective methane production of each sample. It can be noticed from the data, that the maximum SMY_P for WW is obtained for the sample pretreated at 125 °C, with the remaining samples providing yields 25-31% lower than that. In the case of OP, pretreatment at 200 °C produced the sample that ultimately had the highest methane yield, while a similar but slightly lower value was obtained for the sample pretreated at 150 °C. As far as CGW and JW are concerned, maximum methane yields for both substrates were obtained for those samples produced at 150 °C. Interestingly, for CGW the yields corresponding to 75, 175 and 200 °C are all within a 3-7% range lower, and only the value corresponding to 125 °C is 17% lower. Among the four substrates, the case of JW is the only one in which a definite trend can be observed, characterized by an initial increase from 75 to 150 °C and a subsequent decrease from 150 to 200 °C.

BI data corroborate the above mentioned results, with the samples providing maximum SMY_P being associated with higher BI values, thus showing that they were biodegradable to a higher degree compared with the remaining samples. The biodegradability/biodegradation degree for each pretreated sample is also manifested by the data presented in the last column of Table 4-4, which refers to the amount of COD removed, equivalent to methane production. These calculations were made considering that 350 mL of produced methane correspond to 1 g of COD removed (Shahriari et al., 2013). The COD-equivalent values are in agreement with the remaining data, demonstrating that a higher amount of organic matter was removed for the samples having the highest SMY_P.

All the above suggest that when dealing with substrates such as those used in the present study, pretreatment temperatures in the range between 125 and 150 °C are probable to give more positive results. The data also show that, in the majority of cases, the temperatures at which maximum methane yields are achieved do not coincide with the temperatures at which maximum solubilization (Table 4-3) is obtained. Similar observations are made in the study conducted by Jackowiak et al. (2011a), where the maximum methane yield for MW-pretreated wheat straw was also obtained at a temperature of 150 °C, while the highest levels of solubilization were achieved at 180 °C. The disruption of the materials matrices, induced by MW heating, leads to an increase in the fraction available for biodegradation. Nevertheless, while a portion of that fraction may remain on the solid material, another portion is released in the liquid phase and thus removed from the solid matrix, leading to a reduction in the matter effectively available to microbial populations. This may also explain the fact that, as was similarly noticed in a previous study (Fernández-Cegrí et al., 2012), in most cases (except for the JW-sample produced at 150 °C), SMY_P of pretreated samples are lower than the respective SMY of raw substrates. The latter values are 446.2, 268.0, 258.7

and 446.0 mL $CH_{4,\ STP}$/g VS for WW, CGW, OP and JW, respectively. These results are consistent with the results obtained in a previous study (Sapci, 2013), in which methane yields of MW-pretreated winter wheat, spring wheat, oat straw and barley straw, were significantly lower that the methane yields of the untreated materials. The breakage of chemical bonds can at the same time cause the release or/and formation of inhibiting and complex recalcitrant compounds, such as phenols (Haghighi Mood et al., 2013; Marin et al., 2010). Additionally, thermal treatment at higher temperatures often induces the development of Maillard reactions, leading to the formation of certain compounds called melanoidins, which are quite difficult to be degraded and can therefore inhibit the anaerobic digestion process. A good indicator that manifests the occurrence of such reactions is the color of the samples, which tends to turn darker for more intense phenomena (Ariunbaatar et al., 2014). In the present study, a color change was indeed observed for increasing pretreatment temperatures, with it being more evident especially at 150, 175 and 200 °C. Consequently, the probable presence of recalcitrant and/or inhibitory compounds, such as those described above, may also have contributed to the lower SMY_P values at certain temperatures, while it most likely also is the reason for the earlier mentioned delayed methane production for JW samples obtained after pretreatment at 175 and 200 °C. Sapci (2013) was led to a similar conclusion when examining various agricultural straws for biogas production after MW pretreatment.

Interestingly, when observing the data obtained for SMY_{Raw} it can be seen that not all maximum values are found at the same temperature at which maximum SMY_P are noticed. In fact, maximum values for the former parameter are found at 75, 150, 150 and 175 °C, for WW, CGW, OP and JW, respectively. The SMY_{Raw} are expressed on the basis of the mass of raw material that corresponds to the mass of pretreated material actually used, therefore some differences due to composition parameters (moisture and VS contents) are expected. Nevertheless, it may be said that these differences are acceptable, especially for CGW, WW and JW considering that the two temperatures at which the peaks for SMY_P and SMY_{Raw} are observed, are, if not equal (CGW), at least not very distant. The only slightly bigger temperature gap is noticed in the case of OP. In addition, it is worth mentioning that when comparing these values (SMY_{Raw}) with the earlier mentioned SMY obtained for the untreated substrates, it is noticed that the former are all lower than the latter for WW, CGW and JW. However, for OP the methane yield obtained for the sample produced at 150 °C is higher than the value reported for the untreated sample (258.7 mL $CH_{4,\ STP}$/g VS). This suggests that a pretreatment temperature of 150 °C may indeed be worth considering as the most suitable for OP.

4.4.2.2 pH and TPH

In order to better interpret the results of BMP assays, samples of the digestion slurry were weekly taken for determining pH (Fig. 4-7) and TPH (Fig. 4-8) concentrations inside the reactors.

As it can be seen in Fig. 4-7, in most assays, specifically those containing WW, CGW and OP samples, pH values on day 1 are found increased compared with the initially adjusted value of 7.8. On the contrary, for the assays containing JW samples, all pH values determined on day 1 were found beneath 7.8. Moreover, lower initial pH is associated with lower pretreatment temperatures. This suggests that JW-samples obtained at lower temperatures contained a higher amount of degradable matter in comparison with the samples produced at higher temperatures, resulting in a higher quantity of acids being released and in turn, in lower pH. Another factor affecting the latter parameter may also be the acidic character of

these particular samples, which is indicated by the data in Fig. 4-5 as well. After one week of incubation, a general decrease can be noticed for all assays, most likely due to the accumulation of organic acids (Hosseini Koupaie and Eskicioglu, 2015) in the initial stages of the process, whereas after day 7, pH profiles show similar patterns for all assays, except for those containing JW-samples. In fact, while Fig. 4-7a, 4-7b, and 4-7c, show some fluctuations within a relatively close range, in Fig. 4-7d pH appears to be increasing with time. Nevertheless, in the last three samplings all assays show a stable behavior.

Weekly values for TPH concentrations in the digestion slurries are depicted in Fig. 4-8. It is observed that the assays corresponding to higher pretreatment temperatures are generally associated with more elevated TPH concentrations. The values can be considered to follow a constant behavior throughout the incubation period, but only for the assays containing WW, CGW and OP samples. On the contrary, Fig. 4-8d shows that during the first weeks of degradation of JW-samples, TPH being released are found at significantly higher levels, compared to the other assays. Moreover, the presence of these compounds at such concentrations coincides with the manifestation of lower pH values, which was mentioned earlier. This suggests that specifically for JW-samples, the most significant amount of organic material was degraded during the first week of incubation, thus causing the enhanced release of phenolic compounds and the lower pH levels. In contrast, for the remaining samples, the respective data show a more uniform tendency in the development of anaerobic degradation throughout the duration of incubation.

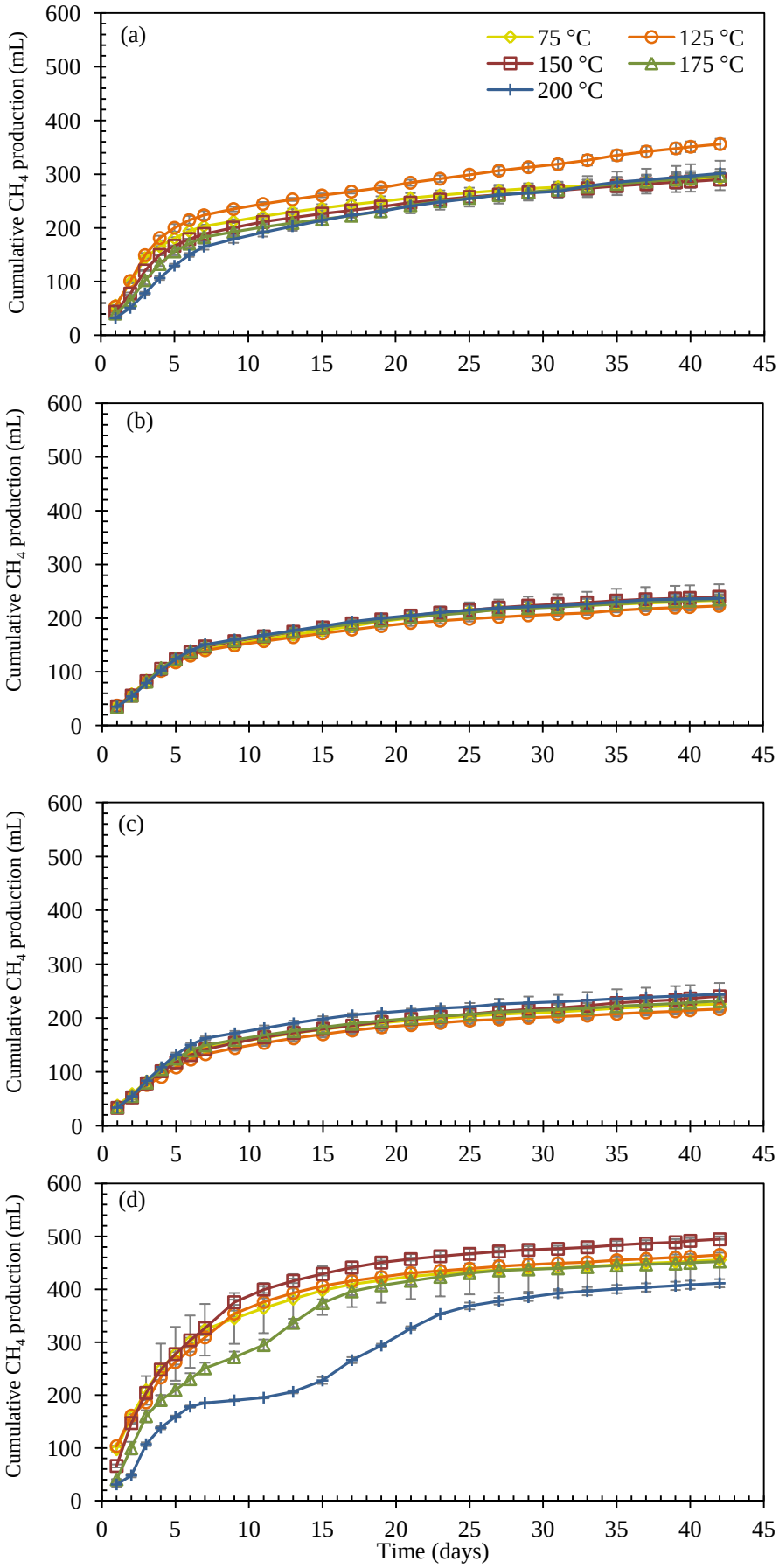


Figure 4-6: Cumulative methane production during BMP assays for (a) WW, (b) CGW, (c) OP and (d) JW, pretreated at different temperatures [75 °C (◇), 125 °C (○), 150 °C (□), 175 °C (△), 200 °C (+)], (error bars represent standard deviation)

Table 4-5: Theoretical methane potentials (TMP), Specific methane yields (SMY), Biodegradability indices (BI), Theoretical oxygen demands (TOD) and COD-equivalents for CH_4 production, for MW-pretreated samples

Substrates	Pretreatment temperature (°C)	TMP ($mLCH_{4,\ STP}/gVS$)	SMY_P [a] ($mLCH_{4,\ STP}/gVS_P$)	SMY_{Raw} [a] ($mLCH_{4,\ STP}/gVS_{Raw}$)	BI [a] (%)	TOD (mg O_2/g VS_P)	COD removed through CH_4 production [a](mgCOD/gVS_P)
WW	75	687.22	180.8 ± 14.3	156.2 ± 12.4	26.3 ± 2.1	1963	516.4 ± 41.0
	125	691.40	256.8 ± 11.8	154.0 ± 7.1	37.1 ± 1.7	1975	733.7 ± 33.6
	150	693.81	177.7 ± 23.7	144.6 ± 19.3	25.6 ± 3.4	1982	507.7 ± 67.8
	175	734.44	186.2 ± 10.2	131.8 ± 7.2	25.3 ± 1.4	2098	531.9 ± 29.0
	200	789.21	191.5 ± 27.9	134.2 ± 19.6	24.3 ± 3.5	2255	547.2 ± 79.7
CGW	75	586.48	218.8 ± 23.6	171.6 ± 18.5	37.3 ± 4.0	1676	625.1 ± 67.3
	125	534.38	193.7 ± 8.5	164.1 ± 7.2	36.3 ± 1.6	1527	553.5 ± 24.4
	150	568.48	234.5 ± 55.9	171.6 ± 40.9	41.3 ± 9.8	1624	670.1 ± 159.7
	175	568.89	222.7 ± 32.2	162.3 ± 23.5	39.1 ± 5.7	1625	636.2 ± 92.1
	200	604.32	228.5 ± 16.9	138.1 ± 10.2	37.8 ± 2.8	1727	653.0 ± 48.2
OP	75	700.10	202.3 ± 30.3	224.4 ± 33.6	28.9 ± 4.3	2000	577.9 ± 86.5
	125	770.53	185.5 ± 13.4	229.7 ± 16.6	24.1 ± 1.7	2202	530.1 ± 38.3
	150	779.33	236.9 ± 59.2	268.8 ± 67.2	30.4 ± 7.6	2227	676.9 ± 169.2
	175	794.78	214.9 ± 24.5	182.5 ± 20.8	27.0 ± 3.1	2271	613.9 ± 70.1
	200	807.28	244.5 ± 3.4	201.9 ± 2.8	30.3 ± 0.4	2307	698.7 ± 9.6
JW	75	540.72	376.5 ± 52.6	147.1 ± 20.5	69.6 ± 9.7	1545	1076 ± 150.3
	125	551.41	387.4 ± 5.1	137.2 ± 1.8	70.3 ± 0.9	1575	1107 ± 14.6
	150	550.85	451.5 ± 39.9	166.0 ± 14.7	82.0 ± 7.2	1574	1290 ± 113.9
	175	619.79	372.3 ± 2.6	174.4 ± 1.2	60.1 ± 0.4	1771	1064 ± 7.4
	200	634.40	322.0 ± 9.3	103.3 ± 3.0	50.8 ± 1.5	1813	920.1 ± 26.6

[a] Values are expressed as average ± standard deviation

Table 4-6: Kinetic modeling parameters for BMP assays, for MW-pretreated samples at five different temperatures

Parameters	WW					CGW					OP					JW				
	75	125	150	175	200	75	125	150	175	200	75	125	150	175	200	75	125	150	175	200
B_0	271.3	319.0	268.9	274.7	286.8	223.2	209.7	228.4	222.6	226.7	213.7	205.9	224.5	217.9	232.5	438.4	449.8	479.5	453.2	447.8
k	0.1999	0.1628	0.1649	0.1345	0.1025	0.1311	0.1427	0.1318	0.1410	0.1371	0.1489	0.1364	0.1284	0.1492	0.1503	0.1929	0.1738	0.1679	0.1152	0.0624
RSS	4664	12111	4413	5617	2999	1988	1919	2099	1877	1665	1611	1073	2163	1593	1361	3904	3233	1815	3307	12584
R^2	0.9495	0.9182	0.9584	0.9545	0.9803	0.9752	0.9719	0.9754	0.9764	0.9803	0.9770	0.9843	0.9742	0.9788	0.9845	0.9839	0.9882	0.9947	0.9912	0.9655

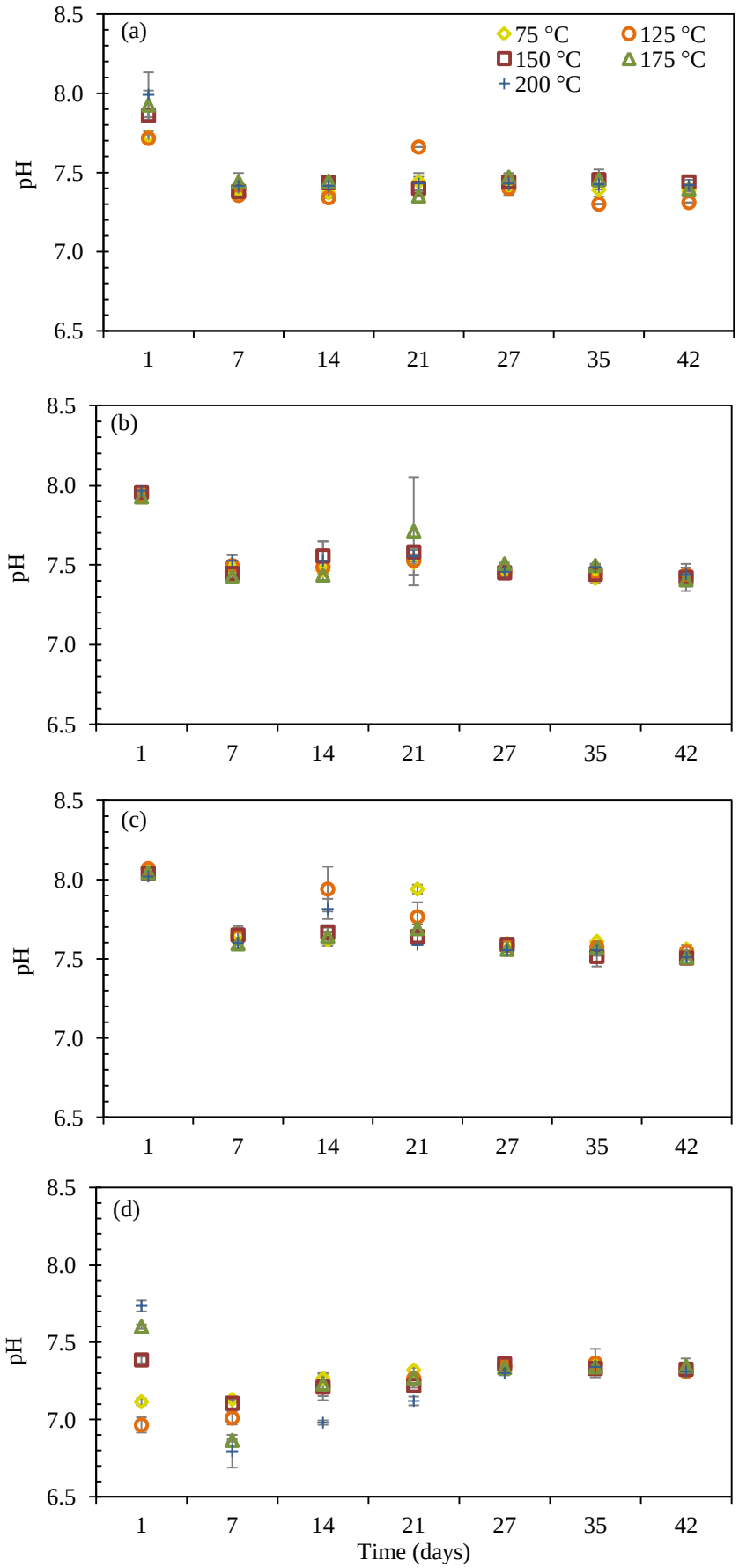


Figure 4-7: pH variation during BMP assays for (a) WW, (b) CGW, (c) OP and (d) JW, pretreated at different temperatures [75 °C (◊), 125 °C (○), 150 °C (□), 175 °C (Δ), 200 °C (+)], (error bars represent standard deviation)

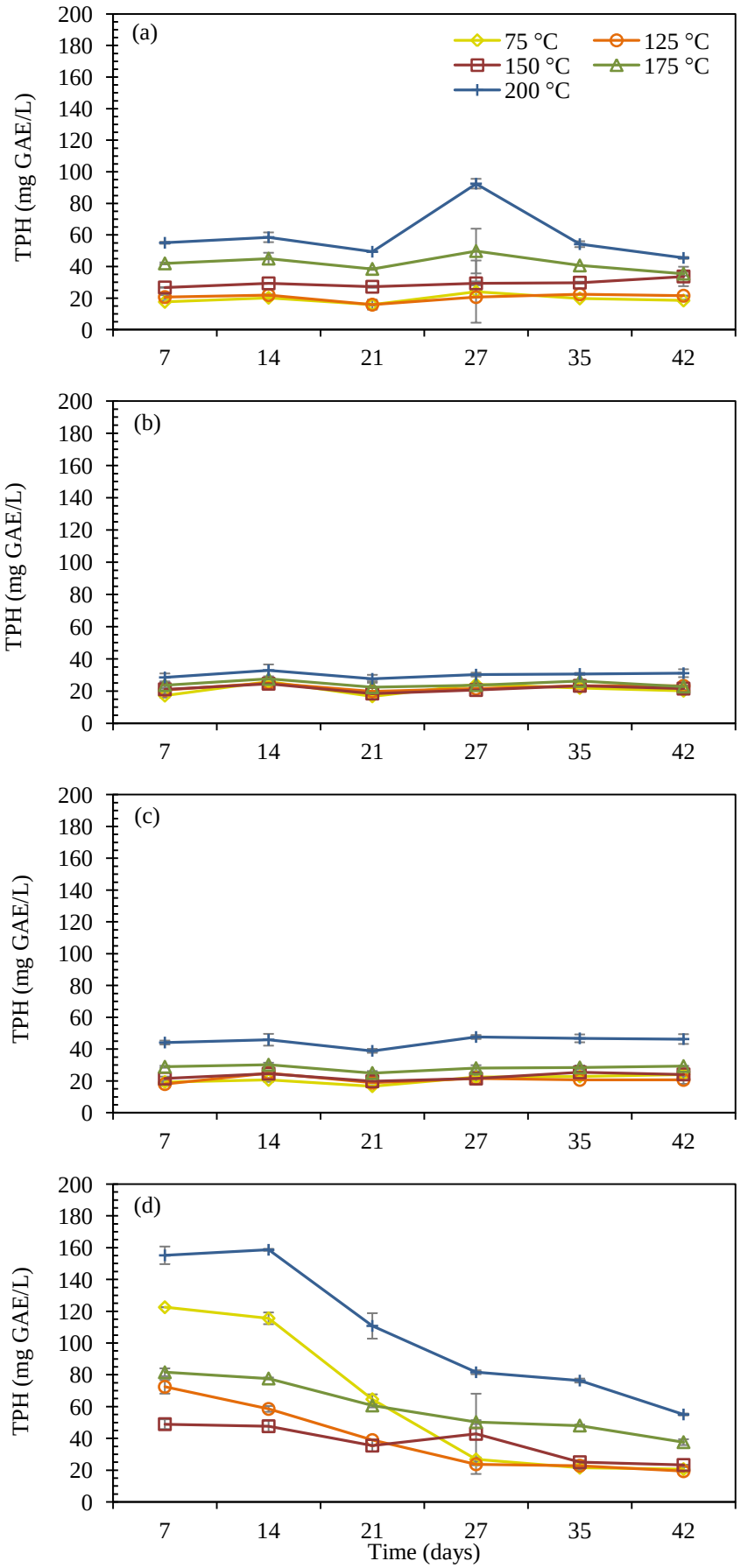


Figure 4-8: TPH variation during BMP assays for (a) WW, (b) CGW, (c) OP and (d) JW, pretreated at different temperatures [75 °C (◇), 125 °C (○), 150 °C (□), 175 °C (△), 200 °C (+)], (error bars represent standard deviation)

4.4.2.3 Kinetic modeling

The results obtained by fitting the methane production data to the first-order exponential model are presented in Table 4-6. The model was found to describe the experimental data at an acceptable level (>0.91) for all samples. The data referring to the rate constant (k) suggest that MW pretreatment did not affect the anaerobic digestion kinetics of all substrates in the same manner. More specifically, for WW and JW, k values are noticed to have a decreasing trend as pretreatment temperature increases. This indicates that samples produced at higher temperatures were probably more difficult to degrade, due to the eventual presence of recalcitrant and/or inhibitory compounds, as it was described earlier, especially for JW, resulting in a slower process, hence the lower k values. Indeed, maximum methane productions (B_0) in combination with the results presented in Fig. 4-6 reveal that the digestion process was mostly retarded rather than prevented. In fact, except for the JW-samples obtained at 175 and 200 °C, the data referring to the remaining WW- and JW-samples suggest that simply more time was required for completely degrading them, albeit without the responsible microbes suffering from significant impediments. On the contrary, no specific tendency is observed for CGW, since all rate constants are found on similar levels. Finally, for OP it is noticed that k values are characterized by an initial decrease for the temperature range between 75 and 150 °C, where the minimum value is found, followed by a subsequent increase until 200 °C, where the maximum value is found. The above mentioned behaviors suggest that in the cases of the two latter substrates, the kinetics of anaerobic digestion of the different MW-pretreated samples were probably similarly affected by the likely presence of recalcitrant and inhibitory compounds.

4.4.3 Energy considerations

The energy requirements for MW pretreatment of the investigated substrates at different temperatures, as well as the energy recovered in the form of heat and methane were calculated in order to provide the energy balance presented in Table 4-6. The specific method being used in the present study for MW pretreatment of the substrates provides for the use of four vessels at every run. Therefore, the calculations regarding specific energy consumption (E_C) and specific energy released in the form of heat (E_Q) were made considering the amount of substrate corresponding to four vessels. Moreover, the specific energy production in the form of methane was calculated using the quantity of gas being produced from each pretreated sample at STP conditions.

A negative energy balance is observed at all pretreatment temperatures, for all investigated substrates. At the same time, the ratio between the energy input and the energy output (E_i/E_o) is higher than unity and in the ranges of 5.2–7.4, 4.4–4.6, 4.3–4.8 and 5.4–8.5, for WW, CGW, OP and JW, respectively. All the above signify that the MW pretreatment process adopted in this study has high energy requirements, which are not balanced by the amount of energy that could potentially be recovered in the form of methane and heat. Negative balances were also obtained in previous studies for MW pretreatment of sludge (Appels et al., 2013; Houtmeyers et al., 2014) and lignocellulosic organic fractions of municipal solid waste (Pecorini et al., 2016). Similarly, Jackowiak et al. (2011a), Passos et al. (2013) and Hu et al. (2012), who studied MW pretreatment of wheat straw, microalgae and cattail, respectively, when determining the ratio between the energy consumed for pretreatment and the energy recovered, found values far greater than unity. However, it is

worth mentioning that in the latter studies, the energy recovered included only the amount corresponding to the produced methane, whereas in the present study it also includes the energy produced in the form of heat. Obviously, energy consumption is found more elevated at increasing pretreatment temperatures. At the same time, higher temperatures also result in larger amounts of energy produced in the form of heat. Considering all the above, it would appear that the MW pretreatment process used in the present study is not economically feasible under the applied conditions. Therefore, some adjustments aiming at reducing the amount of energy being consumed during pretreatment would seem appropriate. Since exposure time is an important parameter as far as energy consumption is concerned, the application of holding times lower than 5 min and/or heating rates higher than 10 °C/min would be worth investigating. Moreover, increasing the density of the pretreatment slurry (solid to liquid ratio) could eventually improve the efficiency of the process. This would ensure a better exploitation of the energy being consumed during pretreatment, by maximizing the amount of substrate being treated. Passos et al. (2013) also suggested that higher biomass concentrations during MW pretreatment may result in an improved energy balance.

Table 4-7: Energy balance

Substrates	Pretreatment temperature (°C)	E_C	E_M	E_Q	E_T	E_i/E_o
WW	75	142069	5592	13488	-122989	7.4
	125	211711	5513	30347	-175850	5.9
	150	246531	5175	38777	-202579	5.6
	175	281352	4719	47207	-229426	5.4
	200	316173	4806	55637	-255730	5.2
CGW	75	48987	6143	4565	-38279	4.6
	125	73000	5876	10272	-56852	4.5
	150	85007	6145	13125	-65737	4.4
	175	97014	5809	15978	-75226	4.5
	200	109020	4945	18831	-85244	4.6
OP	75	70264	8033	6627	-55604	4.8
	125	104708	8225	14912	-81571	4.5
	150	121929	9624	19054	-93251	4.3
	175	139151	6532	23196	-109422	4.7
	200	156373	7228	27338	-121806	4.5
JW	75	233621	5265	22247	-206109	8.5
	125	348141	4913	50055	-293173	6.3
	150	405401	5943	63960	-335498	5.8
	175	462661	6244	77864	-378552	5.5
	200	519921	3699	91768	-424454	5.4

4.5 Conclusions

This study aimed at evaluating the effect of microwave (MW) pretreatment on the solubilization and the degradability of four lignocellulosic agroindustrial waste, namely winery waste (WW), cotton gin waste (CGW), olive pomace (OP) and juice industry waste (JW). It was demonstrated that operational parameters such as solid to liquid ratio, heating rate and holding time at a targeted temperature did not particularly affect the solubilization of the investigated substrates. On the other hand, the variation in pretreatment temperature had a major effect on material solubilization. Moreover, it was concluded that the final effect of MW pretreatment may vary among substrates, depending on their specific characteristics in combination with the conditions applied. The results obtained for WW suggested that during MW pretreatment the most easily degradable matter of this substrate was solubilized and transferred to the liquid phase, while the portion retained in the solid phase, although discrete in quantity, was probably less prone to degradation. The probable presence of recalcitrant compounds may have also contributed to the latter fact resulting in a slower digestion process. As far as JW is concerned, pretreatment seems to have caused the solubilization of most of the organic matter available on this substrate. Nevertheless, methane production data indicated that the portion being retained on the solid matrix had a high degree of biodegradability, with the samples obtained at lower pretreatment temperatures probably containing a higher amount of easily degradable matter. On the other hand, the results referring to CGW and OP lead to the conclusion that MW pretreatment exerted the most significant effect on the solid fraction of these two substrates. In fact, high solids retention and low solubilization both suggested that only a low portion of easily degradable matter was released in the liquid phase, while probably another portion was made more available due to the removal of inhibiting factors. However, the relatively low biodegradability levels are indicative of the presence of either recalcitrant (particularly in the case of CGW) or/and inhibitory compounds (particularly in the case of OP) on the solid samples as well. This seems to be especially true for OP, which appears to have been subjected to most structural changes, among investigated substrates. Ultimately, the application of MW treatment prior to anaerobic digestion did not lead to enhanced methane production from the investigated substrates. Nevertheless, it may be concluded that, in case MW irradiation was going to be used as a pretreatment, those samples produced at temperatures ranging from 125 to 150 °C would have a higher probability of being suitable for methane production. Moreover, in order to improve the energy efficiency of the process, a combination of such temperatures with lower exposure times and higher solid to liquid ratios would be worth investigating.

4.6 References

Altaş, L. (2009). Inhibitory effect of heavy metals on methane-producing anaerobic granular sludge. *Journal of Hazardous Materials*, *162*(2–3), 1551–1556.

Appels, L., Houtmeyers, S., Degrève, J., Van Impe, J., Dewil, R. (2013). Influence of microwave pre-treatment on sludge solubilization and pilot scale semi-continuous anaerobic digestion. *Bioresource Technology*, *128*, 598–603.

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., Lens, P.N.L. (2014). Pretreatment methods to enhance anaerobic digestion of organic solid waste. *Applied Energy*, *123*, 143–156.

Beszédes, S., László, Z., Szabó, G., Hodúr, C. (2011). Effects of microwave pretreatments on the anaerobic digestion of food industrial sewage sludge. *Environmental Progress & Sustainable Energy, 30*(3), 486–492.

Bougrier, C., Delgenès, J.P., Carrère, H. (2008). Effects of thermal treatments on five different waste activated sludge samples solubilisation, physical properties and anaerobic digestion. *Chemical Engineering Journal*, *139*(2), 236–244.

Chen, W.H., Ye, S.C., Sheen, H.K. (2012). Hydrothermal carbonization of sugarcane bagasse via wet torrefaction in association with microwave heating. *Bioresource Technology*, *118*, 195–203.

De Souza, R.O.M.A. (2015). Theoretical Aspects of Microwave Irradiation Practices. In Production of Biofuels and Chemicals with Microwave, in: Z. Fang, R.L. Smith, X. Qi (Eds.), Production of Biofuels and Chemicals with Microwave. Springer, Netherlands, pp. 3-16.

Eskicioglu, C., Prorot, A., Marin, J., Droste, R.L., Kennedy, K.J. (2008). Synergetic pretreatment of sewage sludge by microwave irradiation in presence of H_2O_2 for enhanced anaerobic digestion. *Water Research, 42*(18), 4674–4682.

Fernández-Cegrí, V., De la Rubia, M.A., Raposo, F., Borja, R. (2012). Effect of hydrothermal pretreatment of sunflower oil cake on biomethane potential focusing on fibre composition. *Bioresource Technology, 123*, 424–429.

Haghighi Mood, S., Hossein Golfeshan, A., Tabatabaei, M., Salehi Jouzani, G., Najafi, G.H., Gholami, M., Ardjmand, M. (2013). Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. *Renewable and Sustainable Energy Reviews, 27*, 77–93.

Hendriks, A.T.W.M., Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology, 100*, 10–18.

Hosseini Koupaie, E., Eskicioglu, C. (2015). Below and above boiling point comparison of microwave irradiation and conductive heating for municipal sludge digestion under identical heating/cooling profiles. *Bioresource Technology*, *187*, 235–245.

Hosseini Koupaie, E., Eskicioglu, C. (2016). Conventional heating vs. microwave sludge pretreatment comparison under identical heating/cooling profiles for thermophilic advanced anaerobic digestion. *Waste Management*, *53*, 182–195.

Houtmeyers, S., Degrève, J., Willems, K., Dewil, R., Appels, L. (2014). Comparing the influence of low power ultrasonic and microwave pre-treatments on the solubilisation and semi-continuous anaerobic digestion of waste activated sludge. *Bioresource Technology*, *171*, 44–49.

Hu, Z.H., Yue, Z.B., Yu, H. Q., Liu, S.Y., Harada, H., Li, Y.Y. (2012). Mechanisms of microwave irradiation pretreatment for enhancing anaerobic digestion of cattail by rumen microorganisms. *Applied Energy*, *93*, 229–236.

Huang, Y.F., Chen, W.R., Chiueh, P.T., Kuan, W.H., Lo, S.L. (2012). Microwave torrefaction of rice straw and pennisetum. *Bioresource Technology*, *123*, 1–7.

Jackowiak, D., Bassard, D., Pauss, A., Ribeiro, T. (2011a). Optimisation of a microwave pretreatment of wheat straw for methane production. *Bioresource Technology*, *102*(12), 6750–6756.

Jackowiak, D., Frigon, J.C., Ribeiro, T., Pauss, A., Guiot, S. (2011b). Enhancing solubilisation and methane production kinetic of switchgrass by microwave pretreatment. *Bioresource Technology*, *102*(3), 3535–3540.

Kim, T.H., Ryu, H.J., Oh, K.K. (2016). Low acid hydrothermal fractionation of Giant Miscanthus for production of xylose-rich hydrolysate and furfural. *Bioresource Technology*, *218*, 367–372.

Kratchanova, M., Pavlova, E., Panchev, I. (2004). The effect of microwave heating of fresh orange peels on the fruit tissue and quality of extracted pectin. *Carbohydrate Polymers*, *56*(2), 181–185.

Kuglarz, M., Karakashev, D., Angelidaki, I. (2013). Microwave and thermal pretreatment as methods for increasing the biogas potential of secondary sludge from municipal wastewater treatment plants. *Bioresource Technology*, *134*, 290–297.

La Cara, F., Ionata, E., Del Monaco, G., Marcolongo, L., Gonçalves, M.R., Marques, I.P. (2012). Olive mill wastewater anaerobically digested: Phenolic compounds with antiradical activity. *Chemical Engineering Transactions*, *27*, 325–330.

Li, H., Qu, Y., Yang, Y., Chang, S., Xu, J. (2016). Microwave irradiation – A green and efficient way to pretreat biomass. *Bioresource Technology*, *199*, 34–41.

Liu, X., Wang, W., Gao, X., Zhou, Y., Shen, R. (2012). Effect of thermal pretreatment on the physical and chemical properties of municipal biomass waste. *Waste Management*, *32*(2), 249–255.

Ma, J., Duong, T.H., Smits, M., Verstraete, W., Carballa, M. (2011). Enhanced biomethanation of kitchen waste by different pre-treatments. *Bioresource Technology*, *102*(2), 592–599.

Marculescu, C., Ciuta, S. (2013). Wine industry waste thermal processing for derived fuel properties improvement. *Renewable Energy*, *57*, 645–652.

Marin, J., Kennedy, K.J., Eskicioglu, C. (2010). Effect of microwave irradiation on anaerobic degradability of model kitchen waste. *Waste Management*, *30*(10), 1772–1779.

Merino-Pérez, O., Martínez-Palou, R., Labidi, J., Luque, R. (2015). Microwave-assisted pretreatment of lignocellulosic biomass to produce biofuels and value-added products, in: Z. Fang, R.L. Smith, X. Qi (Eds.), Production of Biofuels and Chemicals with Microwave. Springer, Netherlands, pp. 197-224.

Mollekopf, N., Treppe, K., Dixit, O., Bauch, J., Führlich, T. (2011). Simultaneous drying and modification of properties of starch using vacuum microwave treatment, *Drying Technology*, *29*, 599-605.

Nain, M. Z., Jawed, M. (2006). Performance of anaerobic reactors at low organic load subjected to sudden change in feed substrate types. *Journal of Chemical Technology & Biotechnology*, *81*, 958–965.

Negro, V., Ruggeri, B., Mancini, G., Fino, D. (2016). Recovery of D-limonene through moderate temperature extraction and pyrolytic products from orange peels. *Journal of Chemical Technology & Biotechnology*.

Oliveira, M., Duarte, E. (2016). Integrated approach to winery waste: waste generation and data consolidation. *Frontiers of Environmental Science & Engineering*, *10*(1), 168–176.

Passos, F., Solé, M., García, J., Ferrer, I. (2013). Biogas production from microalgae grown in wastewater: Effect of microwave pretreatment. *Applied Energy*, *108*, 168–175.

Pecorini, I., Baldi, F., Carnevale, E.A., Corti, A. (2016). Biochemical methane potential tests of different autoclaved and microwaved lignocellulosic organic fractions of municipal solid waste. *Waste Management*, *56*, 143-150.

Pellera F.-M., Gidarakos E. (2014), Effect of microwave pretreatment on methane potential of lignocellulosic materials, *5th International Symposium on Energy from Biomass and Waste, VENICE 2014*, San Servolo, Venice, Italy, 17 – 20 November.

Pellera, F.-M., Gidarakos, E. (2016). Effect of substrate to inoculum ratio and inoculum type on the biochemical methane potential of solid agroindustrial waste. *Journal of Environmental Chemical Engineering*, *4*(3), 3217–3229.

Placido, J., Capareda, S. (2013). Evaluation of ligninolytic enzymes, ultrasonication and liquid hot water as pretreatments for bioethanol production from cotton gin trash, in: *ASABE Annual International Meeting*. pp. 203–208.

Raposo, F., Fernández-Cegrí, V., De la Rubia, M.A., Borja, R., Béline, F., Cavinato, C., Demirer, G., Fernández, B., Fernández-Polanco, M., Frigon, J.C., Ganesh, R., Kaparaju, P., Koubova, J., Méndez, R., Menin, G., Peene, A., Scherer, P., Torrijos, M.. Uellendahl, H.. Wierinck, I., de Wilde, V. (2011). Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. *Journal of Chemical Technology & Biotechnology*, *86*, 1088-1098.

Rincón, B., Bujalance, L., Fermoso, F.G., Martín, A., Borja, R. (2013). Biochemical methane potential of two-phase olive mill solid waste: Influence of thermal pretreatment on the process kinetics. *Bioresource Technology*, *140*, 249–255.

Rincón, B., Fermoso, F.G., Borja, R. (2012). Olive oil mill waste treatment: improving the sustainability of the olive oil industry with anaerobic digestion technology, INTECH Open Access Publisher.

Ruiz, B., Flotats, X. (2014). Citrus essential oils and their influence on the anaerobic digestion process: an overview. *Waste Management*, *34*(11), 2063–79.

Sapci, Z. (2013). The effect of microwave pretreatment on biogas production from agricultural straws. *Bioresource Technology*, *128*, 487–494.

Sapci, Z., Morken, J., Linjordet, R. (2013). An investigation of the enhancement of biogas yields from lignocellulosic material using two pretreatment methods: Microwave irradiation and steam explosion. *BioResources*, *8*(2), 1976–1985.

Shahriari, H., Warith, M., Hamoda, M., Kennedy, K. (2013). Evaluation of single vs. staged mesophilic anaerobic digestion of kitchen waste with and without microwave pretreatment. *Journal of Environmental Management*, *125*, 74–84.

Shahriari, H., Warith, M., Hamoda, M., Kennedy, K.J. (2012). Anaerobic digestion of organic fraction of municipal solid waste combining two pretreatment modalities, high temperature microwave and hydrogen peroxide. *Waste Management*, *32*(1), 41–52.

Singh, R., Krishna, B. B., Kumar, J., Bhaskar, T. (2016). Opportunities for utilization of non-conventional energy sources for biomass pretreatment. *Bioresource Technology*, *199*, 398–407.

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, *299*, 152–178.

Tyagi, V.K., Lo, S.-L., Rajpal, A. (2014). Chemically coupled microwave and ultrasonic pre-hydrolysis of pulp and paper mill waste-activated sludge: effect on sludge solubilisation and anaerobic digestion. *Environmental Science and Pollution Research International*, *21*(9), 6205–6217.

Wilkins, M.R., Suryawati, L., Maness, N.O., Chrz, D. (2007). Ethanol production by Saccharomyces cerevisiae and Kluyveromyces marxianus in the presence of orange-peel oil. *World Journal of Microbiology and Biotechnology*, *23*(8), 1161–1168.

Zhao, X., Zhang, L., Liu, D. (2012). Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. *Biofuels, Bioproducts and Biorefining*, *6*(4), 465–482.

CHAPTER 5

Chemical pretreatment of solid agroindustrial waste

This chapter investigates the effect of different chemical pretreatments on the solubilization and the degradability of different solid agroindustrial waste, namely winery waste, cotton gin waste, olive pomace and juice industry waste. Eight different reagents were investigated, i.e. sodium hydroxide (NaOH), sodium bicarbonate ($NaHCO_3$), sodium chloride (NaCl), citric acid (H_3Cit), acetic acid (AcOH), hydrogen peroxide (H_2O_2), acetone (Me_2CO) and ethanol (EtOH), under three condition sets resulting in treatments of varying intensity, depending on process duration, reagent dosage and temperature. Results indicated that chemical pretreatment under more severe conditions is more effective on the solubilization of lignocellulosic substrates, such as those of the present study and among the investigated reagents, H_3Cit, H_2O_2, and EtOH appeared to be the most effective to this regard. At the same time, although chemical pretreatment in general did not improve the methane potential of the substrates, moderate to high severity conditions were found to generally be the most satisfactory in terms of methane production from pretreated materials. In fact, moderate severity treatments using EtOH for winery waste, H_3Cit for olive pomace and H_2O_2 for juice industry waste and a high severity treatment with EtOH for cotton gin waste, resulted in maximum specific methane yield values. Ultimately, the impact of pretreatment parameters on the different substrates seems to be dependent on their characteristics, in combination with the specific mode of action of each reagent. The overall energy balance of such a system could probably be improved by using lower operating powers and higher solid to liquid ratios.

5.1 Introduction

Anaerobic digestion is a biological process, in which a microbial consortium degrades organic substrates in the absence of oxygen. This process is comprised of four main steps, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis, and results in the production of biogas, mainly composed of CH_4 and CO_2, and digestate. Anaerobic digestion has been widely used as an organic waste stabilization method, while lately it has been intensively studied as a promising alternative to traditional energy production technologies, due to its limited environmental impacts (Ariunbaatar et al., 2014; Zheng et al., 2014). This technology is characterized by a high potential for energy recovery, which makes it more efficient in terms of energy generation from organic materials, compared with other biological and thermo-chemical processes. The use of more sustainable energy sources instead of fossil fuels has nowadays become necessary, in order to effectively reduce greenhouse gas emissions. Anaerobic digestion represents a viable option for such a purpose, since it captures and utilizes the methane that would otherwise be naturally produced through the decomposition of organic materials deposited in landfills, and ultimately be released in the atmosphere (Bolado-Rodríguez et al., 2016; Song et al., 2014; Zheng et al., 2014).

Agricultural and agroindustrial waste and by-products represent viable feedstock for anaerobic digestion systems. Their use for such purpose is considered advantageous, since they are highly available in large amounts, while they can also be characterized as renewable and low cost resources (Fernández-Cegrí et al., 2012; Zhao et al., 2014; Zheng et al., 2014). Agroindustrial activities are particularly important in Mediterranean countries, since they represent a significant sector of the economy. Among the most widespread and profitable activities of this region, are the wine and olive oil production industries, as well as the citrus fruits, especially oranges, and cotton processing activities, with all of them resulting in the generation of large amounts of waste materials (Pellera and Gidarakos, 2016). However, the performance of anaerobic digestion of such substrates is often limited, due to their complex lignocellulosic composition. Cellulose, hemicellulose and lignin are the main components of lignocellulosic materials and among them, lignin is the most resistant to biodegradation, constituting the barrier preventing access of the microbes to cellulose (Fernández-Cegrí et al., 2012). The main structural and compositional characteristics of lignocellulosic biomass, which affect their degradability, are cellulose crystallinity, accessible surface area, degree of cellulose polymerization, presence of lignin and hemicellulose, and degree of hemicellulose acetylation. In order to overcome these obstacles, treatment is frequently applied prior to anaerobic digestion of such substrates (Zheng et al., 2014). The objective of any pretreatment method is to disrupt the complex structure of lignocellulosic materials, by reducing the crystallinity as well as the degree of polymerization of cellulose, partially polymerizing and removing hemicellulose, altering and removing lignin and increasing the surface area and porosity of the materials (Behera et al., 2014; Singh et al., 2015). A pretreatment process should be able to achieve an improvement in the digestibility of the treated material, while minimizing environmental pollution, having low energy requirements and limiting the production of potentially inhibiting degradation products, such as organic acids, furan derivatives and phenol compounds (Banerjee et al., 2016; Bolado-Rodríguez et al., 2016). Pretreatment methods, depending on their basic mode of action, can primarily be categorized as physical, chemical and biological, with each category including several separate technologies (Bolado-Rodríguez et al., 2016).

Compared with the other methods, chemical pretreatments are considered very promising, since they can be quite effective in degrading more complex-structured substrates

(Behera et al., 2014; Song et al., 2014). Such methods can be performed by applying a variety of chemical processes of different natures. Chemical pretreatments with alkaline reagents involve the use of compounds such as sodium hydroxide, calcium hydroxide and aqueous ammonia (Liew et al., 2011; López González et al., 2013; Pellera et al., 2016; Sambusiti et al., 2013; Song et al., 2014), while in pretreatments with acid reagents both inorganic and organic acids, such as sulfuric acid, hydrochloric acid, phosphoric acid, acetic acid, citric acid, oxalic acid and maleic acid, are used (Amnuaycheewa et al., 2016; Assawamongkholsiri et al., 2013; Lim et al., 2013; Monlau et al., 2013; Scordia et al., 2011; Song et al., 2014; Tian et al., 2016). Oxidative treatments include ozonation (Ariunbaatar et al., 2014) and treatment with peroxides, with their majority particularly focusing on hydrogen peroxide (Monlau et al., 2012; Silverstein et al., 2007; Song et al., 2014). Other types of chemical pretreatments can utilize organic solvents (Kabir et al., 2014), as well as inorganic salts (Banerjee et al., 2016; Kang et al., 2013; Liu et al., 2009). The effectiveness of such pretreatments on different substrates, is highly dependent on the type of substrate, as well as on the type of method being used. In fact, different results will be obtained when treating different materials with the same pretreatment, as a result of the complexity and variability in lignocellulosic structures (Kang et al., 2013; Sambusiti et al., 2013; Zheng et al., 2014). At the same time, variations will also be observed in the results obtained through different pretreatments of the same substrate, since each method acts on different parts of the material (Song et al., 2014). Consequently, the investigation of various combinations of pretreatment methods and substrates is very useful for better understanding the particular effects of different treatments on specific types of materials. The present study makes a significant contribution to this challenging topic.

This study investigates the effect of chemical pretreatment on four of the most widespread solid agroindustrial waste of the Mediterranean region, namely winery waste (WW), cotton gin waste (CGW), olive pomace (OP) and juice industry waste (JW). The main objective was to determine the impact of such a treatment on the solubilization of these materials, as well as on their degradability under anaerobic conditions for methane production. For this purpose, a number of batch assays were conducted, in which different reagent dosages, process durations and temperatures were adopted. Pretreatment was applied using eight different chemical reagents, i.e. NaOH, $NaHCO_3$, NaCl, H_3Cit, AcOH, H_2O_2, Me_2CO and EtOH, in order to also determine the influence of different reagent natures (alkaline, acidic, saline, oxidative, organic) on the final results. Materials solubilization was assessed by analyzing the liquid fractions obtained after pretreatment for soluble chemical oxygen demand and total phenols concentrations, while Biochemical Methane Potential (BMP) assays were adopted for determining the methane potential of solid pretreated samples.

5.2 Materials and methods

5.2.1 Substrates and inoculum

Four agroindustrial waste typical of the Mediterranean area were used in the present study. More specifically, winery waste (WW), composed of grape skins, seeds and stalks, cotton gin waste (CGW) comprising of cotton fiber, stalks, bur and leaves, olive pomace (OP), which is the solid waste obtained in three-phase olive mills and juice industry waste (JW) comprised of orange peels. Sample handling was not the same for the four substrates, due to their different characteristics. For WW and JW, the materials were initially separated in batches, placed in

zip-lock bags and stored at -20 °C. One day before each use, appropriate amounts were transferred to 4 °C and on the day of the experiment they were comminuted without drying using a food processor. On the other hand, CGW was immediately dried at 60 °C and then comminuted to a particle size less than 500 μm, using a universal cutting mill, while OP was immediately stored at -20 °C without size reduction. The full characterization of the four substrates has been performed in a previous study (Pellera and Gidarakos, 2016) (Chapter 3).

The inoculum used in this study consisted of mesophilic anaerobic sludge with total solids (TS) and volatile solids (VS) contents of 2.02% and 1.35%, respectively, (VS/TS=0.67), and it was obtained from an anaerobic digester situated in the Municipal Wastewater Treatment Facility of Chania, Crete.

5.2.2 Chemical pretreatment

Chemical pretreatment was conducted by using eight different reagents, i.e. sodium hydroxide (NaOH), sodium bicarbonate ($NaHCO_3$), citric acid (H_3Cit), acetic acid (AcOH), sodium chloride (NaCl), hydrogen peroxide (H_2O_2), acetone (Me_2CO) and ethanol (EtOH). Moreover, the effect of operating parameters, such as reagent dosage, process duration and process temperature was evaluated by investigating three different condition sets (Sets A, B and C) for each reagent. The specific experimental conditions for each set were selected on the basis of the results of a preliminary study (Pellera et al., 2016), in which the alkaline (NaOH) pretreatment of olive pomace was investigated by testing five values for reagent dosage (0–4 mmol/gVS) and five values for process duration (1–24 h) at two different temperatures (25 and 90 °C). The final selected conditions can be seen in Table 5-1.

During the experimental procedure, initially, appropriate amounts of substrates and reagent solutions (1 gVS/100 mL) were introduced in 250 mL glass flasks. Subsequently, the flasks were covered with an aluminum foil and were kept at each appropriate temperature for the predetermined time periods. In order to maintain a constant temperature, either an incubator (for the process conducted at 25 °C) or an oven (for the processes conducted at 60 and 90 °C) were used. After the end of the pretreatment process, the final pH of the slurries was measured and the samples were then centrifuged at 3,900 rpm for 15 min, in order to separately collect the solid and liquid fractions. The latter were filtered through a 0.45 μm pore size membrane filter, for sCOD and TPH concentrations determination, while the former were stored at -20 °C until further use. Moreover, a portion of each solid sample was dried at 60 °C for further analyses, which included the determination of TS and VS contents, as well as elemental (CHNS) and fiber composition. All experiments were performed in triplicate.

Table 5-1: Chemical pretreatment experimental conditions

Condition sets	Reagent dosage (mmol/$gVS_{substrate}$)	Process duration (h)	Process temperature (°C)
Set A	0.25	16	25
Set B	0.5	8	60
Set C	1	4	90

5.2.3 Biochemical methane potential assays

The experimental apparatus for BMP (biochemical methane potential) assays consisted of 250 mL conical flasks covered with rubber stoppers. Three PVC (Polyvinyl chloride) tubes were inserted in the stoppers, which allowed N_2 flushing in the flasks, daily methane measurement and weekly sampling for pH measurement and TPH determination.

The working volume for the BMP assays was set to 100 mL, the inoculum quantity was the same for all assays, i.e. 15 gVS/L, while the substrate to inoculum ratio (SIR) on a VS basis ($gVS_{substrate}/gVS_{inoculum}$) was 0.5 for WW and JW and 0.25 for CGW and OP. These values were chosen on the basis of the results of a previous study (Pellera and Gidarakos, 2016) (Chapter 3). Blank assays (SIR=0), containing only the inoculum were also performed, in order to determine the residual methane potential of the inoculum and to then be able to calculate the net methane potential of each substrate.

BMP assays were carried out by firstly introducing the inoculum and substrates in the flasks, in appropriate amounts and by subsequently adding deionized water to the mixture, if needed, in order to bring the total volume to approximately 100 mL. After adjusting the pH of the mixture at 7.8±0.05, the flasks were covered with the rubber stoppers and finally flushed with N_2 for 2 min. The reactors were finally placed in an incubator set at 35 °C. Methane production was measured daily for the first seven days of incubation and subsequently every two days. BMP assays were terminated when methane production was undetectable or less than 5% of the total amount. All assays were performed in duplicate.

5.2.4 Analytical methods

TS and VS contents were determined according to APHA (American Public Health Association) method 2540G. Elemental analysis (C, H, N, S) of the substrates was performed using an EA300 Euro Vector elemental analyzer, via flash combustion at 1,020 °C. The oxygen content was determined by difference, considering the VS content of each sample. pH was determined using a portable pH-meter. The sCOD concentrations in the liquid fractions obtained after pretreatment were determined through APHA method 5220C, while TPH concentrations were determined according to Folin-Ciocalteu's method, on the basis of the procedure described by Singleton et al. (1999). Briefly, 40 μL of sample were placed in glass cuvettes, into which 3.16 mL of deionized water and 200 μL of Folin-Ciocalteu reagent were then added. After mixing using a vortex mixer, the cuvettes were left for 4 minutes and subsequently 600 μL of sodium carbonate solution (20%) were added to the mixture. Finally, the solutions were once again mixed and left for 2 h at 20 °C. The absorbance of each solution was determined at 765 nm. The final TPH concentrations are expressed in gallic acid equivalents (GAE). Fiber analysis, i.e. the determination of NDF (Neutral Detergent Fiber), ADF (Acid Detergent Fiber) and ADL (Acid Detergent Lignin), was based on the method described by Fernández-Cegrí et al. (2012). Methane production was determined by means of volume displacement using an 11.2% KOH solution, as it was done in previous studies (Altaş, 2009; Nain and Jawed, 2006). More specifically, each BMP reactor was connected to an inverted bottle containing the alkaline solution. Subsequently, biogas was released to flow inside the bottle, in order to remove CO_2 and H_2S by absorption and leave only CH_4. The volume of CH_4 being transferred to the bottle caused the displacement of an equal amount of KOH solution, which was then quantified using a graduated cylinder.

5.3 Data analysis

5.3.1 Pretreatment mass yields

The yield of the chemical pretreatment process in terms of mass recovery was estimated by calculating three different mass yield values for each pretreated sample, namely Y_{Wet}, Y_{TS} and Y_{VS}. More specifically, the calculations were made by using Equation 1 and considering the initial mass of substrate (m_i) and the mass of sample obtained after each investigated pretreatment (m_p), with the values being expressed in three different ways, i.e. a) $gWet_{pretreated\ sample}/gWet_{raw\ sample}$ for Y_{Wet}, b) $gTS_{pretreated\ sample}/gTS_{raw\ sample}$ for Y_{TS} and c) $gVS_{pretreated\ sample}/gVS_{raw\ sample}$ for Y_{VS}.

$$Y = \frac{m_p}{m_i} \tag{1}$$

5.3.2 Theoretical methane potential

The Theoretical Methane Potential (TMP) of the chemically pretreated samples at Standard Temperature and Pressure (STP) conditions was estimated through their elemental composition and the stoichiometry of the degradation reaction (Equation 2), using Equation 3 (Lesteur et al., 2010):

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right)H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right)CO_2 + dNH_3 + eH_2S \tag{2}$$

$$TMP\left[\text{mL CH}_{4,\text{STP}}/\text{g VS}\right] = 22.4 \cdot \left[\frac{\left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)}{12a + b + 16c + 14d + 32e}\right] \cdot 1000 \tag{3}$$

5.3.3 Theoretical oxygen demand

The Theoretical Oxygen Demand (TOD) of the four substrates in their untreated (raw) form, as well as after pretreatment at different conditions, was estimated through their elemental composition and the stoichiometry of the oxidation reaction (Equation 4), using Equation 5 (Raposo et al., 2011):

$$C_aH_bO_cN_dS_e + \left(a + \frac{b}{4} - \frac{c}{2} - \frac{3d}{4}\right)O_2 \rightarrow aCO_2 + \left(\frac{b}{2} - \frac{3d}{2}\right)H_2O + dNH_3 \tag{4}$$

$$TOD\left[\text{mg O}_2/\text{g VS}\right] = \frac{\left(2a + \frac{b}{2} - c - \frac{3d}{2}\right) \cdot 16}{12a + b + 16c + 14d} \cdot 1000 \tag{5}$$

5.3.4 Specific Methane Yield

The Specific Methane Yield (SMY) of each chemically pretreated sample, resulting after the end of digestion, was obtained by subtracting the ultimate cumulative methane production of the blank assay (mL CH_4) from the ultimate cumulative methane production of each assay containing the pretreated samples, and by subsequently dividing it either by the total amount of VS of the pretreated samples being added to each reactor, or by the corresponding amount of VS of the raw substrates, calculated on the basis of the mass yields of the pretreatment process. These values were then converted to STP conditions. This way, the SMY was expressed in two different ways, namely SMY_P (mL $CH_{4,\ STP}$/g VS_P) and SMY_{Raw} (mL $CH_{4,\ STP}$/g VS_{Raw}), respectively.

5.3.5 Solubilization

COD solubilization (%) was calculated using the TOD (mg O_2/g VS) of the raw substrates and the sCOD (mg O_2/g VS) values determined for each substrate at the different pretreatment conditions, according to Equation 6.

$$\text{COD solubilization}\,[\%] = \frac{sCOD}{TOD} \cdot 100 \tag{6}$$

5.3.6 Biodegradability Index

The Biodegradability Index (BI) was calculated using the TMP (mL $CH_{4,\ STP}$/g VS) and the SMY_P (mL $CH_{4,\ STP}$/g VS_P) of each pretreated sample, as it is shown in Equation 7.

$$BI\,[\%] = \frac{SMY_P}{TMP} \cdot 100 \tag{7}$$

5.3.7 Kinetic modeling

Methane production was modeled by fitting the data with two kinetic models through non-linear regression, using the Solver tool of Microsoft Office Excel. The goodness of fit was evaluated by taking into consideration both the Residual Sum of Squares (RSS) and the R-square (R^2) values. The first-order exponential and the transference (reaction-curve) models were used (Donoso-Bravo et al., 2010), which are described by Equations 8 and 9, respectively.

$$B = P \cdot \left[1 - \exp(-kt)\right] \tag{8}$$

$$B = P \cdot \left\{1 - \exp\left[-\frac{R_m \cdot (t - \lambda)}{P}\right]\right\} \tag{9}$$

where, t is time (d), B is the cumulative methane production at time t (mL CH_4), P is the methane production potential (mL CH_4), R_m is the maximum methane production rate (mL CH_4/d), k is the rate constant (d^{-1}) and λ is the lag phase (d).

5.3.8 Energy calculations

The specific energy consumption, E_C (kJ/kg VS), resulting from the pretreatment process was calculated according to Equation 10 (Kuglarz et al., 2013).

$$E_C = \frac{P_W \cdot t_E}{m} \tag{10}$$

where, P_W is the power of the oven (888 W), t_E is the exposure time (s) and m is the mass of VS of raw substrate being subjected to pretreatment (kg VS).

The specific energy corresponding to the methane quantity produced from the pretreated samples, E_M (kJ/kg VS), was calculated according to Equation 11 (Passos et al., 2013).

$$E_M = \frac{SMY_{Raw} \cdot \xi}{1000} \tag{11}$$

where, ξ is the lower heating value of methane (35800 kJ/m^3 CH_4).

Considering that pretreatment temperatures of 60 and 90 °C are higher than the temperature of the mesophilic anaerobic digestion (35 °C), the pretreated samples would require an intermediate cooling procedure, which would result in the release of energy in the form of heat. The specific energy corresponding to the potential recovery of the latter amount of energy, E_Q (kJ/kg VS), was calculated according to Equation 12 (Ma et al., 2011).

$$E_Q = \frac{\left[m_s \cdot C_{P,s} \cdot \Delta T + m_w \cdot C_{P,w} \cdot \Delta T\right]}{m} \tag{12}$$

where m_s is the mass (kg) of dry solids of substrate being subjected to pretreatment, m_w is the mass (kg) of water in the vessel during pretreatment (including the water contained in the substrate), $C_{P,s}$ and $C_{P,w}$ are the specific heat capacities of solids (1.95 kJ/kg °C) and water (4.18 kJ/kg °C), respectively and ΔT is the temperature difference (°C) between the temperature of the slurry at the end of pretreatment and the temperature of the mesophilic anaerobic digestion.

The specific energy profit of the pretreatment, E_T (kJ/kg VS), was calculated considering E_M, E_Q and E_C, according to Equation 13 (Kuglarz et al., 2013).

$$E_T = E_M + E_Q - E_C \tag{13}$$

with the sum of E_M and E_Q comprising the specific energy output, E_o (kJ/kg VS), and E_C constituting the specific energy input E_i (kJ/kg VS).

In order to be able to provide an energy balance for the chemical pretreatment process applied in the present study, all the above mentioned energy parameters were expressed as kJ per kg of VS of raw (untreated) substrate, aiming at a more accurate approach.

5.4 Results and discussion

5.4.1 Effect of pretreatment on solubilization

Fig. 5-1 and Table 5-2 show the results regarding sCOD solubilization during chemical pretreatment of the substrates, while Fig. 5-2 presents the corresponding data referring to TPH release. It is generally noticed that increasing the severity of pretreatment, mainly in terms of temperature and reagent dosage leads to higher material solubilization. Specifically, for all reagents, treatments B and C showed higher organic matter release compared with treatment A.

It seems that pretreatment has a positive effect on the solubilization of OP (Fig. 5-1c) already by applying a moderate severity process. In fact, for most reagents, a more pronounced difference in sCOD solubilization is observed between treatments A and B, compared with the difference between treatments B and C. There are only three exceptions (NaOH, H_3Cit and H_2O_2), for which the observed differences are similar. Conversely, in the case of CGW (Fig. 5-1b) a severity level corresponding to treatment C is able of achieving a quite higher degree of solubilization for this material, compared with the two lower-severity treatments (A and B). This is true for all reagents except for EtOH, for which treatment B is the one actually causing a more marked effect on sCOD release. As far as WW (Fig. 5-1a) and JW (Fig. 5-1d) are concerned, only for some of the examined reagents (i.e. $NaHCO_3$, NaCl and AcOH for WW and NaOH, $NaHCO_3$, H_3Cit and H_2O_2 for JW), the treatments with the highest severity (C) caused a more enhanced solubilization of these substrates, with this being more evident for JW. Regarding the remaining reagents in each case, the increase in severity from A to B and from B to C influences solubilization to a similar degree when using NaOH, H_3Cit and H_2O_2 for WW and NaCl and Me_2CO for JW. Finally, a decreased difference between treatments B and C is shown when using Me_2CO and EtOH with WW and AcOH and EtOH with JW.

Similar observations can generally be made regarding TPH release (Fig. 5-2). Nevertheless, in this case, the impact being exerted by the variation in pretreatment conditions from B to C, appears to be more significant.

When comparing the impact of different reagents on substrate solubilization, it is noticed that the application of the treatments at milder conditions does not allow significant differences to be observed between reagents. In fact, similar sCOD values are obtained for all A treatments, for all substrates. Conversely, the different impact of each reagent is more visible as the treatment severity increases from A to B, while further increase from B to C, causes not only a more pronounced difference between reagents, but also a change in the order concerning their effectiveness. Differences in substrates solubilization with the use of different reagents may be attributed to their properties and the manner in which they affect such substrates. In fact, alkaline, acid and oxidative pretreatments, all cause an increase in the accessible surface area of lignocellulosic materials. However, while the action of alkaline reagents is concentrated more on lignin and less on hemicellulose solubilization, acid reagents act more on the structure of cellulose and hemicellulose (Zheng et al., 2014). More specifically, alkaline pretreatments are known to cause swelling to lignocellulosic materials, as a result of solvation and saphonication reactions. In contrast acid pretreatments lead to the disruption of covalent bonds, hydrogen bonds, and Van der Waals forces connecting the various components of such materials (Song et al., 2014). In treatments using H_2O_2, the strong oxidative action of the reagent, as well as the release of hydroxyl radicals help not only disrupt lignin and hemicellulose structures, but also make a portion of cellulose more

available to microorganisms (Zheng et al. 2014). On the other hand, organic solvents, such as EtOH can cause partial lignin hydrolysis, as well as hemicellulose solubilization, due to their impact on internal lignin bonds and bonds between lignin and hemicellulose (Behera et al., 2014; Kabir et al., 2014). Finally, the use of inorganic salts, such as NaCl has resulted quite effective for hemicellulose degradation (Liu et al., 2009) and removal of the loosely bound portion of lignin (Banerjee et al., 2016). Ultimately, H_3Cit, H_2O_2 and EtOH appeared as the most effective of the eight tested reagents in solubilizing the studied substrates. Consequently, treatments B and C, using these reagents, were adopted for producing the samples for subsequent BMP assays.

The changes in the pH of the slurries at the end of each pretreatment are depicted in Fig. 5-3, where hollow markers represent the initial pH of each solution. After pretreatment was concluded, the final pH of the slurries containing CGW and OP was reduced in the cases of NaOH, $NaHCO_3$ and NaCl, while it was increased for H_3Cit, AcOH, H_2O_2, Me_2CO and EtOH (except for OP with treatment A). Decreased pH values after chemical pretreatment have also been observed in previous studies where NaOH (Sambusiti et al., 2013) and $Ca(OH)_2$ (Fernandes et al., 2009) were used as reagents. This phenomenon is generally attributed to the release of substances, such as organic acids and phenolic compounds, as a result of lignin degradation (Bolado-Rodríguez et al., 2016). On the other hand, increased pH values were found by Zhao et al. (2010), Fernandes et al. (2009) and Zhao et al. (2014) after pretreatment with acetic-propionic acid, maleic acid and acetic acid, respectively. This pH increase could be associated to the consumption of hydrogen ions in the hydrolysis reaction being developed during pretreatment (Zhao et al. 2010). In contrast with the above mentioned results, for WW and JW, pH was found decreased in all treatments, except for those where the two acids (H_3Cit, AcOH) were used. Consequently, in these two cases, pH reduction for H_2O_2, Me_2CO and EtOH treatments, may be attributed to the nature of WW and JW, since both of them are materials of acidic character.

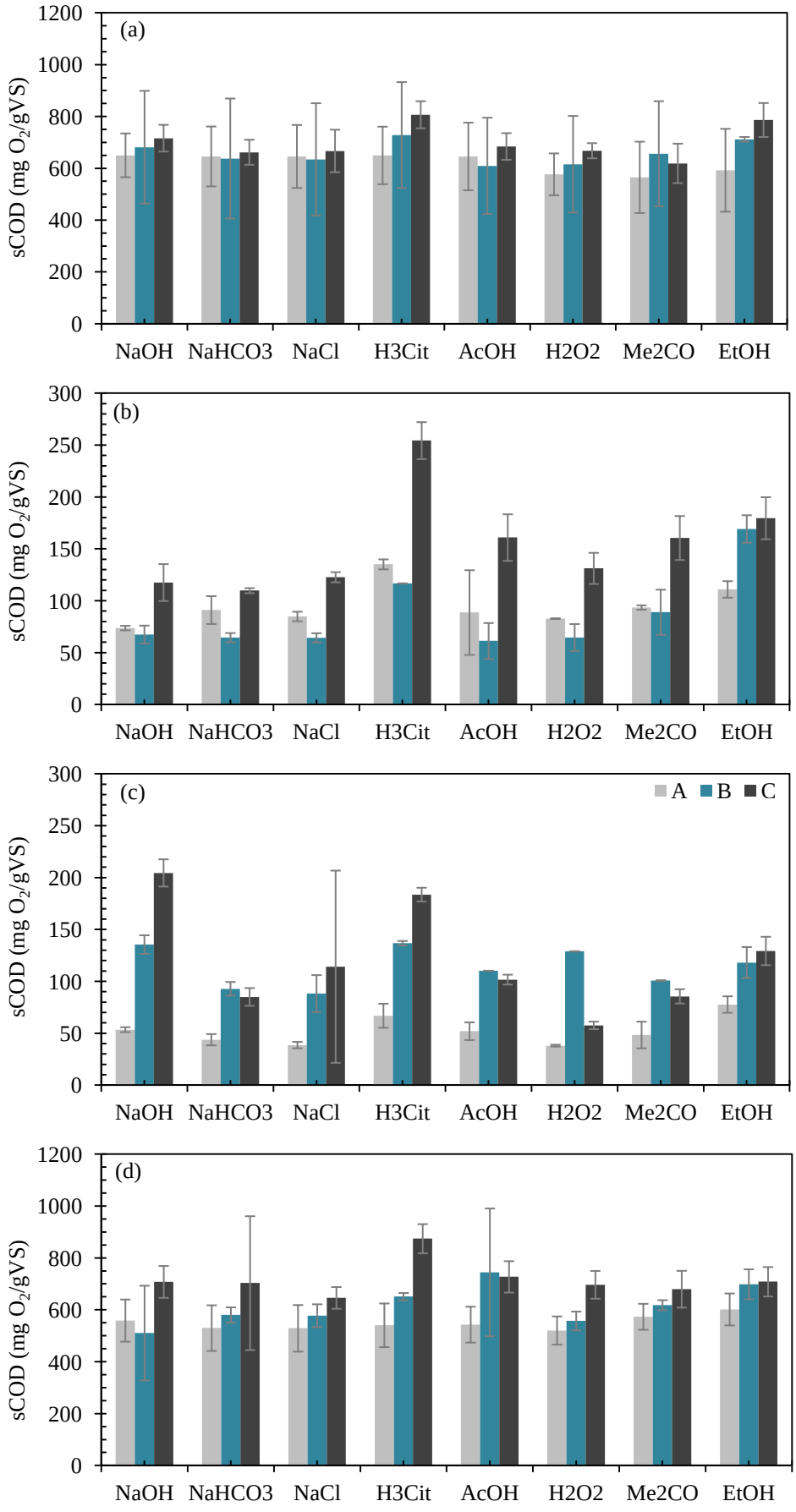


Figure 5-1: sCOD concentrations in the slurries after pretreatment with eight different reagents at three condition sets [A (), B (), C ()], for (a) WW, (b) CGW, (c) OP and (d) JW (error bars represent standard deviation)

Table 5-2: COD solubilization (%) after chemical pretreatment of substrates

Substrate	Condition sets	COD solubilization (%)							
		NaOH	$NaHCO_3$	NaCl	H_3Cit	AcOH	H_2O_2	Me_2CO	EtOH
WW	A	46.5 ± 2.7	46.2 ± 1.6	46.2 ± 3.9	46.5 ± 2.7	46.2 ± 3.9	41.2 ± 1.6	40.4 ± 4.2	53.9 ± 1.4
	B	48.7 ± 15.6	45.6 ± 16.6	45.4 ± 15.5	52.1 ± 14.6	43.6 ± 13.3	44.0 ± 13.3	46.9 ± 14.5	50.9 ± 0.6
	C	51.2 ± 3.7	47.3 ± 3.5	47.7 ± 5.9	57.7 ± 3.7	48.9 ± 3.7	47.8 ± 2.1	44.3 ± 5.5	56.2 ± 4.7
CGW	A	6.58 ± 0.19	8.13 ± 1.20	7.58 ± 0.41	12.1 ± 0.43	7.92 ± 3.64	7.40 ± 0.01	8.35 ± 0.19	9.91 ± 0.71
	B	6.02 ± 0.77	5.76 ± 0.40	5.75 ± 0.39	10.4 ± 0.0	5.47 ± 1.55	5.76 ± 1.17	7.94 ± 1.94	15.1 ± 1.2
	C	10.5 ± 1.6	9.80 ± 0.22	11.0 ± 0.4	22.7 ± 1.6	14.4 ± 2.0	11.7 ± 1.3	14.5 ± 2.1	16.0 ± 1.8
OP	A	3.15 ± 0.14	2.58 ± 0.32	2.27 ± 0.18	3.94 ± 0.68	3.06 ± 0.50	2.24 ± 0.05	2.85 ± 0.76	4.58 ± 0.47
	B	7.99 ± 0.53	5.48 ± 0.39	5.20 ± 1.05	8.07 ± 0.13	6.50 ± 0.01	7.61 ± 0.01	5.94 ± 0.00	6.97 ± 0.88
	C	12.1 ± 0.8	5.01 ± 0.50	6.73 ± 5.47	10.8 ± 0.4	6.00 ± 0.28	3.52 ± 0.21	5.05 ± 0.40	7.63 ± 0.81
JW	A	43.3 ± 6.3	41.0 ± 6.8	40.9 ± 6.9	41.8 ± 6.5	42.0 ± 5.3	40.3 ± 4.2	44.4 ± 3.9	46.4 ± 4.8
	B	39.4 ± 14.1	44.8 ± 2.3	44.6 ± 3.4	50.3 ± 1.1	57.5 ± 19.0	43.0 ± 2.8	47.7 ± 1.5	53.9 ± 4.5
	C	54.6 ± 4.7	54.3 ± 3.5	49.9 ± 3.2	67.5 ± 4.3	56.1 ± 4.7	53.8 ± 4.1	52.5 ± 5.5	54.7 ± 4.4

All values are expressed as average ± standard deviation

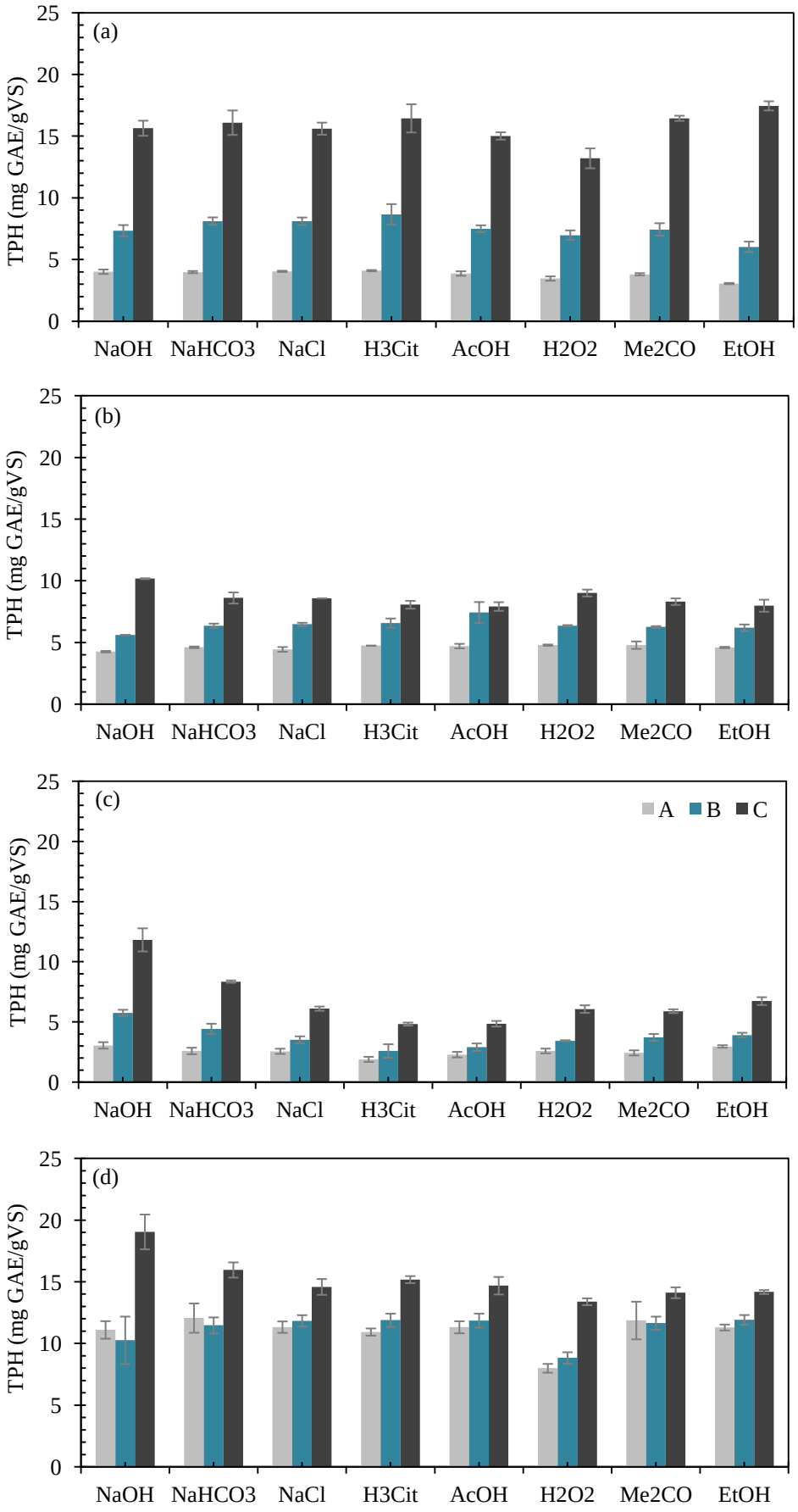


Figure 5-2: TPH concentrations in the slurries after pretreatment with eight different reagents at three condition sets [A (▒), B (■), C (■)], for (a) WW, (b) CGW, (c) OP and (d) JW (error bars represent standard deviation)

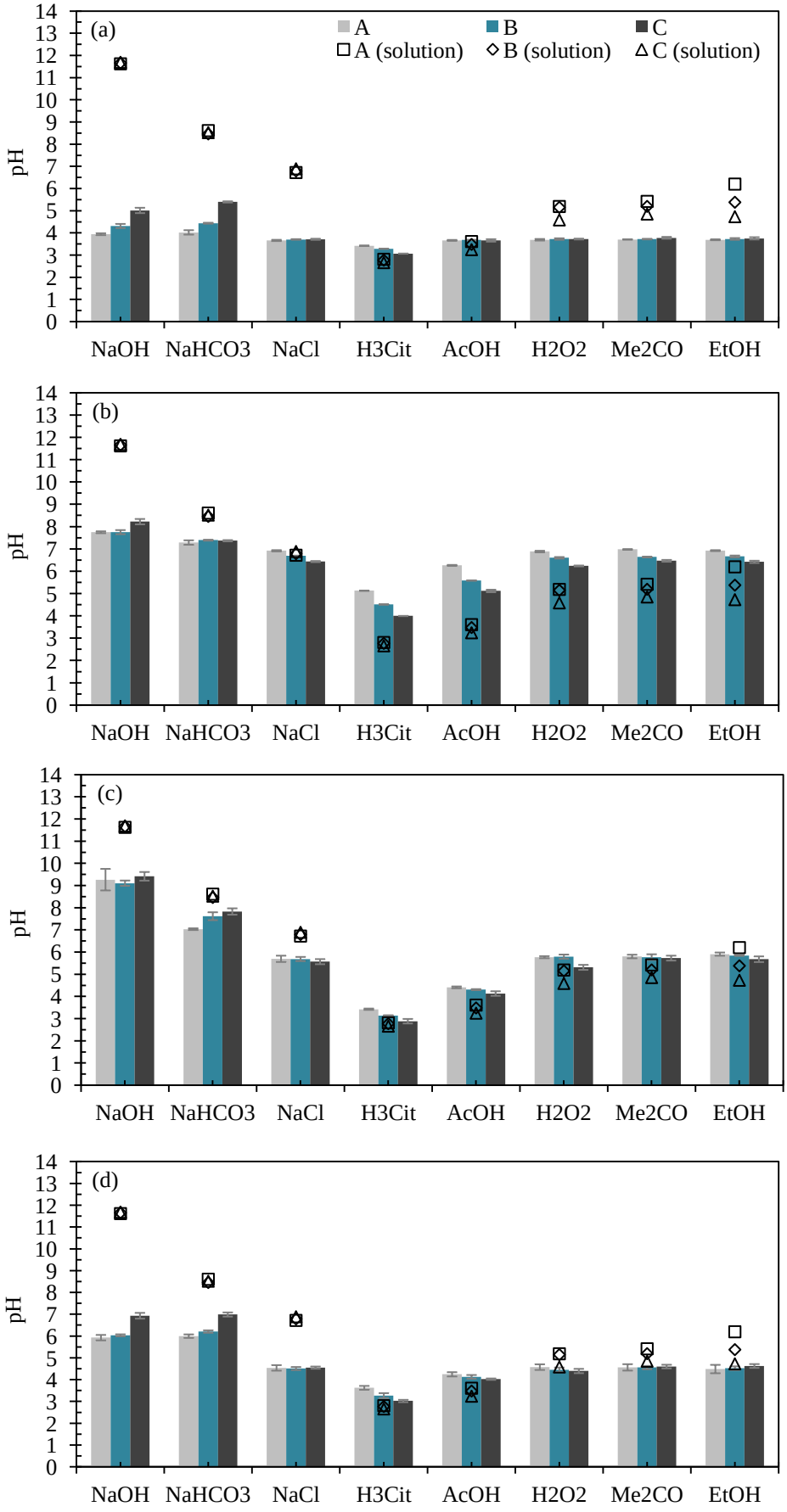


Figure 5-3: pH of the slurries after pretreatment with eight different reagents at three condition sets [A (■, □), B (■, ◊), C (■, Δ)], for (a) WW, (b) CGW, (c) OP and (d) JW (error bars represent standard deviation)

5.4.2 Moisture, VS contents and elemental composition of chemically pretreated samples

The composition of chemically pretreated samples is presented in Table 5-3. Results show that chemical pretreatment led to the production of samples with higher moisture contents, i.e. lower TS contents, compared with the untreated substrates. This suggests that due to the nature of the pretreatment process, some moisture quantity originating from each reagent solution being used, was probably retained within the mass of the samples in the form of free moisture, thus resulting in higher contents. The most significant variation was noticed for CGW with an increase ranging from 38 to 49%, while the substrate which was affected the least is OP, with an increase between 0.4 and 9%. As far as VS/TS is concerned, these values are also found increased after pretreatment, with the least affected substrate in this case being JW. Different types of reagents do not seem to have caused significantly different effects regarding this parameter. Nevertheless, increasing the severity of pretreatment (form B to C) resulted in generally stable values characterized by a slightly increasing trend for all reagents. Tian et al. (2016) also noticed higher values for VS/TS after chemical pretreatment of corn stover with H_2SO_4, AcOH and H_3PO_4, with the increase in acid concentration resulting in a stable behavior. Moreover, in two other studies (Asadieraghi et al., 2014; Jiang et al., 2013) the authors found increased volatile matter contents for lignocellulosic materials being chemically treated with different acids.

Elemental analysis of pretreated samples revealed uniformity in the general effect of pretreatment on the elemental composition of the substrates. In fact, C and H contents of samples obtained after chemical pretreatment were found increased in comparison with the respective values of the untreated substrates. In contrast, O contents were reduced, while the variation of N contents was dependent on each material. These results are in agreement with those found by Lim et al. (2013), Jiang et al. (2013) and Asadieraghi et al. (2014), who in their respective studies also observed increased C and H contents, as well as decreased O contents, after the application of chemical treatments. Moreover, it is noticed that, depending on the substrate, chemical pretreatment impacted each element at a different degree. In fact, for all three reagents being investigated, H content was the most affected by pretreatment in the cases of WW, CGW and JW, while in the case of OP, N content sustained the most significant variation. Nevertheless, when comparing the different types of reagents it is evident that the H and C contents of WW were affected the most by H_2O_2 and EtOH treatments, respectively, independently of severity, while the N content was particularly impacted by the H_3Cit-C and H_2O_2-C treatments. Similarly, H_2O_2-C and EtOH-C treatments exerted the most pronounced effect on C and H contents of JW, while H_2O_2-B and EtOH-B had the most significant effect on N content. H_3Cit was the reagent causing the most important effect on C and H contents of CGW, while both H_2O_2 and EtOH had an analogous result on its N content. In the case of OP, N was much impacted by H_3Cit and H_2O_2, H by EtOH and C by the H_2O_2-C and EtOH-B treatments. C/N and C/H values were affected accordingly, as a result of the different degree of impact of each pretreatment on each element. Specifically, C/H was found reduced, while C/N was found more elevated for pretreated samples compared with untreated substrates. A few exceptions were observed for C/N, i.e. WW-samples obtained after H_3Cit-C and H_2O_2-C treatments and the JW-sample generated through the H_3Cit-C treatment. All the above mentioned variations in the elemental composition of the pretreated materials may be explained by the fact that during pretreatment, a portion of the organic compounds present on the substrates matrices was released in the liquid fraction, while leaving another portion still on the solid fraction. This, depending on the

composition of the latter substances, would have possibly increased the percentages of some of the elements for the solid fraction. Furthermore, the variation in C/H in particular, has previously been associated with such phenomena (Lim et al., 2013; Jiang et al., 2013) and strongly encourages this supposition. As far as pretreatment severity is concerned, the variation in reagent dosage and process temperature led to the development of coinciding trends for C and H contents with all investigated reagents and for all substrates. More specifically, the use of H_3Cit and EtOH at increasing severities results in a decreasing trend for WW and OP, while the exact opposite is observed for CGW and JW. On the other hand, when using H_2O_2, C and H contents are decreased only for WW, while for the remaining substrates they are increased. Regarding N content, the observed behavior seems to be dependent on each substrate, since the only cases in which these trends coincide with those of C and H contents are those of CGW and OP for H_3Cit, those of CGW and JW for H_2O_2 and that of JW for EtOH. Finally, while the trends in C/N values are found to agree with those of N contents for all reagents and substrates, those of C/H follow different patterns. In fact, the H_3Cit treatments resulted in a decreasing trend for all substrates, the H_2O_2 treatments caused a decrease for WW, no detectable change for CGW and an increase for OP and JW, while the EtOH treatments led to increasing values for WW and CGW and to decreasing values for OP and JW. Song et al. (2012) observed a decrease in the total carbon content and an increase in the total nitrogen content of rice straw after chemical pretreatment with increasing reagent concentration, while in another study (Song et al., 2014), in which seven different reagents were used on corn straw, the authors observed a decreasing trend for C contents and C/N values, as they increased the concentrations of the reagents. The results of the present study are found to partially agree with these literature results.

It is worth mentioning that S contents are not included in ultimate analysis results, since all values were below the detection limit of the instrument (0.01%).

Table 5-3: Composition of chemically pretreated samples used in BMP assays

Substrates	Reagents	Condition sets	M (%) [a]	VS/TS	Elemental analysis					
					C (%) [b]	H (%) [b]	O (%) [b]	N (%) [b]	C/N	C/H
WW	- (raw)	-	71.9	0.920	45.9 ± 4.2	5.95 ± 1.17	38.3± 5.3	1.80 ± 0.31	25.5 ± 5.5	7.83 ± 0.97
	H_3Cit	B	78.3	0.985	57.9 ± 0.8	8.34 ± 0.13	30.5± 0.2	1.84 ± 0.40	32.2 ± 7.4	6.94 ± 0.20
		C	80.3	0.983	56.1 ± 0.1	8.38 ± 0.03	31.5± 0.1	2.34 ± 0.01	24.0 ± 0.1	6.69 ± 0.04
	H_2O_2	B	80.0	0.981	57.1 ± 0.2	8.72 ± 0.27	30.7± 0.8	1.60 ± 0.29	36.2 ± 6.5	6.55 ± 0.18
		C	81.2	0.984	56.1 ± 0.3	8.51 ± 0.30	31.4± 0.6	2.38 ± 0.01	23.6 ± 0.1	6.59 ± 0.20
	EtOH	B	83.7	0.979	58.6 ± 0.0	8.60 ± 0.17	28.8± 0.2	1.83 ± 0.00	32.1 ± 0.0	6.82 ± 0.13
		C	80.1	0.983	58.1 ± 0.5	8.04 ± 0.07	30.5± 1.0	1.61 ± 0.35	37.1 ± 7.9	7.23 ± 0.00
CGW	- (raw)	-	29.4	0.750	32.8 ± 1.2	4.40 ± 0.22	36.4± 1.4	1.40 ± 0.20	23.4 ± 3.8	7.46 ± 014
	H_3Cit	B	75.1	0.777	42.6 ± 1.4	7.39 ± 0.39	26.9± 1.8	0.84 ± 0.04	50.4 ± 0.6	5.76 ± 0.11
		C	72.9	0.858	44.7 ± 0.5	7.99 ± 0.15	32.2± 0.3	0.90 ± 0.02	49.8 ± 1.5	5.60 ± 0.16
	H_2O_2	B	69.4	0.759	40.9 ± 0.2	7.48 ± 0.03	26.9± 0.2	0.60 ± 0.00	68.7 ± 0.2	5.47 ± 0.05
		C	67.5	0.813	44.0 ± 0.1	7.84 ± 0.08	28.6± 0.4	0.77 ± 0.14	58.2 ± 10.5	5.62 ± 0.04
	EtOH	B	75.5	0.821	40.5 ± 1.6	7.24 ± 0.16	33.5± 1.9	0.79 ± 0.13	52.0 ± 6.5	5.60 ± 0.10
		C	78.7	0.835	41.1 ± 3.2	7.25 ± 0.54	34.4± 3.7	0.67 ± 0.03	61.5 ± 2.2	5.67 ± 0.02
OP	- (raw)	-	46.5	0.977	54.2 ± 3.2	7.53 ±1.14	34.5± 5.0	2.09 ± 0.07	25.9 ± 2.4	7.24 ± 0.68
	H_3Cit	B	48.5	0.993	57.3 ± 2.1	9.47 ± 0.46	31.8± 3.2	0.72 ± 0.63	85.4 ± 28.3	6.05 ± 0.08
		C	46.9	0.998	56.1 ± 2.2	9.14 ± 0.50	34.3± 2.9	0.28 ± 0.11	216 ± 80	6.13 ± 0.10
	H_2O_2	B	54.3	0.989	57.4 ± 1.7	9.61 ± 0.32	31.2± 2.3	0.65 ± 0.21	92.5 ± 27.2	5.97 ± 0.02
		C	50.9	0.992	60.2 ± 2.2	10.3 ± 0.2	28.4± 2.4	0.35 ± 0.00	172 ± 7	5.83 ± 0.07
	EtOH	B	49.6	0.992	61.0 ± 1.4	10.7 ± 0.2	27.0± 1.4	0.43 ± 0.18	155 ± 66	5.68 ± 0.04
		C	55.7	0.991	59.1 ± 0.2	10.2 ± 0.0	29.3± 0.2	0.50 ± 0.03	118 ± 7	5.76 ± 0.00
JW	- (raw)	-	78.8	0.965	45.3 ± 0.1	6.29 ± 0.89	44.3± 1.2	0.90 ± 0.15	50.3 ± 6.9	7.28 ± 0.80
	H_3Cit	B	92.7	0.959	46.6 ± 0.2	7.76 ± 0.02	40.8± 0.2	0.77 ± 0.02	60.4 ± 1.0	6.01 ± 0.00
		C	94.8	0.979	48.3 ± 0.7	7.96 ± 0.12	40.6± 0.8	0.99 ± 0.04	48.9 ± 2.9	6.06 ± 0.00
	H_2O_2	B	91.2	0.962	46.3 ± 0.2	7.41 ± 0.24	41.9± 0.4	0.65 ± 0.00	70.9 ± 0.8	6.24 ± 0.18
		C	94.5	0.966	49.5 ± 3.6	8.19 ± 0.80	38.0± 4.5	0.92 ± 0.07	53.8 ± 0.0	6.05 ± 0.15
	EtOH	B	90.9	0.967	46.1 ± 0.1	7.56 ± 0.22	42.4± 0.3	0.68 ± 0.01	67.9 ± 0.7	6.10 ± 0.16
		C	93.2	0.967	49.0 ± 0.0	8.88 ± 0.05	38.0± 0.1	0.90 ± 0.08	54.4 ± 4.9	5.52 ± 0.04

[a] wet basis, [b] dry basis, Elemental analysis values are expressed as average ± standard deviation

5.4.3 Pretreatment mass yields

As it can be seen in Table 5-4, the mass yields of the chemical pretreatment process were calculated in three different ways, namely Y_{Wet}, Y_{TS} and Y_{VS}, in order to be able to express this parameter on a wet sample, a total solids and a volatile solids basis, respectively. As it is immediately made evident by Y_{Wet} values greater than unity, chemical pretreatment results in final samples of higher mass compared with the untreated substrates. This may be explained by the higher moisture retention within the mass of each material during the pretreatment process, as it was observed earlier (paragraph 5.4.2). Y_{TS} and Y_{VS} values for each separate sample are very close to each other however in most cases the latter are greater than the former. This indicates that the solids being retained in each pretreated sample, are for the most part comprised of degradable matter. Furthermore, it is observed that solids retention in the majority of cases usually diminishes with the application of processes of higher severity. Similar results have also been observed in previous studies involving chemical pretreatment of materials of similar nature. Silverstein et al. (2007) found that solids retention diminished after treating cotton stalks with H_2SO_4, NaOH and H_2O_2 at increasing reagent concentrations and reaction time, while Park et al. (2015) made a similar observation when increasing the concentrations of HNO_3, H_2SO_4, NaOH and $Ca(OH)_2$ during pretreatment of rice straw. An analogous trend was also noticed by Kang et al. (2013) when treating Miscanthus straw with five inorganic salts at increasing temperature, concentration and pretreatment time. Nevertheless, in the present study there are also some cases in which opposite observations are true, i.e. solids retention increases. Specifically, these are the EtOH treatment of WW, the H_2O_2 treatment of CGW and the H_3Cit and H_2O_2 treatments of OP. As far as the latter substrate is concerned, another interesting observation is the fact that both Y_{TS} and Y_{VS} values are slightly greater than unity. This is indicative of an increase in the TS and VS contents of these specific pretreated samples. The experimental procedure followed during pretreatment did not include rinsing the solid fractions recovered after centrifugation and filtration. This implies that whichever substances were contained in the free moisture being retained within the mass of the pretreated samples (as mentioned in paragraph 5.4.2) would have not been removed from the solid fraction, thus eventually contributing to the final solids contents of the samples. These substances may include not only residues from the reagents being used during pretreatment, but also condensed degradation compounds. In fact, it has been reported that the soluble compounds (e.g. phenols, furans etc.) being generated after the disruption of lignocellulosic structures, i.e. the solubilization of lignin and hemicellulose and the breakage of their internal bonds, can often be condensed through the appropriate reactions, sometimes even with each other, at both higher and lower temperatures, including room temperature (Klinke et al., 2004; López-Gonzáles et al., 2013; Scordia et al., 2011). The above mentioned phenomena would have occurred for all samples to some extent, however, the results indicate that they may have been more pronounced in the case of OP, probably due to its specific characteristics and composition, which includes compounds such as sugars, volatile acids, polyphenols, polyalcohols, proteins and pigments (Karantonis et al., 2008).

Table 5-4: Mass yields of chemically pretreated samples used in BMP assays

Substrates	Reagents	Condition sets	Mass yields		
			Y_{Wet}	Y_{TS}	Y_{VS}
WW	H_3Cit	B	1.17	0.91	0.97
		C	1.04	0.73	0.78
	H_2O_2	B	1.19	0.84	0.90
		C	0.99	0.66	0.71
	EtOH	B	1.29	0.75	0.80
		C	1.15	0.81	0.87
CGW	H_3Cit	B	1.77	0.63	0.65
		C	1.26	0.48	0.55
	H_2O_2	B	1.64	0.71	0.72
		C	1.54	0.71	0.77
	EtOH	B	1.88	0.65	0.71
		C	1.91	0.58	0.64
OP	H_3Cit	B	1.12	1.07	1.09
		C	1.19	1.18	1.21
	H_2O_2	B	1.20	1.03	1.04
		C	1.21	1.11	1.13
	EtOH	B	1.28	1.21	1.23
		C	1.32	1.09	1.11
JW	H_3Cit	B	1.49	0.51	0.51
		C	1.50	0.37	0.38
	H_2O_2	B	1.73	0.72	0.71
		C	1.78	0.46	0.46
	EtOH	B	1.50	0.64	0.64
		C	1.66	0.54	0.54

5.4.4 Effect of pretreatment on substrate degradability

5.4.4.1 Methane production during BMP assays

On the basis of the results of the pretreatment procedure, only six out of the twenty-four samples obtained for each substrate (WW, CGW, OP and JW) were chosen for BMP assays. Namely, the samples resulting after pretreatment with H_3Cit, H_2O_2 and EtOH with treatments B and C were chosen. This selection was made, in order to test one sample of each reagent category (with the exception of alkalines), i.e. one acid, one oxidant and one organic solvent, and specifically those for which higher sCOD solubilization was achieved. Alakline reagents served more for comparison purposes, as alkaline processes are the most widely adopted in literature among chemical pretreatments.

Fig. 5-4 shows the cumulative methane production of the above mentioned samples, while Table 5-5 presents the specific methane yields (SMY) of the pretreated samples, as well as the biodegradability indices (BI) calculated based on the TMP of each pretreated sample.

SMY are expressed on the basis of both the added amount of VS and the amount of VS corresponding to the untreated (raw) substrate.

As it can be seen from the results, milder treatments (B) had a more positive outcome, compared with the more severe treatments (C) when H_3Cit and H_2O_2 were used, while the opposite is true in the cases where EtOH was the reagent of choice. There are only two exceptions to the above mentioned observations, where the opposite is noticed. Specifically, for WW the EtOH-B treatment yielded a higher methane potential than the EtOH-C treatment, while the H_3Cit-C treatment prevailed the H_3Cit-B treatment in the case of CGW. In a similar study, Monlau et al. (2012) compared different pretreatments of sunflower stalks and observed that an intermediate pretreatment temperature around 55 °C was more effective than 80 °C, for methane production, when using H_2O_2, as well as alkaline reagents.

When comparing the three reagents for each separate treatment, it is noticed that the EtOH-treated WW samples provide the highest methane yields for both B- and C-treatments, while for OP, the samples treated with H_3Cit prevail at both condition sets, although for the latter substrate, for treatment C the difference from the EtOH-treated sample is only slight. In the cases of CGW and JW on the other hand, treatments H_2O_2-B and EtOH-C provided the materials with the highest methane yields for each condition set, with the respective values being quite close. Similar results have been observed in previous studies as well. In fact, when Amnuaycheewa et al. (2016) compared various organic acids for the pretreatment of rice straw, they found that citric acid was the one providing the best results concerning the enhancement of biogas production. Moreover, Kabir et al. (2014) have reported that organic acids and low molecular weight alcohols are produced during anaerobic digestion as intermediate products; therefore their eventual presence not only does not cause inhibition to the process, since they are easily degradable, but may also be beneficial to it. Consequently, it can be assumed that any eventual residues of H_3Cit and EtOH remained on the substrates after pretreatment, probably did not have such a negative effect on methane production, as the residues of the other reagents may have had, hence the more positive results observed for these substrates. Overall, the highest methane yields (SMY_P, $mLCH_{4,\,STP}/gVS_P$) were obtained after the EtOH-B, EtOH-C, H_3Cit-B and H_2O_2-B pretreatments for WW, CGW, OP and JW, respectively. A similar behavior can be observed when referring to the SMY expressed as SMY_{Raw} ($mLCH_{4,\,STP}/gVS_{Raw}$), as well as to the corresponding BI (%) values. There are only two exceptions in which SMY_{Raw} follow the opposite trends compared with SMY_P. More specifically, this is noticed for the two H_3Cit-treated OP-samples and the two EtOH-treated JW-samples. Interestingly, in these same cases BI trends are also found to disagree with SMY_P trends, while being in accordance with SMY_{Raw} trends. The only additional case in which there is an absolute disagreement between SMY and BI is for the H_3Cit-treated WW-samples, for which however the difference in degradability can be considered negligible. The fact that SMY are mostly in accordance with BI values corroborate the supposition regarding the effects of these specific pretreatments on the investigated substrates, i.e. that higher methane potentials are linked to higher biodegradability levels.

It is worth mentioning that while SMY_{Raw} are lower than SMY_P for WW, CGW and JW, in the case of OP the opposite is seen. This is related to the high mass yield values observed for this substrate. Moreover, when comparing the aforementioned results with the SMY of the untreated substrates (Pellera and Gidarakos, 2016) (Chapter 3), namely 446.2, 268.0, 258.7 and 446.0 mL $CH_{4,\,STP}$/g VS for WW, CGW, OP and JW, respectively, it is evident that pretreated materials present generally lower methane potentials, with the exception of the CGW-sample generated after the EtOH-C treatment, for which the SMY_P seems to exceed the one obtained for the untreated substrate. Reduced methane potentials after chemical

pretreatment were observed in a previous study as well (Pellera et al., 2016) and may be attributed to the loss of a portion of degradable matter initially present on the solid matrix of the substrates and its transfer to the liquid phase as a result of pretreatment (Fernández-Cegrí et al., 2012).

Ultimately, the obtained results reveal that different pretreatment severities have different effects on the degradability of the produced materials. In fact, while for WW moderate severity processes seem to generally be more appropriate for subsequent methane production, in the case of CGW this is true only when using H_2O_2, with more severe conditions being more fruitful for the latter substrate when using H_3Cit and EtOH. As far as OP and JW are concerned, moderate conditions appear to be more suitable when applying H_3Cit and H_2O_2 treatments, while the EtOH treatments give more satisfactory results when adopted at a higher severity.

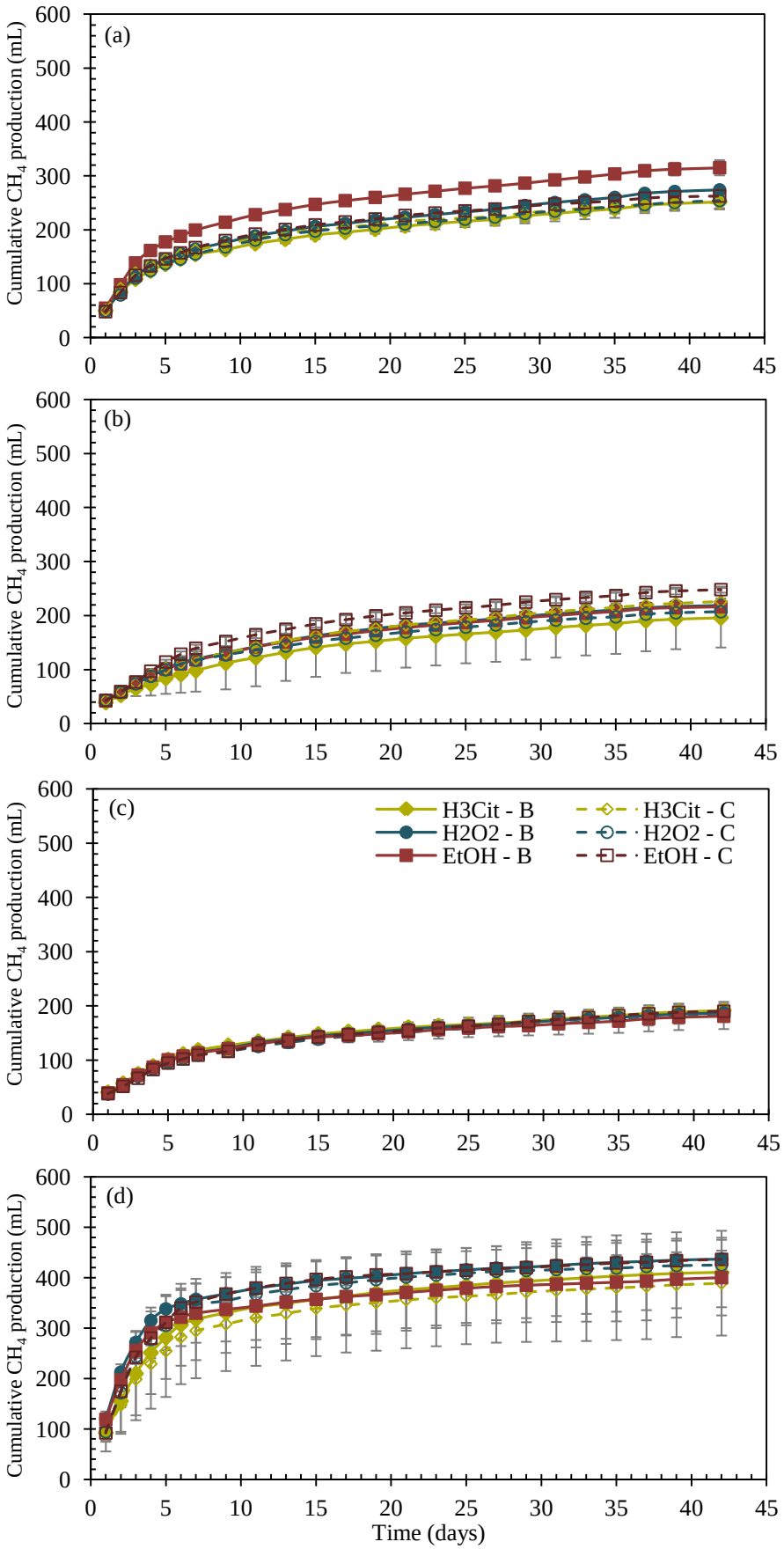


Figure 5-4:Cumulative methane production of samples pretreated with three different reagents at two condition sets [H_3Cit-B (-◆-), H_3Cit-C (-◇-), H_2O_2-B (-●-), H_2O_2-C (-○-), EtOH-B (-■-), EtOH-C (-□-)], for (a) WW, (b) CGW, (c) OP and (d) JW (error bars represent standard deviation)

Table 5-5: Theoretical methane potentials (TMP), Specific methane yields (SMY) and Biodegradability indices (BI) of chemically pretreated samples used in BMP assays

Substrates	Reagents	Condition sets	TMP (mL $CH_{4,\ STP}$/g VS)	SMY_P [a] (mL $CH_{4,\ STP}$/g VS_P)	SMY_{Raw} [a] (mL $CH_{4,\ STP}$/g VS_{Raw})	BI [a] (%)
WW	H_3Cit	B	665.6	163.7 ± 16.5	159.1 ± 16.0	24.6 ± 2.5
		C	644.6	163.0 ± 15.7	126.7 ± 12.2	25.3 ± 2.4
	H_2O_2	B	672.8	190.2 ± 6.7	171.3 ± 6.1	28.3 ± 1.0
		C	648.5	166.0 ± 16.4	117.5 ± 11.6	25.6 ± 2.5
	EtOH	B	690.8	239.5 ± 16.9	190.7 ± 13.5	34.7 ± 2.4
		C	662.3	176.6 ± 11.0	153.4 ± 9.6	26.7 ± 1.7
CGW	H_3Cit	B	650.0	147.1 ± 132.0	95.4 ± 85.6	22.6 ± 20.3
		C	610.0	265.2 ± 12.9	146.5 ± 7.1	43.5 ± 2.1
	H_2O_2	B	649.9	247.5 ± 6.0	178.0 ± 4.3	38.1 ± 0.9
		C	646.6	220.2 ± 5.2	168.7 ± 4.0	34.0 ± 0.8
	EtOH	B	559.6	241.5 ± 3.2	172.5 ± 2.3	43.2 ± 0.6
		C	554.1	318.0 ± 13.8	204.7 ± 8.9	57.4 ± 2.5
OP	H_3Cit	B	689.1	183.2 ± 5.0	200.1 ± 5.5	26.6 ± 0.7
		C	658.5	179.9 ± 2.8	217.3 ± 3.3	27.3 ± 0.4
	H_2O_2	B	699.9	172.3 ± 40.8	178.7 ± 42.3	24.6 ± 5.8
		C	754.5	156.2 ± 60.1	176.3 ± 67.8	20.7 ± 8.0
	EtOH	B	779.6	157.5 ± 4.0	193.0 ± 4.9	20.2 ± 0.5
		C	739.4	179.0 ± 3.4	197.8 ± 3.7	24.2 ± 0.5
JW	H_3Cit	B	526.4	353.5 ± 82.5	180.4 ± 42.1	67.2 ± 15.7
		C	537.2	334.3 ± 141.2	126.1 ± 53.2	62.2 ± 26.3
	H_2O_2	B	508.1	385.6 ± 8.4	275.0 ± 6.0	75.9 ± 1.7
		C	572.3	371.3 ± 4.7	171.9 ± 2.2	64.9 ± 0.8
	EtOH	B	505.9	332.8 ± 89.6	214.3 ± 57.7	65.8 ± 17.7
		C	586.8	384.5 ± 20.9	206.6 ± 11.2	65.5 ± 3.6

[a] Values are expressed as average ± standard deviation

5.4.4.2 pH and TPH during BMP assays

During the BMP assays weekly samples of the digestion slurry were withdrawn from the reactors, in order to determine the pH (Fig. 5-5) and TPH (Fig. 5-6) concentrations in their interior.

As it is shown in Fig. 5-5, pH values on day 1 of incubation are found decreased compared with the initially adjusted value of 7.8, with this phenomenon being particularly pronounced for the assays corresponding to JW-samples. In fact, in these particular cases pH reaches values as low as 6.75. After the first week of incubation, a further decrease in pH can generally be noticed (until day 15), most likely due to the accumulation of organic acids during the initial stages of the digestion process (Liew et al., 2011; Song et al., 2014). This observation is true for all assays except for those containing JW-samples, for which the pH increases instead, showing a tendency towards the stabilization of the systems. Afterwards, beyond day 7, pH profiles tend to show similar patterns among substrates, with a slight initial increasing trend (after day 15) and subsequent small fluctuations around a close range of values. On the other hand, JW-assays show a continuous increasing trend with time. This latter behavior of pH can be attributed to the consumption of the previously produced acids (Song et al., 2012). Results following patterns similar to those observed in the present study were also found in the studies conducted by Zhang et al. (2011), Song et al. (2012), Song et al. (2014) and Liew et al. (2011). No significant differences can be noticed regarding pH, nor between treatments using different reagents or between treatments of different severity. This suggests that the reagent residues that eventually remained on the pretreated samples were most probably in a quantity that apparently did not exert an inhibitory effect on anaerobic digestion.

The results regarding the variation of TPH concentrations in the digestion slurries are depicted in Fig. 5-6. An initial increasing behavior is observed during the first weeks of incubation for all substrates. More specifically, for the assays corresponding to WW (Fig. 5-6a), CGW (Fig. 5-6b) and OP (Fig. 5-6c), most peak values are found on day 15, with some others being noticed later during digestion (days 21 and 27). On the other hand, in the case of JW-samples (Fig. 5-6d) TPH concentrations reach their maximum within the first week of incubation (day 7). Interestingly, the higher presence of these compounds coincides with the manifestation of lower pH values, which was mentioned earlier. This suggests that a significant amount of degradable organic material was consumed during these periods, thus causing the more pronounced release of phenolic compounds and organic acids and in turn the lower pH levels. Following this initial period of higher TPH levels, a continuous decreasing trend is developed, especially after day 27, with similar variation patterns being observed for all assays, corroborating the stabilization tendency inferred by the earlier discussed corresponding pH values. Furthermore, it is noted that there are no significant variations among different treatments confirming the absence of particularly inhibitory factors.

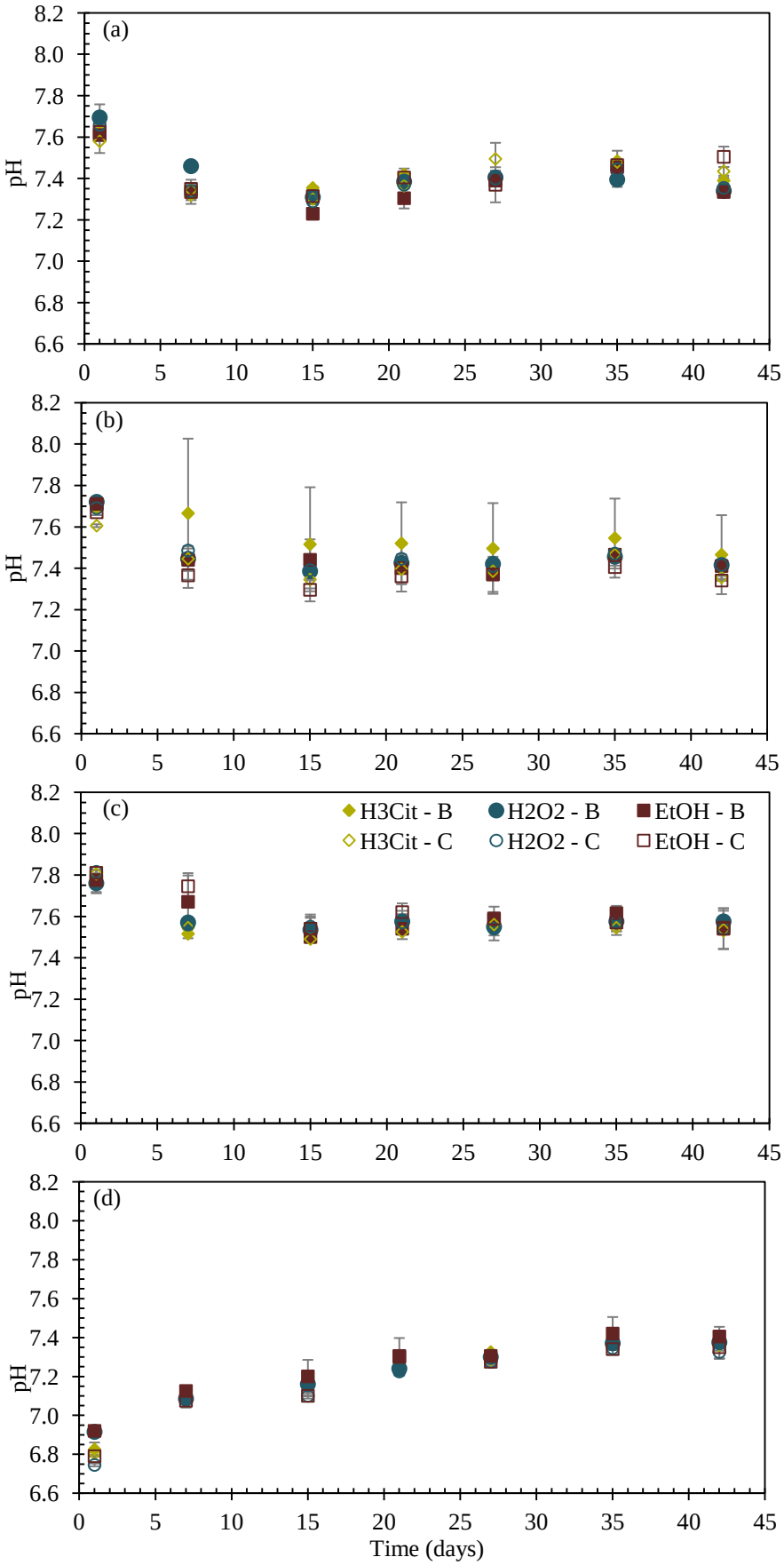


Figure 5-5: pH variation during BMP assays for samples pretreated with three different reagents at two condition sets [H_3Cit-B (-◆-), H_3Cit-C (- ◇ -), H_2O_2-B (-●-), H_2O_2-C (- ○ -), EtOH-B (-■-), EtOH-C (- □ -)], for (a) WW, (b) CGW, (c) OP and (d) JW (error bars represent standard deviation)

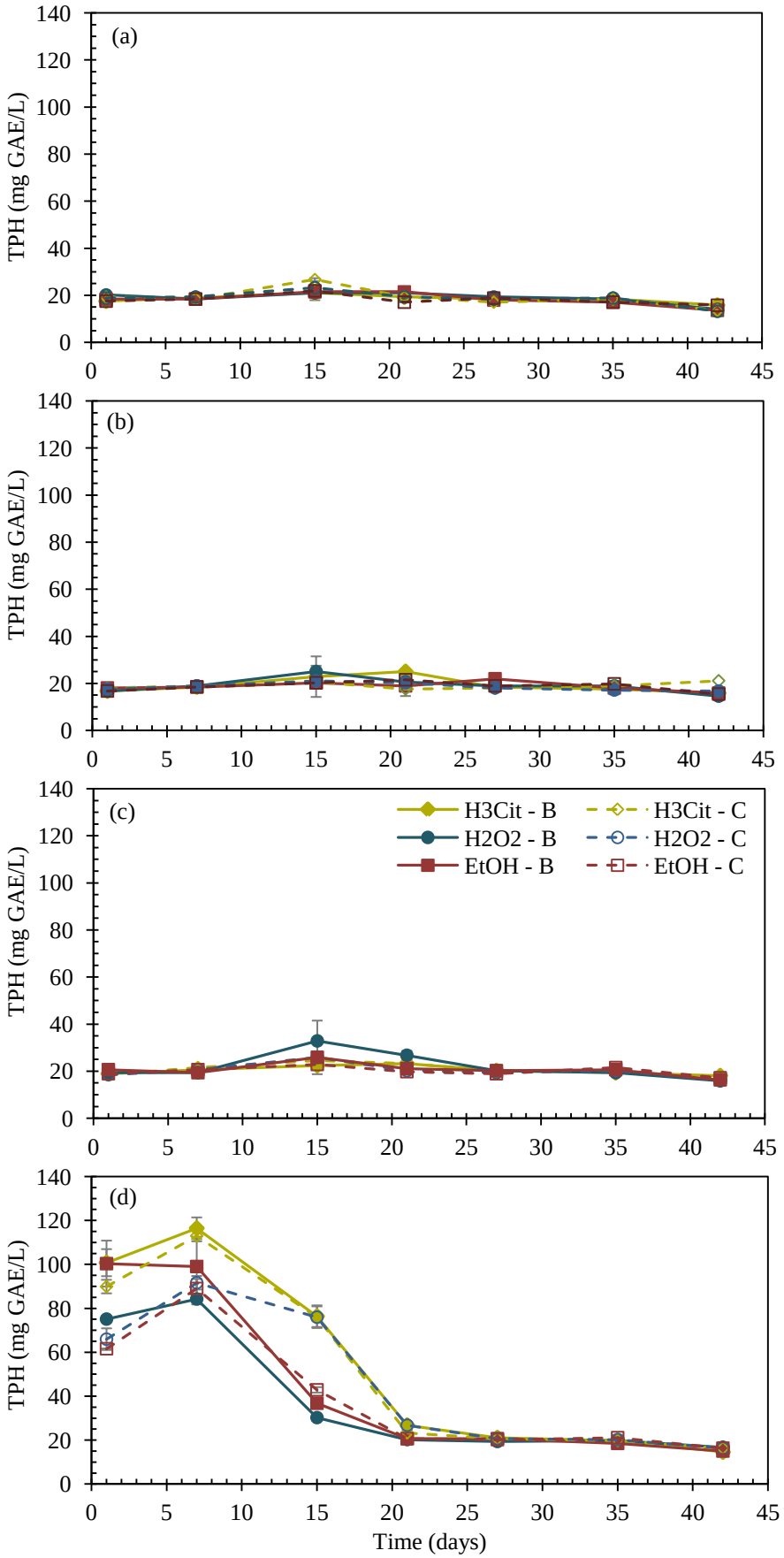


Figure 5-6: TPH variation during BMP assays for samples pretreated with three different reagents at two condition sets [H_3Cit-B (-◆-), H_3Cit-C (- ◇ -), H_2O_2-B (-●-), H_2O_2-C (- ○ -), EtOH-B (-■-), EtOH-C (- □ -)], for (a) WW, (b) CGW, (c) OP and (d) JW (error bars represent standard deviation)

5.4.4.3 Kinetic modeling of BMP data

The results obtained by fitting the methane production data to both the first-order exponential and the transference models are presented in Table 5-6. Data fitting for both models resulted in the same values for P and R^2, hence the combined presentation of the data. The models were found to describe the experimental data at an acceptable level for all samples. Chemical pretreatment appears to have affected the anaerobic digestion kinetics of each investigated substrate differently. Indeed, the rate constant (k) values obtained for WW and CGW seem to generally follow an increasing trend with the variation in pretreatment severity from B to C, for all reagents being used. These results imply the development of a digestion process at a slightly faster rate with an increase in the severity of pretreatment. The opposite is observed for OP and JW, suggesting that a more intense pretreatment may retard digestion in these cases. López-Gonzáles et al. (2013), who focused on thermo-chemical pretreatment of press mud, also obtained lower k values for the data corresponding to higher severities. As far as the maximum methane production rate (R_m) is concerned, although the highest values correspond to the samples with the maximum SMY and BI, the trends followed by the data of these three parameters do not always coincide. In fact, when comparing the results presented in Table 5-5 with those of Table 5-6, it can be noticed than the behavior of R_m in certain cases coincides with the behavior of either the SMY or the BI. These findings suggest that the maximum methane production rate for a specific substrate is evidently in correlation with its biodegradability.

5.4.5 Fiber composition of chemically pretreated samples

In order to better understand the effect of the pretreatments on each substrate, the fiber composition of the chemically pretreated samples with the highest SMY was determined. Table 5-7 presents the NDF, ADF and ADL contents of the pretreated samples, as well as of the respective raw substrates, expressed as a percentage of TS. Additionally, the ADL content was also expressed as a percentage of the ADF content. NDF includes hemicellulose, cellulose and lignin, ADF includes cellulose and lignin, while ADL represents lignin. As it can be seen from the results, in all cases the pretreatments seem to have produced samples with an increased NDF content compared with the untreated substrates. In other words, this suggests that the pretreatments in addition to causing the partial disruption of the substrates matrices, also induced the release of the easily degradable organic matter of each substrate, thus leaving solid samples with higher fiber contents. The ADF contents were also higher after pretreatment, while the ADL contents (%TS) were decreased for WW and OP and increased for CGW and JW. Nevertheless, when ADL values are expressed as a percentage of the ADF contents, they are found reduced in all cases, except for JW. These results would imply that all lignocellulosic components were affected by pretreatment to some degree. However, in the case of WW, hemicellulose and lignin appear to have been affected the most, while for CGW, OP and JW, this is the case for cellulose and lignin. Moreover, for CGW and OP, lignin in particular, seems to have suffered a higher reduction, while for JW, results indicate a more pronounced reduction for cellulose. Previous studies, where chemical pretreatments using H_3Cit (Amnuaycheewa et al., 2016), H_2O_2 (Song et al., 2012; Song et al., 2014) and EtOH (Kabir et al., 2014) were applied to lignocellulosic substrates, have also examined the fiber composition of pretreated samples. Both Amnuaycheewa et al. (2016) and Song et al. (2012) observed decreased cellulose and lignin contents for pretreated rice straw, while a similar tendency was observed in another study treating corn stover (Song et al.,

2014). On the other hand, in the study conducted by Kabir et al. (2014) a decrease in the hemicellulose and lignin contents of forest residues was combined with an increase in their cellulose content.

Table 5-6: Kinetic modeling parameters for BMP assays

Substrates	Reagents	Condition sets	Modeling parameters			
			P	*k*	*Rm*	R^2
WW	H_3Cit	B	227.8	0.1614	36.76	0.9276
		C	230.7	0.1838	42.41	0.9342
	H_2O_2	B	248.5	0.1537	38.19	0.9343
		C	232.1	0.1609	37.35	0.9472
	EtOH	B	289.5	0.1683	48.71	0.9496
		C	244.3	0.1659	40.54	0.9579
CGW	H_3Cit	B	165.0	0.1039	17.14	0.8431
		C	212.6	0.1120	23.82	0.9624
	H_2O_2	B	207.2	0.1186	24.57	0.9657
		C	193.2	0.1254	24.24	0.9533
	EtOH	B	203.9	0.1192	24.30	0.9620
		C	234.8	0.1175	27.59	0.9769
OP	H_3Cit	B	175.7	0.1542	27.09	0.9544
		C	175.2	0.1324	23.20	0.9537
	H_2O_2	B	172.9	0.1476	25.54	0.9614
		C	167.9	0.1454	24.40	0.9541
	EtOH	B	166.2	0.1624	26.98	0.9541
		C	176.4	0.1301	22.96	0.9561
JW	H_3Cit	B	387.9	0.2442	94.71	0.9730
		C	367.3	0.2341	85.98	0.9736
	H_2O_2	B	414.3	0.3296	136.53	0.9635
		C	408.4	0.2687	109.76	0.9809
	EtOH	B	376.6	0.3473	130.81	0.9564
		C	417.7	0.2666	111.34	0.9863

Table 5-7: Fiber composition chemically pretreated samples with the highest SMY

		NDF (%TS)	ADF (%TS)	ADL (%TS)	ADL (%ADF)
WW	Raw	43.2	41.5	39.2	94.6
	EtOH-B	61.6	60.2	39.1	65.0
CGW	Raw	62.1	60.3	44.6	73.9
	EtOH-C	84.5	72.3	52.6	72.8
OP	Raw	64.5	50.4	46.3	91.8
	H_3Cit-B	82.3	58.1	40.7	70.1
JW	Raw	19.6	14.3	2.1	14.6
	H_2O_2-B	45.7	26.4	15.9	60.3

5.4.6 Energy considerations

The energy requirements for chemical pretreatment of the investigated substrates at different conditions, as well as the energy recovered in the form of heat and methane were calculated in order to provide the energy balance presented in Table 5-8. It is noted that, the calculations regarding specific energy consumption (E_C) and specific energy released in the form of heat (E_Q) were made considering the maximum amount of substrate being introduced in the oven at each run of the pretreatment experiments. Specifically, a maximum of 21 flasks could be used at the same time, with each one containing 1 g of VS of substrate. Moreover, the specific energy production in the form of methane was calculated using the quantity of gas being produced from each pretreated sample at STP conditions.

It is evident that energy consumption is higher for B-treatments, due to their longer duration, i.e. 8 h compared with 4 h corresponding to C-treatments. On the other hand, larger amounts of energy produced in the form of heat are obtained from C-treatments, which are conducted at a higher temperature. Moreover, it is observed that for all reagents and at both condition sets, a negative energy balance is obtained, for all investigated substrates. Similar negative specific energy profits were also obtained by Kuglarz et al. (2013), when investigating thermal treatment of sludge at temperatures between 30 and 100 °C. Additionally, the values obtained for the energy ratio Ei/Eo, are higher than unity and between 5.1 and 5.4 for B-treatments and around 1.2 for C-treatments. All the above lead to the conclusion that the amount of energy that could potentially be recovered in the form of methane and heat, could not balance the energy requirements of the pretreatment processes that were applied under the specific conditions being adopted in this study. However, pretreatments performed at a higher temperature and a shorter duration (C-treatments) resulted in a more encouraging outcome, since both E_T and Ei/Eo are significantly lower for these treatments. A similar observation was also made by Passos et al. (2013) when determining the ratio between the energy consumed for thermal pretreatment of microalgae at 55, 75 and 95 °C, and the energy recovered from methane production. The energy ratios they obtained were greater than unity, albeit characterized by a decreasing trend with an increase in temperature. Taking into consideration the above observations, it can be concluded that the pretreatment processes used in the present study are not economically attractive when conducted under the applied conditions. Nevertheless, if appropriate adjustments were made, energy consumption could be reduced, thus improving the overall energy balance. More specifically, the use of a different heating system, with a lower operating power, should be considered. At the same time, increasing the density of the pretreatment slurry, i.e. the solid to liquid ratio, is worth investigating, since it would ensure a better exploitation of the energy being consumed during pretreatment, by maximizing the amount of substrate being treated. In fact, in a previous study involving low temperature thermal pretreatment of microalgal biomass (Passos et al., 2013), the authors obtained an improved energy balance when applying scenarios with more concentrated biomass.

Table 5-8: Energy balance

Substrates	Reagents	Condition sets	E_C	E_M	E_Q	E_T	E_i/ E_o
WW	H_3Cit	B	1217829	5694	226666	-985468	5.2
		C	608914	4535	498666	-105714	1.2
	H_2O_2	B	1217829	6132	226666	-985031	5.2
		C	608914	4207	498666	-106041	1.2
	EtOH	B	1217829	6828	226666	-984335	5.2
		C	608914	5492	498666	-104757	1.2
CGW	H_3Cit	B	1217829	3414	222036	-992379	5.4
		C	608914	5246	488478	-115190	1.2
	H_2O_2	B	1217829	6371	222036	-989422	5.3
		C	608914	6041	488478	-114395	1.2
	EtOH	B	1217829	6175	222036	-989618	5.3
		C	608914	7329	488478	-113107	1.2
OP	H_3Cit	B	1217829	7164	222451	-988213	5.3
		C	608914	7779	489393	-111743	1.2
	H_2O_2	B	1217829	6398	222451	-988980	5.3
		C	608914	6311	489393	-113211	1.2
	EtOH	B	1217829	6908	222451	-988469	5.3
		C	608914	7083	489393	-112439	1.2
JW	H_3Cit	B	1217829	6458	228947	-982424	5.2
		C	608914	4477	503684	-100754	1.2
	H_2O_2	B	1217829	9844	228947	-979037	5.1
		C	608914	6153	503684	-99077	1.2
	EtOH	B	1217829	7674	228947	-981208	5.1
		C	608914	7395	503684	-97836	1.2

5.5 Conclusions

This study aimed at evaluating the influence of chemical pretreatment on the solubilization and the degradability of four solid agroindustrial waste, namely winery waste (WW), cotton gin waste (CGW), olive pomace (OP) and juice industry waste (JW). In order to investigate the effect of different chemical natures, eight reagents were used, specifically NaOH, $NaHCO_3$, NaCl, H_3Cit, AcOH, H_2O_2, Me_2CO and EtOH, while for evaluating the impact of the process severity, pretreatment was conducted under three condition sets, by varying process duration, reagent dosage and temperature. Substrate solubilization was clearly positively associated with pretreatment severity, with H_3Cit, H_2O_2, and EtOH generally exhibiting higher levels of effectiveness. It can be inferred that the effect of the pretreatment processes at different severities, is highly dependent not only on the mode of action of each reagent, but also on the substrate being treated. Fiber analysis confirmed that the chemical pretreatments in use, indeed altered the structure of the investigated substrates, resulting in

cellulose and hemicellulose solubilization and in the breakage of the bonds connecting them to lignin. Ultimately, chemical pretreatment resulted in lower methane potentials compared with untreated substrates. Nevertheless, moderate to high severity conditions are recommended when treating substrates such as those investigated in the present study, for subsequent methane production. More specifically, while a moderate severity seems to be generally preferable when applying H_3Cit and H_2O_2 treatments, EtOH treatments would eventually require more severe conditions, in order to be more effective. In conclusion, the most effective options for each substrate, in terms of methane production, seemed to be moderate severity EtOH-, H_3Cit- and H_2O_2- treatments for WW, OP and JW, respectively, and a high severity EtOH-treatment for CGW. Energy calculations results showed high energy consumption for chemical pretreatment and suggested that the overall energy balance could be improved by using a heating system with a lower operating power, as well as a higher solid to liquid ratio in the pretreatment slurry.

5.6 References

Altaş, L. (2009). Inhibitory effect of heavy metals on methane-producing anaerobic granular sludge. *Journal of Hazardous Materials*, *162*(2–3), 1551–1556.

Amnuaycheewa, P., Hengaroonprasan, R., Rattanaporn, K., Kirdponpattara, S., Cheenkachorn, K., Sriariyanun, M. (2016). Enhancing enzymatic hydrolysis and biogas production from rice straw by pretreatment with organic acids. *Industrial Crops and Products*, *87*, 247–254.

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., Lens, P.N.L. (2014). Pretreatment methods to enhance anaerobic digestion of organic solid waste. *Applied Energy*, *123*, 143–156.

Asadieraghi, M., Wan Daud, W.M.A. (2014). Characterization of lignocellulosic biomass thermal degradation and physiochemical structure: Effects of demineralization by diverse acid solutions. *Energy Conversion and Management*, *82*, 71–82.

Assawamongkholsiri, T., Reungsang, A., Pattra, S. (2013). Effect of acid, heat and combined acid-heat pretreatments of anaerobic sludge on hydrogen production by anaerobic mixed cultures. *International Journal of Hydrogen Energy*, *38*(14), 6146–6153.

Banerjee, D., Mukherjee, S., Pal, S., Khowala, S. (2016). Enhanced saccharification efficiency of lignocellulosic biomass of mustard stalk and straw by salt pretreatment. *Industrial Crops and Products*, *80*, 42–49

Behera, S., Arora, R., Nandhagopal, N., Kumar, S. (2014). Importance of chemical pretreatment for bioconversion of lignocellulosic biomass. *Renewable and Sustainable Energy Reviews*, *36*, 91–106.

Bolado-Rodríguez, S., Toquero, C., Martín-Juárez, J., Travaini, R., García-Encina, P.A. (2016). Effect of thermal, acid, alkaline and alkaline-peroxide pretreatments on the biochemical methane potential and kinetics of the anaerobic digestion of wheat straw and sugarcane bagasse. *Bioresource Technology*, *201*, 182–190.

Donoso-Bravo, A., Pérez-Elvira, S.I., Fdz-Polanco, F. (2010). Application of simplified models for anaerobic biodegradability tests. Evaluation of pre-treatment processes. *Chemical Engineering Journal*, *160*(2), 607–614.

Fernandes, T.V., Klaasse Bos, G.J., Zeeman, G., Sanders, J.P.M., van Lier, J.B. (2009). Effects of thermo-chemical pre-treatment on anaerobic biodegradability and hydrolysis of lignocellulosic biomass. *Bioresource Technology*, *100*(9), 2575–2579.

Fernández-Cegrí, V., De la Rubia, M.A., Raposo, F., Borja, R. (2012). Effect of hydrothermal pretreatment of sunflower oil cake on biomethane potential focusing on fibre composition. *Bioresource Technology*, *123*, 424–429.

Jiang, L., Hu, S., Sun, L., Su, S., Xu, K., He, L., Xiang, J. (2013). Influence of different demineralization treatments on physicochemical structure and thermal degradation of biomass. *Bioresource Technology*, *146*, 254–260.

Kabir, M.M., Rajendran, K., Taherzadeh, M.J., Sárvári Horváth, I. (2014). Experimental and economical evaluation of bioconversion of forest residues to biogas using organosolv pretreatment. *Bioresource Technology, 178*, 201–208.

Kang, K.E., Park, D.-H., Jeong, G.-T. (2013). Effects of inorganic salts on pretreatment of Miscanthus straw. *Bioresource Technology, 132*, 160–165.

Karantonis, H.C., Tsantila, N., Stamatakis, G., Samiotaki, M., Panayotou, G., Antonopoulou, S., Demopoulos, C.A. (2008). Bioactive polar lipids in olive oil, pomace and waste byproducts. *Journal of Food Biochemistry*, *32*, 443–459.

Klinke, H.B., Thomsen, A.B., Ahring, B.K. (2004). Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Applied Microbiology and Biotechnology*, *66*(1), 10–26.

Kuglarz, M., Karakashev, D., Angelidaki, I. (2013). Microwave and thermal pretreatment as methods for increasing the biogas potential of secondary sludge from municipal wastewater treatment plants. *Bioresource Technology, 134*, 290–297.

Lesteur, M., Bellon-Maurel, V., Gonzalez, C., Latrille, E., Roger, J.M., Junqua, G., Steyer, J. P. (2010). Alternative methods for determining anaerobic biodegradability: a review. *Process Biochemistry, 45*(4), 431-440.

Liew, L.N., Shi, J., Li, Y. (2011). Enhancing the solid-state anaerobic digestion of fallen leaves through simultaneous alkaline treatment. *Bioresource Technology*, *102*(19), 8828–8834.

Lim, W.-S., Kim, J.-Y., Kim, H.-Y., Choi, J.-W., Choi, I.-G., Lee, J.-W. (2013). Structural properties of pretreated biomass from different acid pretreatments and their effects on simultaneous saccharification and ethanol fermentation. *Bioresource Technology, 139*, 214–219.

Liu, L., Sun, J., Cai, C., Wang, S., Pei, H., Zhang, J. (2009). Corn stover pretreatment by inorganic salts and its effects on hemicellulose and cellulose degradation. *Bioresource Technology*, *100*(23), 5865–5871.

López González, L. M., Vervaeren, H., Pereda Reyes, I., Dumoulin, A., Romero Romero, O., Dewulf, J. (2013). Thermo-chemical pre-treatment to solubilize and improve anaerobic biodegradability of press mud. *Bioresource Technology*, *131*, 250–257.

Ma, J., Duong, T.H., Smits, M., Verstraete, W., Carballa, M. (2011). Enhanced biomethanation of kitchen waste by different pre-treatments. *Bioresource Technology*, *102*(2), 592–599.

Monlau, F., Barakat, A., Steyer, J.P., Carrere, H. (2012). Comparison of seven types of thermo-chemical pretreatments on the structural features and anaerobic digestion of sunflower stalks. *Bioresource Technology*, *120*, 241–247.

Monlau, F., Latrille, E., Da Costa, A.C., Steyer, J.P., Carrère, H. (2013). Enhancement of methane production from sunflower oil cakes by dilute acid pretreatment. *Applied Energy*, *102*, 1105–1113.

Nain, M. Z., Jawed, M. (2006). Performance of anaerobic reactors at low organic load subjected to sudden change in feed substrate types. *Journal of Chemical Technology & Biotechnology*, *81*, 958–965.

Park, G.W., Kim, I., Jung, K., Seo, C., Han, J.I., Chang, H.N., Kim, Y.C. (2015). Enhancement of volatile fatty acids production from rice straw via anaerobic digestion with chemical pretreatment. *Bioprocess and biosystems engineering*, *38*(8), 1623-1627.

Passos, F., García, J., Ferrer, I. (2013). Impact of low temperature pretreatment on the anaerobic digestion of microalgal biomass. *Bioresource Technology*, *138*, 79–86.

Pellera, F.-M., Gidarakos, E. (2016). Effect of substrate to inoculum ratio and inoculum type on the biochemical methane potential of solid agroindustrial waste. *Journal of Environmental Chemical Engineering*, *4*(3), 3217–3229.

Pellera, F.-M., Santori, S., Pomi, R., Polettini, A., Gidarakos, E. (2016). Effect of alkaline pretreatment on anaerobic digestion of olive mill solid waste. *Waste Management*, *58*, 160-168.

Raposo, F., Fernández-Cegrí, V., De la Rubia, M.A., Borja, R., Béline, F., Cavinato, C., Demirer, G., Fernández, B., Fernández-Polanco, M., Frigon, J.C., Ganesh, R., Kaparaju, P., Koubova, J., Méndez, R., Menin, G., Peene, A., Scherer, P., Torrijos, M.. Uellendahl, H.. Wierinck, I., de Wilde, V. (2011). Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. *Journal of Chemical Technology & Biotechnology*, *86*, 1088-1098.

Sambusiti, C., Monlau, F., Ficara, E., Carrère, H., Malpei, F. (2013). A comparison of different pre-treatments to increase methane production from two agricultural substrates. *Applied Energy*, *104*, 62–70.

Scordia, D., Cosentino, S.L., Lee, J.W., Jeffries, T.W. (2011). Dilute oxalic acid pretreatment for biorefining giant reed (Arundo donax L.). *Biomass and Bioenergy*, *35*(7), 3018–3024.

Silverstein, R.A., Chen, Y., Sharma-Shivappa, R.R., Boyette, M.D., Osborne, J. (2007). A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. *Bioresource Technology*, *98*(16), 3000–3011.

Singh, J., Suhag, M., Dhaka, A. (2015). Augmented digestion of lignocellulose by steam explosion , acid and alkaline pretreatment methods : A review. *Carbohydrate Polymers*, *117*, 624–631.

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, *299*, 152–178.

Song, Z., Yang, G., Guo, Y., Zhang, T. (2012). Comparison of two chemical pretreatments of rice straw for biogas production by anaerobic digestion. *BioResources*, *7*(3), 3223–3236.

Song, Z., Yang, G., Liu, X., Yan, Z., Yuan, Y., Liao, Y. (2014). Comparison of seven chemical pretreatments of corn straw for improving methane yield by anaerobic digestion. *PLoS ONE*, *9*(4), 1–8.

Tian, Y., Zhang, H., Mi, X., Wang, L., Zhang, L. and Ai, Y. (2016). Research on anaerobic digestion of corn stover enhanced by dilute acid pretreatment: Mechanism study and potential utilization in practical application. *Journal of Renewable and Sustainable Energy*, *8*(2), 023103-1–023103-14.

Zhang, Q., Tang, L., Zhang, J., Mao, Z., Jiang, L. (2011). Optimization of thermal-dilute sulfuric acid pretreatment for enhancement of methane production from cassava residues. *Bioresource Technology*, *102*(4), 3958–3965.1

Zhao, R., Zhang, Z., Zhang, R., Li, M., Lei, Z., Utsumi, M., Sugiura, N. (2010). Methane production from rice straw pretreated by a mixture of acetic-propionic acid. *Bioresource Technology*, *101*(3), 990–994.

Zhao, X., Wang, L., Lu, X., Zhang, S. (2014). Pretreatment of corn stover with diluted acetic acid for enhancement of acidogenic fermentation. *Bioresource Technology*, *158*, 12–18.

Zheng, Y., Zhao, J., Xu, F., Li, Y. (2014). Pretreatment of lignocellulosic biomass for enhanced biogas production. *Progress in Energy and Combustion Science*, *42*, 35–53.

CHAPTER 6

Anaerobic digestion of sold agroindustrial waste in semi-continuous mode: Evaluation of mono-digestion and co-digestion systems

The present chapter aimed at investigating the anaerobic digestion of four agroindustrial waste, namely cotton gin waste (CGW), winery waste (WW), olive pomace (OP) and juice industry waste (JW), in semi-continuous mode, conducting not only mono-digestion, but also co-digestion assays, using a synthetic organic fraction (SOF) sample as a co-substrate. These assays were divided into two groups, in which different conditions were applied. Group I investigated the variation in operational parameters, such as the organic loading rate (OLR) and the hydraulic retention time (HRT), while in Group II, the assays were fed with different substrates in a sequential order. Results showed that co-digestion assays resulted in more elevated methane yields compared with mono-digestion assays. An increase of the OLR exerted different effects on methane production, depending on the substrate being fed to the reactors, while halving the HRT led to the achievement of maximum methane yields for all assays, with the latter values corresponding to an OLR of 1.0 gVS/L/d. Further reduction of the HRT coupled to an increase of the OLR caused a significant decrease of methane yields, due to system overloading and possibly washout phenomena, in all cases except for the assays being fed with OP-substrates. The latter variation in operational parameters also resulted in one case of severe system overloading, which led to instability and ultimately failure of the assay being fed with JW-single-substrate. Sequential feeding with different substrates led to a more equilibrated operation, especially for co-digestion systems. Moreover, higher methane yields were observed during the phases corresponding to WW- and JW-substrates, while a similar outcome was also obtained in the cases in which these same substrates were fed to the reactors at process startup.

6.1 Introduction

Agroindustrial activities represent one of the leading sectors of the economy, especially for countries, such as those situated in the Mediterranean region, which are characterized by high production rates of agricultural commodities (Fountoulakis et al., 2008). Processing of agricultural commodities for the production of various food products leads to the generation of large amounts of waste materials. Among the most produced agroindustrial waste in Mediterranean countries are those originating from wine and olive oil production, as well as from citrus fruits (mainly orange) and cotton processing (Pellera and Gidarakos, 2016). The most abundant solid waste generated during wine production is grape marc, which is produced during the crushing, pressing, and draining stages. It is a lignocellulosic material rich in polyphenols and it is usually composed of stalks, skins, seeds and pulp (Díaz et al., 2013; El Achkar et al., 2016; Nogales et al., 2005; Vatai et al., 2009). As far as olive oil extraction is concerned, waste generation depends on the type of system being used. Three-phase systems generate a type of solid waste, which is known as olive pomace and is composed of olive pulp, skin, pieces of kernels and oil (Carlini et al., 2015; Kalderis and Diamadopoulos, 2010). Orange juice manufacturing generates large amounts of solid waste, which are mainly composed of peels (60-65%), while they also contain seeds and membrane residues (Martín et al., 2010; Negro et al., 2016). Such materials have a rich composition, which includes fats, sugars, acids, insoluble carbohydrates, flavonoids, essential oils, phenolic compounds, etc. (Boukroufa et al., 2015). Cotton processing waste are produced during the ginning process and usually account for 10% of the initial seed cotton quantity. These materials are comprised of burs, stalks, leaves, immature cottonseed and other plant materials (Cotton Australia, 2016; Hamawand et al., 2016; Placido and Capareda, 2013). Agroindustrial waste such as those described above, require appropriate management, in order to avoid potential environmental problems related to their disposal. To this regard, the use of these materials for energy production would seem as a suitable solution (Aboudi et al., 2015; Aboudi et al., 2016; Anjum et al., 2016).

The organic fraction of municipal solid waste (OFMSW) is characterized by a highly heterogeneous composition, which depends on several factors, related to seasonal, geographical, lifestyle and cultural differences. These include variations in consumption patterns, type of waste produced, recycling, disposal and collection practices (Alibardi and Cossu, 2015; Li et al., 2011; Ward et al., 2008). However, despite this variability, OFMSW is typically mainly composed of food waste, such as kitchen waste, leftovers from households, restaurants, cafeterias etc., and garden waste, which include lignocellulosic materials, such as grass clippings, leaves, weeds, tree prunings e.tc. (Alibardi and Cossu, 2015; Cesaro and Belgiorno, 2014). Due to its high content in food waste, OFMSW is characterized by high moisture and biodegradable organic matter contents. These characteristics are seen as the main drawbacks in the case that OFMSW is inappropriately managed, since they could pose serious threats to the environment (Alibardi and Cossu, 2015). For this reason, waste management policies, such as the European Landfill Directive (99/31/EC), have lately been focusing on organic waste recovery, rather than disposal (Cesaro and Belgiorno, 2014).

Anaerobic digestion has been recognized as an effective waste management technology and has been used for the treatment of various types of waste, including agricultural and agroindustrial waste, the organic fraction of municipal solid waste, livestock effluents, sludge etc. (Da Ros et al., 2016a). This process involves the biological conversion of the organic matter present in waste materials, through the action of a microbial consortium under anaerobic conditions (Ward et al., 2008). The main product of this process is biogas, which

due to its methane-rich composition, can be used for energy production. In fact, methane obtained through anaerobic digestion has been considered as a renewable energy source and can be an excellent alternative to fossil fuels for heat and electricity generation. This potential for alternative energy production via anaerobic digestion is of great importance, especially when considering the increasing global demand for renewable energy, after the implementation of the European Renewable Energy Directive (2009/28/EC), which sets a 20% target for the overall share of energy from renewable sources. Apart from biogas, anaerobic digestion also results in the production of significant amounts of digestate, which usually has a nutrient-rich composition and, depending on its specific characteristics, may be composted or utilized as a fertilizer or a soil conditioner (Barrantes Leiva et al., 2014; Fitamo et al., 2016; Kim and Oh, 2011; Ward et al., 2008). The efficient performance of an anaerobic digestion system depends on a number of parameters, such as temperature, pH, nutrients content, carbon to nitrogen ratio (C/N), presence of inhibitory compounds etc. Often, during the digestion of certain substrates, some of these parameters may be encountered more or less distant from the optimal conditions. In those cases, the performance of the anaerobic digestion process may be compromised, eventually leading to failure (Esposito et al., 2012). Such phenomena depend on the composition of the substrates and are mostly related to nutrient balance and organic load issues (Mata-Alvarez et al., 2014). Therefore, care should be taken, in order to provide anaerobic digestion systems with feedstocks of a suitable composition. To this purpose, co-digestion is frequently implemented (Fitamo et al., 2016).

Anaerobic co-digestion consists in the combined anaerobic treatment of two or more substrates, which have been mixed together (Astals et al., 2014; Ganesh et al., 2013). The most important parameter in anaerobic co-digestion for obtaining a suitable feedstock composition is the appropriate selection of co-substrates and mixing ratios. In fact, co-substrates are usually chosen in order to have complementary characteristics. This favors the development of synergistic effects, which lead to an improved nutrient balance, often manifested by more appropriate C/N, within the optimal range. Additional benefits of co-digestion include dilution of eventual potentially toxic compounds, adjustment of the moisture content, pH and buffer capacity of the mixture, increased content of biodegradable matter, as well as widening of the range of microbes participating to the process. All the above, ultimately result in the improvement of methane production and digestate stability. Nevertheless, a univocal determination of optimal substrate mixing conditions is not possible, due to the wide variety of potential feedstock materials. Therefore, a targeted investigation should be carried out in each case (Astals et al., 2014; Esposito et al., 2012; Fitamo et al., 2016).

Apart from chemical parameters, operational parameters are also important for the performance of anaerobic digestion systems, with two of the most important being the organic loading rate (OLR) and the hydraulic retention time (HRT) (Aboudi et al., 2015; Gou et al., 2014; Ganesh et al., 2013; Ziganshin et al., 2016). In fact, finding the right balance between these two parameters is decisive for optimizing the efficiency of the process (Aslanzadeh et al., 2014). Evidently, the optimum operational conditions are usually related to the characteristics of the feedstock materials and thus, should be determined for each separate case, either with single-substrates in mono-digestion systems, or mixed-substrates in co-digestion systems. Therefore, continuous research around this subject is necessary, especially when considering the high variability in the type and composition of possible substrates, within each geographical area.

In this study the anaerobic digestion of four agroindustrial waste, namely cotton gin waste (CGW), winery waste (WW), olive pomace (OP) and juice industry waste (JW), under

semi-continuous operation was investigated. Both mono-digestion and co-digestion assays were conducted, with the latter including the use of a synthetic organic fraction (SOF) sample as a co-substrate. These assays were divided into two groups, with different conditions being applied for each of them. The assays of Group I had the objective of investigating the variation in operational parameters, such as the organic loading rate (OLR) and the hydraulic retention time (HRT). On the other hand, the assays of Group II, aimed at evaluating the performance of anaerobic digestion systems being fed with different substrates in a sequential order, at a constant OLR. Moreover, several chemical parameters were monitored during the incubation period for all investigated assays, including pH, total alkalinity, volatile acids, soluble chemical oxygen demand, total ammoniacal nitrogen and total phenols concentrations. Currently, there is lack of studies regarding mono- and co-digestion of CGW, WW, OP and JW, under the conditions evaluated in the present study. In addition, feeding of these substrates in a sequential order, in both mono- and co-digestion modes, had not been studied before.

6.2 Materials and methods

6.2.1 Substrates and inocula

The substrates used in this study included four agroindustrial waste (AW) samples and one synthetic organic fraction (SOF) sample, which was prepared in the laboratory. The former comprised of the waste materials originating from four of the most important agroindustrial activities encountered in the Mediterranean region, namely cotton processing (cotton gin waste, CGW), wine production (winery waste, WW), olive oil production (olive mill solid waste, i.e. olive pomace, OP) and citrus juice production (juice industry waste, JW). After their collection, these samples were stored in zip-lock bags at -20 °C, while prior to their use each one of them was adequately prepared. Specifically, WW and JW were comminuted without drying using a food processor, CGW was dried at 60 °C and then comminuted to a particle size less than 500 μm, using a universal cutting mill, while OP was kept as received. The SOF material was a synthetic sample, which intended to resemble the organic fraction of municipal solid waste (OFMSW). To this purpose, several types of household kitchen waste, including cooked pasta and rice, bread, cheese, vegetables, fruit, meat, coffee grounds, eggshells and paper were mixed together. The components of this substrate are materials that are typically encountered in the Mediterranean diet and their selection was based on previous studies (Bouallagui et al., 2009; Fountoulakis and Manios, 2009; Gomez et al., 2006; Molinuevo-Salces et al., 2012; Montalvo et al., 2012), conducted in relevant geographical areas. In addition to the above mentioned materials, a certain amount of deionized water was also added to the mixture, in order to improve its fluidity. This substrate was characterized regarding total solids (TS) and volatile solids (VS) contents, pH, elemental composition, total phenols (TPH), total ammoniacal nitrogen (TAN), soluble chemical oxygen demand (sCOD) and bulk density. The different components of the SOF sample were comminuted, then mixed and homogenized using a food processor and the final substrate was stored at -20 °C.

In total, eight feeding materials were used in the semi-continuous experiments performed in this study. The first four materials contained separately the four AW (single-substrates), while the remaining were composed of a mixture of each AW and a specific amount of SOF (mixed-substrates), in order to obtain a ratio of AW:SOF equal to 40:60, on a VS basis. This value for the mixing ratio was chosen taking into consideration the results of relevant literature (Anjum et al., 2016; Chen et al., 2014; Fitamo et al., 2016). Additionally,

appropriate amounts of deionized water were added to all eight feeding materials, in order to further improve their fluidity and facilitate the feeding procedure. The preparation of such feeding materials required further mixing and homogenization, after which samples in paste form were obtained. All feeding materials were divided in small portions, stored at -20 °C and one day before each use, an appropriate number of portions was transferred to 4 °C.

The inoculum used in this study consisted of anaerobic sludge originating from a mesophilic anaerobic digester of the Municipal Wastewater Treatment Facility of Chania, Crete and it was characterized by TS and VS contents of 2.31% and 1.62% respectively (VS/TS=0.70), a pH of 7.54 and TPH, TAN, TA and VA concentrations of 43.28 mg/L, 830 mg/L, 3498 mg$CaCO_3$/L and 168.7 mg/L, respectively.

6.2.2 Experimental setup and procedure

Two groups of reactors were used in the present study (Group I and Group II), with each Group including 8 trials (A-1, A-2, A-3, A-4, B-1, B-2, B-3, B-4). For both Groups reactors 'A' were fed with single-substrates, while reactors 'B' were fed with mixed-substrates. Each group served a different scope.

The trials of Group I aimed at investigating the effect of the variation in two operational parameters, i.e. the organic loading rate (OLR) and the hydraulic retention time (HRT), on the anaerobic digestion of one particular feeding material for each trial. More specifically, for this Group the experiment was divided in four phases. During Phase 1 (days 1-20) the OLR was kept constant for all reactors and equal to 1.0 gVS/L/d, while the HRT (HRT_1) was dependent on each feeding material. During Phase 2 (days 21-40) the OLR was increased to 1.3 gVS/L/d and consequently the HRT (HRT_2) was reduced accordingly, due to the variation in the volume of material being fed. In the two subsequent phases, namely Phase 3 (days 41-55) and Phase 4 (days 56-70), the same values as before were adopted for OLR (1.0 and 1.3 gVS/L/d, respectively), while the HRT was respectively halved by feeding the reactors with twice the volume of material. In order to double the volume fed to the reactors while maintaining the same OLR, each feeding material was diluted with an appropriate amount of deionized water. A similar methodology had also been adopted by Ziganshin et al. (2016).

In the trials of Group II the OLR was kept constant (1.0 gVS/L/d) throughout the whole duration of the experiment, while the HRT was passively varied, since the reactors were fed with four different feeding materials in a sequential order (CGW → WW → OP → JW), with each reactor starting from a different material. This order was chosen on the basis of the seasonality of the agricultural commodities from which the investigated waste are derived. As a result of such sequential feeding, for this Group, the experiment was divided into five phases. Phase 1 (days 1-20), Phase 2 (days 21-35), Phase 3 (days 36-50) and Phase 4 (days 51-65) corresponded to the four feeding materials, while in Phase 5 (days 65-70) each reactor was fed again with the material that it had received during Phase 1.

The above mentioned experimental conditions for the reactors of Group I and Group II are summarized in Table 6-1 and Table 6-2, respectively.

The experimental apparatus that was used for the semi-continuous assays consisted of 500 mL conical flasks covered with rubber stoppers. Three PVC (Polyvinyl chloride) tubes were inserted in the stoppers, of which two allowed N_2 flushing in the flasks and methane measurement, while the third was used for sampling and feeding operations.

Semi-continuous assays were carried out by firstly introducing an appropriate amount of inoculum in the flasks, in order to obtain a quantity of 15 gVS/L, and by subsequently adding deionized water to the flasks to bring the total volume to approximately 250 mL (working

volume). After adjusting the pH at 7.8±0.05, by adding small amounts of NaOH (1 M), the flasks were covered with the rubber stoppers and finally flushed with N_2 for at least 2 min. In order to deplete the residual biodegradable organic material present in the anaerobic sludge used as inoculum, the lattter was degassed by incubation at 35°C, until gas production was negligible. A similar procedure has been followed before (Astals et al., 2014; Moraes et al., 2015). Feeding of the reactors began as soon as the degassing period ended. Semi-continuous conditions were maintained by periodically withdrawing and inserting the same volume of digestion slurry and substrate, respectively, according to the operational parameters. Specifically, feeding and sampling operations were performed every five days for a total period of 70 days. The slurry samples taken from the reactors during the experiment were further analyzed in order to determine pH, total alkalinity (TA), volatile acids (VA), sCOD, TAN and TPH concentrations. All analyses except pH were performed on centrifuged samples (13,200 rpm, for 10 min). All trials were carried out in duplicate (a total of 16 reactors per Group), with the results being expressed as means. At the end of the experiment, the digestates obtained from each assay were dried at 60 °C and characterized regarding TS and VS contents, elemental composition and metal concentrations.

6.2.3 Analytical methods

TS and VS contents were determined according to APHA (American Public Health Association) method 2540G. Elemental analysis (C, H, N, S) of the substrates was performed using an EA300 Euro Vector elemental analyzer, via flash combustion at 1,020 °C. Oxygen content was determined by difference, considering the VS content of each sample. Total metals concentrations in digested samples, obtained at the end of the experiment, were determined after acid digestion with HNO_3 and through Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Agilent). pH was determined using a portable pH-meter (PH25, Crison). TA and VA measurements were based on APHA methods 2320B and 2310B, respectively. TAN (NH_4-N) was determined using a test kit (Merck) adopting a method that corresponds to APHA method 4500D. sCOD was determined through APHA method 5220C, while TPH were determined according to Folin-Ciocalteu's method, on the basis of the procedure described by Singleton et al. (1999). Briefly, 40 μL of sample were placed in glass cuvettes, to which 3.16 mL of deionized water and 200 μL of Folin-Ciocalteu reagent were then added. After mixing using a vortex (Genius 3, IKA), the cuvettes were left for 4 minutes and subsequently 600 μL of sodium carbonate solution (20%) were added to the mixture. Finally, the solutions were once again mixed and left for 2 h at 20 °C. The absorbance of each solution was determined at 765 nm. The final TPH concentrations are expressed in gallic acid equivalents (GAE). Methane production was determined by means of volume displacement using an 11.2% KOH solution, as it was done in previous studies (Altaş, 2009; Nain and Jawed, 2006). More specifically, each BMP reactor was connected to an inverted bottle containing the alkaline solution. Subsequently, biogas was released to flow inside the bottle, in order to remove CO_2 and H_2S by absorption and leave only CH_4. The volume of CH_4 being transferred to the bottle caused the displacement of an equal amount of KOH solution, which was then quantified using a graduated cylinder.

Table 6-1: Experimental conditions during semi-continuous operation of reactors of Group I

Assays: Group I	Feeding material	Incubation period (days)							
		1-20		21-40		41-55		56-70	
		OLR (gVS/L/d)	HRT (d)	OLR (gVS/L/d)	HRT (d)	OLR (gVS/L/d)	HRT (d)	OLR (gVS/L/d)	HRT (d)
I-A-1	CGW-M	1.0	147	1.3	114	1.0	74	1.3	57
I-A-2	WW-M	1.0	208	1.3	156	1.0	104	1.3	78
I-A-3	OP-M	1.0	500	1.3	357	1.0	250	1.3	179
I-A-4	JW-M	1.0	179	1.3	132	1.0	89	1.3	66
I-B-1	CGW-C	1.0	167	1.3	132	1.0	83	1.3	66
I-B-2	WW-C	1.0	179	1.3	147	1.0	89	1.3	74
I-B-3	OP-C	1.0	250	1.3	192	1.0	125	1.3	96
I-B-4	JW-C	1.0	167	1.3	132	1.0	83	1.3	66

Table 6-2: Experimental conditions during semi-continuous operation of reactors of Group II

Assays: Group II	OLR (gVS/L/d)	Incubation period (days)									
		1-20		21-35		36-50		51-65		66-70	
		Feeding material	HRT (d)	Feeding material	HRT (d)	Feeding material	HRT (d)	Feeding material	HRT (d)	Feeding material	HRT (d)
II-A-1	1.0	CGW-M	147	WW-M	208	OP-M	500	JW-M	179	CGW-M	147
II-A-2	1.0	WW-M	208	OP-M	500	JW-M	179	CGW-M	147	WW-M	208
II-A-3	1.0	OP-M	500	JW-M	179	CGW-M	147	WW-M	208	OP-M	500
II-A-4	1.0	JW-M	179	CGW-M	147	WW-M	208	OP-M	500	JW-M	179
II-B-1	1.0	CGW-C	167	WW-C	179	OP-C	250	JW-C	167	CGW-C	167
II-B-2	1.0	WW-C	179	OP-C	250	JW-C	167	CGW-C	167	WW-C	179
II-B-3	1.0	OP-C	250	JW-C	167	CGW-C	167	WW-C	179	OP-C	250
II-B-4	1.0	JW-C	167	CGW-C	167	WW-C	179	OP-C	250	JW-C	167

6.3 Data analysis

6.3.1 Theoretical methane potential

The Theoretical Methane Potential (TMP) of the feeding materials at Standard Temperature and Pressure (STP) was estimated through their elemental composition and the stoichiometry of the degradation reaction (Equation 1), using Equation 2 (Lesteur et al., 2010):

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right)H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right)CO_2 + dNH_3 + eH_2S \quad (1)$$

$$TMP\left[\text{mL CH}_{4,\text{STP}}/\text{g VS}\right] = 22.4 \cdot \left[\frac{\left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)}{12a + b + 16c + 14d + 32e}\right] \cdot 1000 \quad (2)$$

6.3.2 Theoretical oxygen demand

The Theoretical Oxygen Demand (TOD) of the feeding materials was estimated through their elemental composition and the stoichiometry of the oxidation reaction (Equation 3), using Equation 4 (Raposo et al., 2011):

$$C_aH_bO_cN_dS_e + \left(a + \frac{b}{4} - \frac{c}{2} - \frac{3d}{4}\right)O_2 \rightarrow aCO_2 + \left(\frac{b}{2} - \frac{3d}{2}\right)H_2O + dNH_3 \quad (3)$$

$$TOD\left[\text{mg O}_2/\text{g VS}\right] = \frac{\left(2a + \frac{b}{2} - c - \frac{3d}{2}\right) \cdot 16}{12a + b + 16c + 14d} \cdot 1000 \quad (4)$$

6.3.3 Free Ammonia Nitrogen

Free Ammonia Nitrogen (FAN) concentrations inside the reactors during semi-continuous assays were calculated combining weekly TAN (NH_4-N) values and their corresponding pH values, using Equation 5 (Fotidis et al., 2013).

$$FAN = \frac{[TAN]}{1 + \frac{10^{-pH}}{K_a}} \quad (5)$$

where, FAN and TAN are the free and total ammonia nitrogen concentrations, respectively, and K_a is the dissociation constant, with a value of $1.12 \cdot 10^{-9}$ at 35 °C.

6.3.4 Methane Yields

Methane production data obtained from the semi-continuous assays were used to calculate the methane yields. Considering that feeding and sampling operations were performed at predetermined time intervals, i.e. every five days, MY for the different feeding periods were calculated by dividing the cumulative methane quantity (in STP conditions) that had been produced during each period, with the amount of feeding material (in g of VS) that was added to the reactors on the first day of that period (Equation 6).

$$MY = \frac{CM}{F} \tag{6}$$

where, *MY* is the methane yield (mL $CH_{4,STP}$/g VS), *CM* is the cumulative methane production (mL $CH_{4,\,STP}$) and *F* is the substrate amount (g VS) fed to each reactor.

6.3.5 Organic loading rate and Hydraulic retention time

Considering that in the present study, specific OLR values were selected for performing the semi-continuous assays, it was necessary to calculate the exact quantities of feeding materials that correspond to these predetermined OLR values. Moreover, based on these quantities and considering the bulk density and VS content of each feeding material, as well as a constant reactor working volume of 250 mL, the respective HRT values were also calculated. This procedure was carried out by using Equations 7 and 8 (Banks et al., 2011).

$$VS_{fm} = OLR \times V_{working} \tag{7}$$

$$HRT = \frac{V_{working}}{V_{fm}} \tag{8}$$

where, $V_{working}$, is the working volume of the anaerobic digestion reactor (L), VS_{fm} is the daily amount of volatile solids of feeding material (gVS/d), *OLR* is the organic loading rate (gVS/L/d), *HRT* is the hydraulic retention time (d) and V_{fm} is the daily addition of feeding material in terms of volume (L/d).

6.3.6 Kinetic modeling

The methane production data obtained for every assay during each feeding period (5 days) of the experiment were fitted to a pseudo-first-order exponential model (Martín et al., 2013) (Equation 9) through non-linear regression, using the Solver tool of Microsoft Office Excel. The goodness of fit was evaluated by taking into consideration both the Residual Sum of Squares (RSS) and the R-square (R^2) values.

$$B = B' \cdot \left[1 - \exp\left(-k' t\right)\right] \tag{9}$$

where, *t* is time (d), *B* is the cumulative methane production at time *t* (mL CH_4), *k'* is the apparent rate constant (d^{-1}) and *B'* is the maximum methane production (mL CH_4).

6.3.7 Energy calculations

The specific energy corresponding to the maximum average methane yields obtained for each separate feeding material during semi-continuous digestion assays, *E* (kJ/kg VS), was calculated according to Equation 10 (Passos et al., 2013).

$$E_M = \frac{MY \cdot \xi}{1000} \quad (10)$$

where, ξ is the lower heating value of methane (35800 kJ/m^3 CH_4).

6.4 Results and discussion

6.4.1 Characteristics of substrates

The characteristics, as well as a description of the composition of the SOF sample are reported in Table 6-3. The SOF sample used in the present study is mainly composed of fruit and vegetables (58.4%) and contains a fair amount of moisture, while having a high VS/TS. This sample is also characterized by an acidic pH.

As far as the eight feeding materials (Table 6-4) are concerned, solids analysis showed higher TS and VS contents for single-substrates compared with mixed-substrates, except for JW-substrates, for which the opposite is true. The results of elemental analysis revealed that the majority of single-substrates have lower C and H contents than their respective mixed-substrates, except for the case of OP-M, which has a higher C content than OP-C. Regarding N contents, those of CGW-M and JW-M are found lower than those of CGW-C and JW-C, respectively, while the opposite happens for WW- and OP- substrates. C/N values are all found within the range that is favorable for anaerobic digestion, which according to literature (Kayhanian, 1999; Weiland, 2010) may vary from 15 to 35. Single-substrates are found on lower levels compared with mixed-substrates, with the latter being near the highest recommended value. The only exception to these observations is the value corresponding to JW-M, which is well above the upper limit of this range. Moreover, the highest TS, VS, C, H and N contents are associated with the two OP-substrates.

Table 6-3: Composition and characteristics of the synthetic organic fraction sample

Fractional Composition [a]	Component	%	Component	%
	Pasta	2.98	Cucumber	4.06
	Rice	2.89	Apple/Pear	2.80
	Bread	3.59	Orange	11.25
	Cheese	0.87	Peach	2.86
	Lettuce	6.56	Banana	4.65
	Zucchini/Eggplant	6.23	Meat	3.69
	Onion	4.31	Coffee	5.44
	Carrot	2.98	Eggshells	0.23
	Tomato	5.18	Paper	2.13
	Potato	7.53	Water	19.78

Proximate analysis[a]	TS (%)	VS (%)	VS/TS
	16.3	15.3	0.94

Ultimate analysis [b]	C (%)	H (%)	O (%)	N (%)	S (%)	C/N
	40.2	5.31	46.8	1.73	<DL	23.3

pH	sCOD (mgO_2/g) [a]	TPH (mg/g) [a]	TAN (mg/g) [a]	Bulk density (kg/L) [a]
4.76	66.15	0.55	$1.93 \cdot 10^{-2}$	1.09

DL: Detection Limit , [a] wb: wet basis , [b] db: dry basis

Table 6-4: Characteristics of feeding materials

Properties	Feeding materials							
	CGW-M	WW-M	OP-M	JW-M	CGW-C	WW-C	OP-C	JW-C
TS (%) [a]	22.2	20.6	45.2	16.2	19.5	18.7	24.1	17.3
VS (%) [a]	15.6	19.5	44.2	15.7	16.5	17.5	23.0	16.3
VS/TS	0.70	0.94	0.98	0.97	0.84	0.94	0.95	0.95
Bulk density (kg/L)	1.09	1.06	1.10	1.04	1.10	1.07	1.07	1.04
Elemental composition [b]								
C (%)	32.8	45.9	54.2	45.3	42.6	47.5	51.8	47.1
H (%)	4.40	5.95	7.53	6.29	7.89	8.82	9.55	8.64
O (%)	31.5	40.7	33.9	44.1	32.5	35.9	32.2	37.5
N (%)	1.40	1.80	2.09	0.90	1.41	1.58	1.67	1.44
S (%)	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL
C/N	23.5	25.5	25.9	50.4	30.3	30.1	31.0	32.8
Empirical formula	$C_{27.4}H_{44.2}O_{19.7}N$	$C_{29.8}H_{46.4}O_{19.8}N$	$C_{30.2}H_{50.4}O_{14.2}N$	$C_{58.8}H_{97.8}O_{42.8}N$	$C_{35.3}H_{78.4}O_{20.2}N$	$C_{35.1}H_{78.4}O_{19.9}N$	$C_{36.2}H_{80.0}O_{16.9}N$	$C_{38.3}H_{84.2}O_{22.9}N$
TOD (mgO_2/gVS)	1268	1339	1710	1301	1678	1692	1885	1635
TMP ($mLCH_{4,STP}/gVS$)	443.9	468.5	598.6	455.2	587.3	592.3	659.7	572.1

DL: Detection Limit , [a] wb: wet basis , [b] db: dry basis

6.4.2 Methane production

The graphs of Fig. 6-1 and Fig. 6-2 present the daily methane production during semi-continuous assays for the two Groups of assays, respectively. In both cases, following the degassing period, methane volumes produced during the first five days of active experiment, i.e. the first days after the beginning of feeding, are quite low compared with the following values, while the production rate seems to also be lower. These results suggest that this initial period served the purpose of acclimating the microbial populations to the feeding materials. Indeed, starting from the next feeding (day 5), the assays appear to be acquiring a more normal behavior. In fact, as time moves forward, the response of the systems to each feeding material is made more distinct.

At first glance, it can be noticed that the behavior of assays I-A-1 and I-B-1 (Fig. 6-1a) is similar to the behavior of assays I-A-3 and I-B-3 (Fig. 6-1c), respectively, while such similarities are also observed for assays I-A-2 and I-B-2 (Fig. 6-1b) and I-A-4 and I-B-4 (Fig. 6-1d), respectively. For the former assays, corresponding to CGW-M, CGW-C, OP-M and OP-C, respectively, an increase in methane production is observed during the first period of Phase 1, with a stabilization trend towards the end of this phase. This is true for both single- and mixed-substrates, while the stabilization trend is particularly evident for CGW-substrates. During Phase 2, the increase in OLR caused an analogous increase in methane production, which kept developing until the end of the phase. Furthermore, it is noted that methane production corresponding to the assays being fed with mixed-substrates (CGW-C and OP-C) is found at higher levels compared with the assays being fed with single-substrates (CGW-M and OP-M). The behavior presented for the remaining assays, corresponding to WW-M, WW-C, JW-M and JW-C, respectively, paints a slightly different picture. After the acclimation period, an initial increase in methane production was observed for the first and second feedings (days 5 and 10, respectively), while for the third feeding a decrease was noticed for both assays I-B-2 and I-B-4, which were being fed with the WW-C and JW-C, respectively, and for assay I-A-4, which was being fed with JW-M. In contrast for assay I-A-2 a slight increase was detected. Once the OLR was raised to 1.3 gVS/L/d, the increasing trend returned and it was maintained for all three consecutive feedings (days 20, 25 and 30) for assays I-B-2 and I-B-4. In the case of assays I-A-2 and I-A-4 however, a decrease was noted after the second feeding (day 25), similarly to what had been observed in Phase 1. It is worth mentioning that, during this whole first part of the experiment (Phase 1 and Phase 2), methane production for the assays containing WW-substrates, i.e. I-A-2 and I-B-2, ranged around similar levels, with the two respective curves almost matching. On the other hand, for the assays containing JW-substrates, i.e. I-A-4 and I-B-4, this is the case only for the first six days of experiment. Afterwards, methane production for assay I-A-4, which was being fed with JW-M, is found at lower levels. Moreover, for the latter assay, during Phase 2, methane production shows signs of a diauxic behavior, with a second peak being eventually observed after the third feeding. This indicates the probable difficulty encountered by microbial populations in degrading this specific substrate. In fact, the development of such a diauxic pattern often indicates the complexity of the degradation process. More specifically, in the present case it is likely that the organic load for this assay, after day 25, eventually became excessive for the microbes to handle in one dose, probably due to the high biodegradability of JW, being a fruit waste (Fonoll et al., 2015). This would have probably caused the degradation of this substrate to be divided in two phases, with the first phase being dedicated to the consumption of the readily and easily degradable matter and the second phase, to the matter requiring more time to be degraded (Cadavid-Rodriguez and Horan, 2012).

After evaluating the results obtained during the first 35 days of the experiment, the moment was considered appropriate for changing the operational conditions of the assays. For this reason, the reactors were left without feeding for the next five days (until day 40), in order to improve the stability of the systems, in preparation for the upcoming variations. Indeed, on day 40 (beginning of Phase 3), the OLR was restored to 1.0 gVS/L/d, while the HRT was reduced to half of that corresponding to Phase 1, while on day 55 (beginning of Phase 4) the OLR was raised again to 1.3 gVS/L/d, and the HRT was reduced to half of that corresponding to Phase 2.

After the initiation of Phase 3 and during both this phase and the next, methane production variation patterns for assays I-A-1, I-A-3, I-B-1 and I-B-3 were similar to those observed in Phases 1 and 2. Nevertheless, the levels of the respective values were found slightly increased in comparison with the first part of the experiment. However, as far as assays I-A-2, I-A-4, I-B-2 and I-B-4 are concerned, several differentiations are noticed. In fact, for the assays being fed with WW-substrates (I-A-2 and I-B-2), the behavior observed in Phase 3 is very similar to the behavior observed in Phase 1. The only difference is that during Phase 3 the assay corresponding to the single-substrate, i.e. I-A-2, had a higher methane production compared with the assay being fed with the mixed-substrate, i.e. I-B-2. This situation seems to be reversed after the first feeding of Phase 4 (day 55), while it returns for the next two feedings. At the same time, while a continuous increase is observed for assay I-A-2, assay I-B-2 is characterized by a quite defined decrease. On the other hand, for the assays being fed with JW-substrates (I-A-4 and I-B-4), a continuous increase is observed only for I-B-4, while for I-A-4, in addition to the reduced values compared with the first part of the experiment, a diauxic pattern is now more visible, with methane production rising again a few days after the peak. Finally, for the latter assay, a dramatic decrease in methane production is observed during the last 15 days of the experiment.

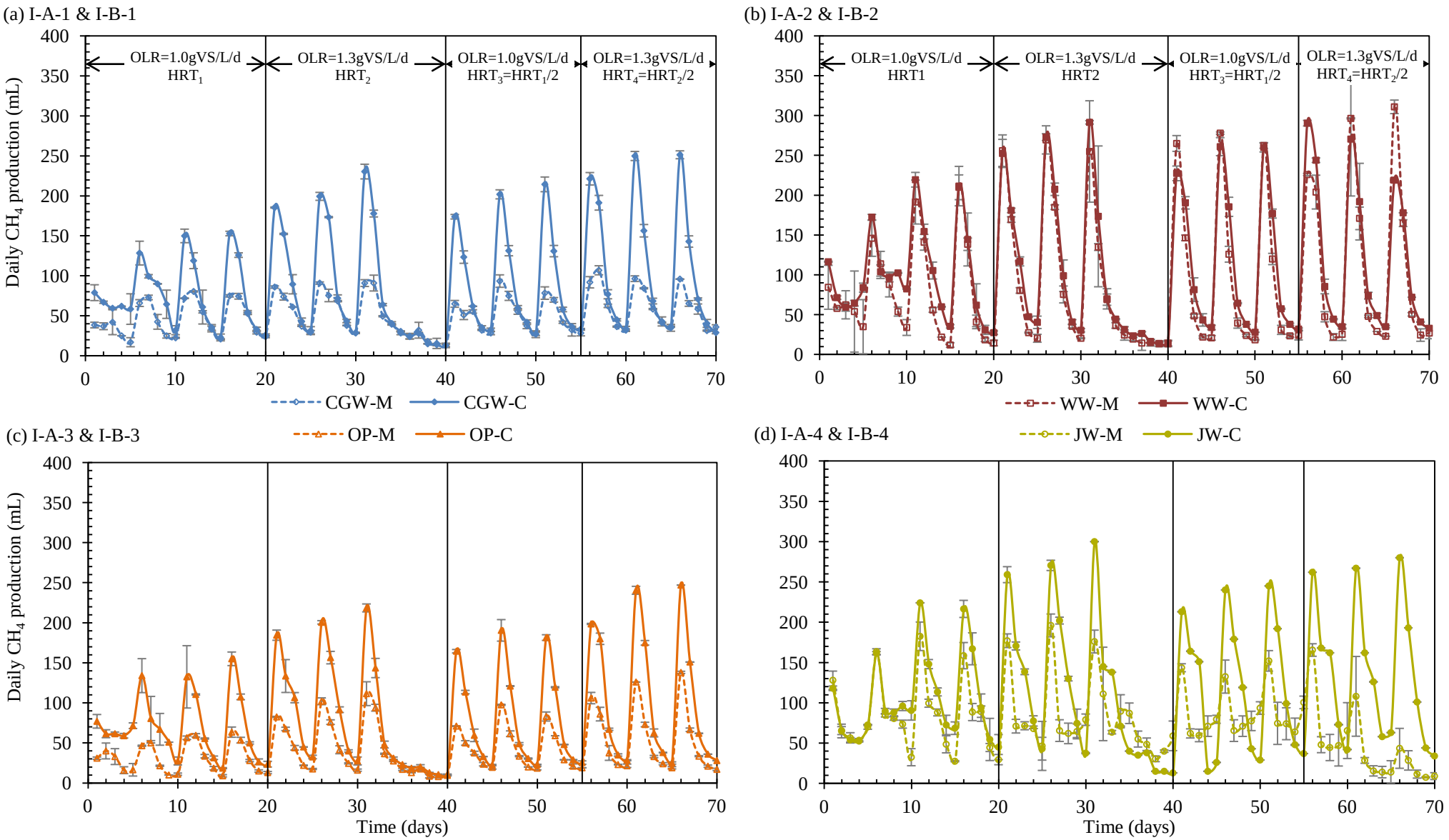


Figure 6-1: Daily methane production (mL) during semi-continuous assays for reactors of Group I [(a) I-A-1, I-B-1, (b) I-A-2, I-B-2, (c) I-A-3, I-B-3, (d) I-A-4, I-B-4] [A-dashed lines, B-continuous lines, CGW-M (- ◊ -), CGW-C (—◆—), WW-M (- □ -), WW-C (—■—), OP-M (- Δ -), OP-C (—▲—), JW-M (- ○ -), JW-C (—●—)]

Regarding the assays of Group II (Fig. 6-2), the operational conditions adopted during the first 20 days of the experiment were the same as those adopted in the assays of Group I, therefore the same observations are valid in this case as well. Moving forward in the experiment, it is noticed that during the time periods in which WW- and JW- substrates were fed to the reactors, methane production was higher not only for mixed- (B-assays), but also for single-substrates (A-assays). Similar patterns showing higher methane production for WW and JW, compared with CGW and OP were also obtained in a previous study (Pellera and Gidarakos, 2016) (Chapter 3) and are mainly attributed to the higher degradability of the former substrates, which is a characteristic of fruit waste in general (Fonoll et al., 2015; Ward et al., 2008). Nevertheless, although in the case of single-substrates the differences observed between different phases, i.e. for different feeding materials, are quite intense, in the case of mixed-substrates on the contrary, methane production is maintained around similar levels, with less intense fluctuations between one phase and the next, i.e. between feeding materials. This is probably related to the fact that the four mixed-substrates, for the most part, contain the same component, i.e. SOF. Therefore, it is logical that they would result in less distant methane production values. Furthermore, it is worth mentioning that, excluding the first days of the experiment, methane volumes being produced in the different assays, but corresponding to the same feeding materials, are found on similar levels, manifesting the immediate response of the microbial population to each new substrate. Indeed, after the end of the first phase of the experiment, the systems appear to have adapted to being fed with these materials. Consequently, no additional acclimation period would be required with each change of feeding material, since the microbial community has already adapted to substrates of similar nature. This is especially true in the case of mixed-substrates, in which due to the higher similarities in their composition, it would be less difficult for microbes to respond to each change of feeding material. The enhanced adaptation of the microbial population to the investigated feeding materials is also revealed by the data obtained after the last feeding (day 65). More specifically, as it was mentioned earlier, after feeding the reactors with four feeding materials (either single- or mixed-substrates) in a sequential order, the last feeding operation was performed using the material that had been fed first to each assay. The results of this operation showed that in all cases, the final values for methane production were higher than their respective initial values (considering the entire Phase 1). This fact suggests a good level of adaptation on behalf of the microbial populations, while it could also be a result of the presence of larger amounts of degradable matter in the reactors, considering that by the end of the experiment, each assay had received fourteen feedings.

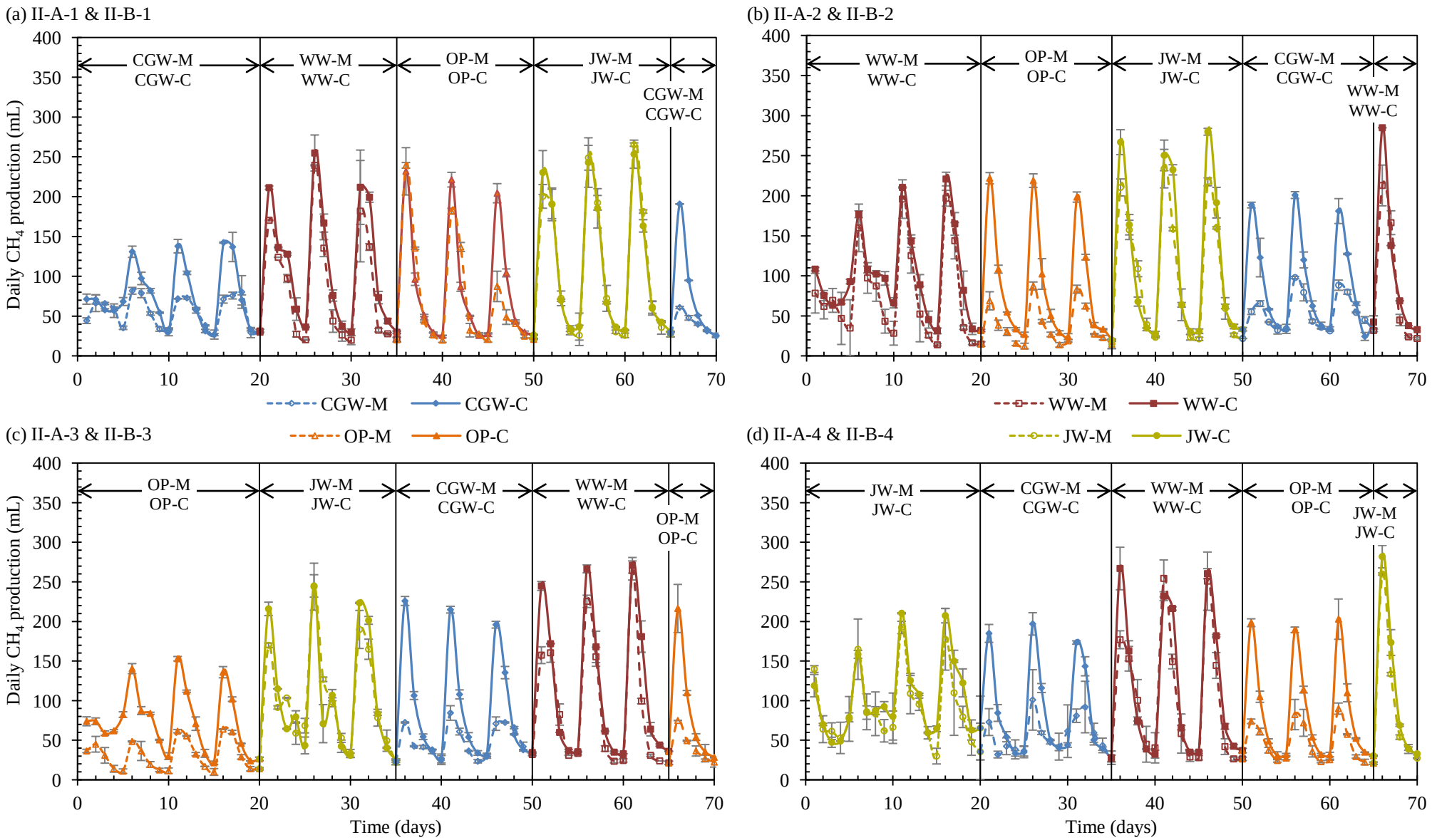


Figure 6-2: Daily methane production (mL) during semi-continuous assays for reactors of Group II [(a) II-A-1, II-B-1, (b) II-A-2, II-B-2, (c) II-A-3, II-B-3, (d) II-A-4, II-B-4] [A-dashed lines, B-continuous lines, CGW-M (- ◊ -), CGW-C (—◆—), WW-M (- □ -), WW-C (—■—), OP-M (- Δ -), OP-C (—▲—), JW-M (- ○ -), JW-C (—●—)]

6.4.3 Methane yields

Methane yields (MY) for the assays of both Group I (Fig. 6-3) and Group II (Fig. 6-5) were calculated based on the data provided by the previously described methane production profiles.

At the beginning of Phase 1, an initial increase in MY was observed between the first and the second feeding operations, in all cases. This increasing trend however, was not maintained until the end of this phase, for any of the assays. In fact, MY kept increasing until the third feeding operation and then decreased on the fourth one, for most of them. On the other hand, for I-B-1 and I-A-2 a slight decrease is observed immediately after the second feeding, while in the case of I-B-3, MY fluctuate between the second and the fourth feeding of Phase 1. The variation in OLR from 1.0 to 1.3 g/L/d at the beginning of Phase 2 led to decreased initial MY values for the assays being fed with CGW- and JW-substrates (Fig. 6-3a and Fig. 6-3d, respectively), while in the cases of the assays corresponding to WW- and OP-substrates (Fig. 6-3b and Fig. 6-3c, respectively) the respective values were found at a slightly higher level. Despite this initial difference however, in all cases an increase was observed for the second feeding, while this trend was maintained only for assays I-A-1, I-B-1, I-A-3 and I-A-4. For the remaining assays the third feeding of Phase 2 was characterized by a lower MY. At this point, it is worth mentioning that for the calculation of this latter MY, although the period lasted from day 31 to day 40, only the data obtained between days 31-35 were used, for consistency with the other calculations. Transitioning to the second part of the experiment, the restoration of the OLR to the previous value, as well as the further reduction of HRT to half, resulted in increased MY in all cases except for the assay being fed with OP-M (Fig. 6-3c). Moving forward into this phase, MY kept increasing for assays I-B-1, I-A-4 and I-B-4 and decreasing for assays I-A-2 and I-B-2, while for assays I-A-1, I-A-3 and I-B-3 values were found fluctuating between the first and the third feedings. Finally, during Phase 4, the combined increase in OLR and decrease in HRT led to a general decreasing trend for MY for the majority of the assays, with the most dramatic effect being observed for I-A-4, containing JW-M. Assays I-A-3 and I-B-3 were characterized by very slight fluctuations in a small range of values, while an increasing trend was observed for assay I-A-2.

In order to make all the above observations more clear, average MY were calculated for each phase of the experiment and were then depicted in Fig. 6-4. It is worth mentioning that the yields corresponding to the first five days of the experiment were not included in the average yields of Phase 1, since, as mentioned before, these first days functioned as an adaptation period and therefore these values were considered not comparable. The effect of each variation in the operational parameters is now more evident in all cases. Specifically, it can be seen that the initial increase in OLR (Phase 2) caused a decrease in MY for the assays containing WW-substrates (I-A-2 and I-B-2) and JW-substrates (I-A-4 and I-B-4) and an increase for the assays containing OP-substrates (I-A-3 and I-B-3). In the case of the assays containing CGW-substrates, I-A-1 presented a decrease, while I-B-1 showed an increase. It seems that in the latter case, co-digestion was beneficial, since it allowed the use of a higher OLR without apparent negative consequences concerning the stability of the process. The decrease in HRT coupled with the restoration to the initial OLR (Phase 3), seems to have caused a more significant effect on MY compared with the variation in OLR alone, leading in all cases to the achievement of higher values. In fact, during this phase (Phase 3), maximum average MY were achieved, as well as most maximum individual MY (i.e. for the separate feedings of each phase). Specifically, maximum average values were 184.9, 347.3, 154.1 and 316.3 mL $CH_{4,\ STP}$/g VS_{added} for I-A-1, I-A-2, I-A-3 and I-A-4, respectively, and 326.7, 411.6,

290.0 and 431.1 mL $CH_{4,\ STP}$/g VS_{added} for I-B-1, I-B-2, I-B-3 and I-B-4, respectively. Further decrease in HRT combined to an increase in OLR (Phase 4) led to a significant reduction in MY, in all cases except for assays I-A-3 and I-B-3, which were being fed with OP-substrates. Indeed, said variations in operational parameters did not cause significant changes in MY for these specific assays. Decreased methane yields especially during Phase 2 and Phase 4 are indicative of eventual inhibition of the anaerobic digestion process and may be attributed to system overloading phenomena, due to the increasing amounts of organic matter being added and possibly accumulated inside the reactors. Moreover, washout of microbial populations should not be excluded, especially during Phase 4, due to the significant reduction in HRT (Fitamo et al., 2016; Gou et al., 2014). Nevertheless, no signs of process inhibition related to other parameters were observed during these two phases for the majority of assays, similarly to what had been reported by Barrantes-Leiva et al. (2014). On the other hand, in the case of the assay I-A-4, overloading most likely led to system instability, which was accompanied by volatile acids accumulation, ultimately resulting in failure of the assay (Chen et al., 2016; Moraes et al., 2015). These findings are consistent with those reported by other authors. Martín et al. (2010) investigated the anaerobic digestion of orange peel waste under varying OLR and they observed that, while methane yield increased for OLR ranging from 1.20 to 3.67 kgCOD/m^3/d, a decrease of this parameter was recorded for values in the range of 3.67-5.10 kgCOD/m^3/d. The same authors, when studying the co-digestion of orange peel waste with residual glycerol (Martín et al., 2013) found that the maximum methane yield was obtained at a substrate load equal to 1.2 gVS/L, while further increase of the latter, led to a reduction in the methane yield. These authors as well, associated their results with an accumulation of volatile organic acids inside the reactors and the development of acidification phenomena, which ultimately inhibited the anaerobic digestion process. In the study conducted by Aboudi et al. (2015), during co-digestion of sugar beet and pig manure, a progressive increase of the OLR from 4.2 to 7.4 g VS/L/d led to increased specific methane production, however further increase up to 12.8 g VS/L/d caused the specific methane production to gradually drop. In another study, Zhang et al. (2013), found reduced methane yields after an increase in OLR, ultimately resulting in anaerobic digestion failure. Furthermore, Aslanzadeh et al. (2014) observed a continuous decrease in methane yields for a sample composed of the organic fraction of municipal solid waste, as they reduced the HRT from 10 to 7 and finally to 5 d, which corresponded to OLR of 2, 3 and 4 gVS/L/d, respectively. Additionally, in the same study, a sample composed of food processing waste was also evaluated and an increase was noticed for a HRT reduction from 10 to 7 d, followed by a decrease when the HRT was set to 5 d. On the other hand, Molinuevo-Salces et al. (2012) reported improved methane yields after a decrease in HRT from 25 to 15 d, corresponding to an increase in OLR from 0.4 to 0.6 gVS/L/d, thus agreeing with the results of the present study referring to the assays containing OP-substrates (I-A-3 and I-B-3).

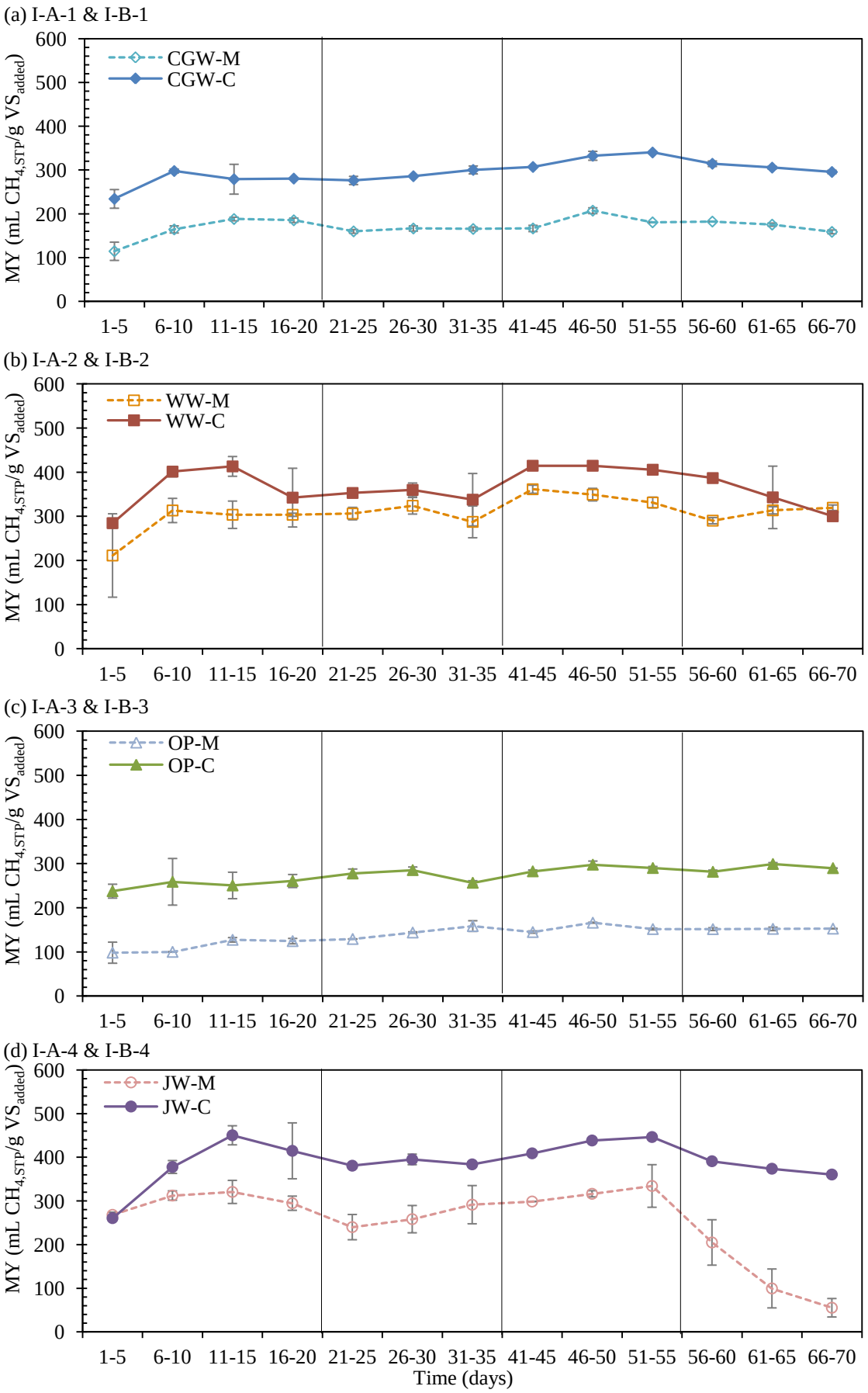


Figure 6-3: Methane Yields (MY) variation during semi-continuous assays for reactors of Group I [CGW-M (- ◊ -), CGW-C (—◆—), WW-M (- □ -), WW-C (—■—), OP-M (- △ -), OP-C (—▲—), JW-M (- ○ -), JW-C (—●—)]

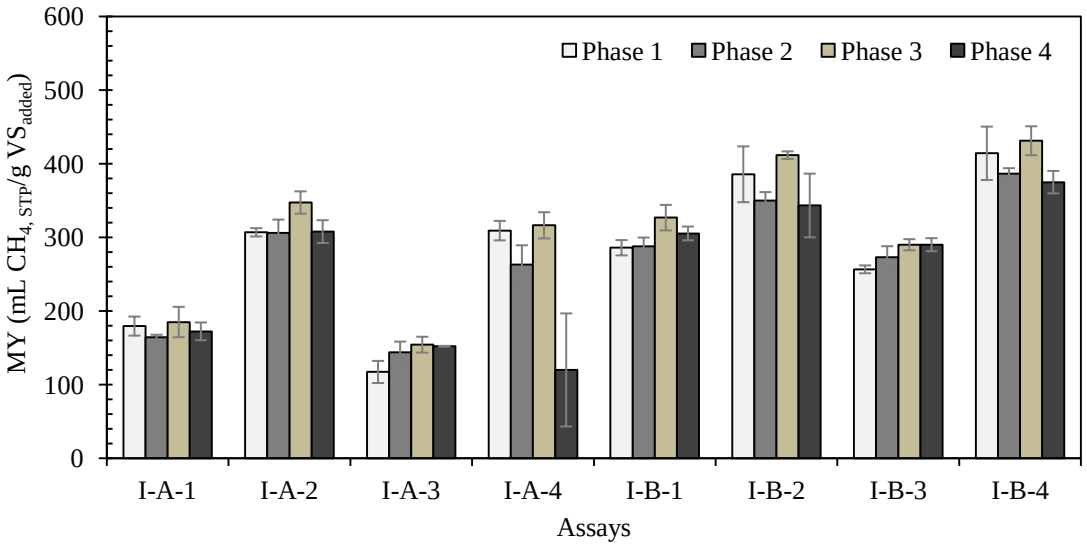


Figure 6-4: Average Methane Yields (MY) (mL $CH_{4,\ STP}$/g VS_{added}) for the assays of Group I

For the assays of Group II (Fig. 6-5), similar variation patterns are observed among assays. More specifically, similarly to what was observed for methane production profiles (Fig. 6-2), depending on the type of feeding material being fed to the reactors, MY values fluctuate between lower and higher levels, moving from one phase to the next. In fact, the gaps between phases are quite pronounced in the case of single-substrates, with the values being doubled or halved for certain assays. This phenomenon is visible for the mixed-substrates as well, but at a lower degree. Furthermore, as for methane production, also for MY, during the phases in which WW- and JW-substrates are fed to the reactors, MY are found higher compared with the phases in which the feeding materials contained CGW- and OP-substrates. As reported earlier (paragraph 6.4.2), this is related to the degree of degradability of each substrate. The average MY obtained for each assay of Group II can be seen in Table 6-5. No extremely high differences can be noticed between assays, nor for single-substrates, or for mixed-substrates. Nevertheless, in the former case II-A-3 and II-A-4 presented the lowest and the highest value, respectively. Contrarily to what was observed for the assays of Group I, no signs of inhibition or acidification were observed for any of the single-substrates assays of Group II. This reveals that sequentially changing the feeding materials had a positive effect on the process, since it apparently led to a more equilibrated environment inside the reactors, preventing such phenomena from occurring. Moreover, it was made evident that, especially in the case of mixed-substrates, feeding the systems with more degradable materials at startup, leads to more satisfactory results. This was demonstrated by the fact that, assays II-B-2 and II-B-4, which had WW-C and JW-C as initial feeding materials, provided higher MY than assays II-B-1 and II-B-3, which were initially fed with CGW-C and OP-C. Overall, the highest MY were obtained for the assays II-A-4 (274 mL $CH_{4,\ STP}$/gVS_{added}) and II-B-4 (363 mL $CH_{4,\ STP}$/gVS_{added}), when referring to single-substrates and mixed-substrates, respectively. It is noted that, similarly to what was applied for the yields of Group I, the yields corresponding to the adaptation period, comprising of the first five days of the experiment, were not included in the average methane yields.

The MY values obtained in the present study, for the assays of both Group I and Group II are found in line with previously reported data (Table 6-6), referring not only to mono-digestion but also to co-digestion investigations.

The results presented in both Table 6-5 and Fig. 6-5 also reveal the positive effect of co-digestion on MY. As a matter of fact, the combined treatment of AW with SOF led to significantly higher MY for the assays of both Group I and Group II, confirming the behavior noticed earlier for methane production data. More specifically, for the assays of Group I, said increase was in the ranges of 59-77%, 12-26% and 88-119%, for the assays being fed with CGW-, WW- and OP-substrates, respectively, considering all four phases of the experiment. For the assays being fed with JW-substrates, MY increased by 34-47% during Phases 1 through 3, while during Phase 4, a 213% increase was recorded, due to the failure of the assay I-A-4. On the other hand, for the assays of Group II, the MY corresponding to mixed-substrates assays were 32-42% higher than those corresponding to single-substrates assays.

A similar behavior, where co-digestion leads to higher methane yields compared with mono-digestion of the same substrates, has been observed before (Aboudi et al., 2016; Barrantes-Leiva et al., 2014; Bayr et al., 2014; Chen et al., 2016; Molinuevo-Salces et al., 2012). This suggests that co-digestion provides more favorable conditions for methane production, since mixed-substrates most likely contain more easily degradable matter compared with single-substrates, with this probably also being related to their higher C/N (Bayr et al., 2014; Chen et al., 2016; Molinuevo-Salces et al., 2012), all being between 30 and 33 (Table 6-4). Similarly, when Panichnumsin et al. (2010) investigated different mixing ratios for the co-digestion of cassava pulp and pig manure, the feedstock having a C/N equal to 33 provided the highest methane yield. Nevertheless, too high C/N may also result in decreased methane yields, due to nutrient deficiency, which prevents the reproduction and growth of anaerobic microbes (Lin et al., 2011). Such a case was reported by Zhang et al. (2013) who obtained a lower methane yield for food liquid waste having a C/N equal to 55.8. In the present study, this was observed for the assay being fed with JW-M, which had a C/N as high as 50.4. In this case, co-digestion improved the conditions for anaerobic digestion by increasing the N content of the substrate.

Table 6-5: Average Methane Yields (MY) (mL $CH_{4,\ STP}$/g VS_{added}) for the assays of Group II

Assays	Average MY (mL $CH_{4,\ STP}$/g VS_{added})
II-A-1	258.9 ± 100.2
II-A-2	255.6 ± 93.3
II-A-3	241.2 ± 109.9
II-A-4	274.2 ± 92.0
II-B-1	342.5 ± 60.5
II-B-2	362.9 ± 50.3
II-B-3	343.4 ± 57.4
II-B-4	363.2 ± 59.7

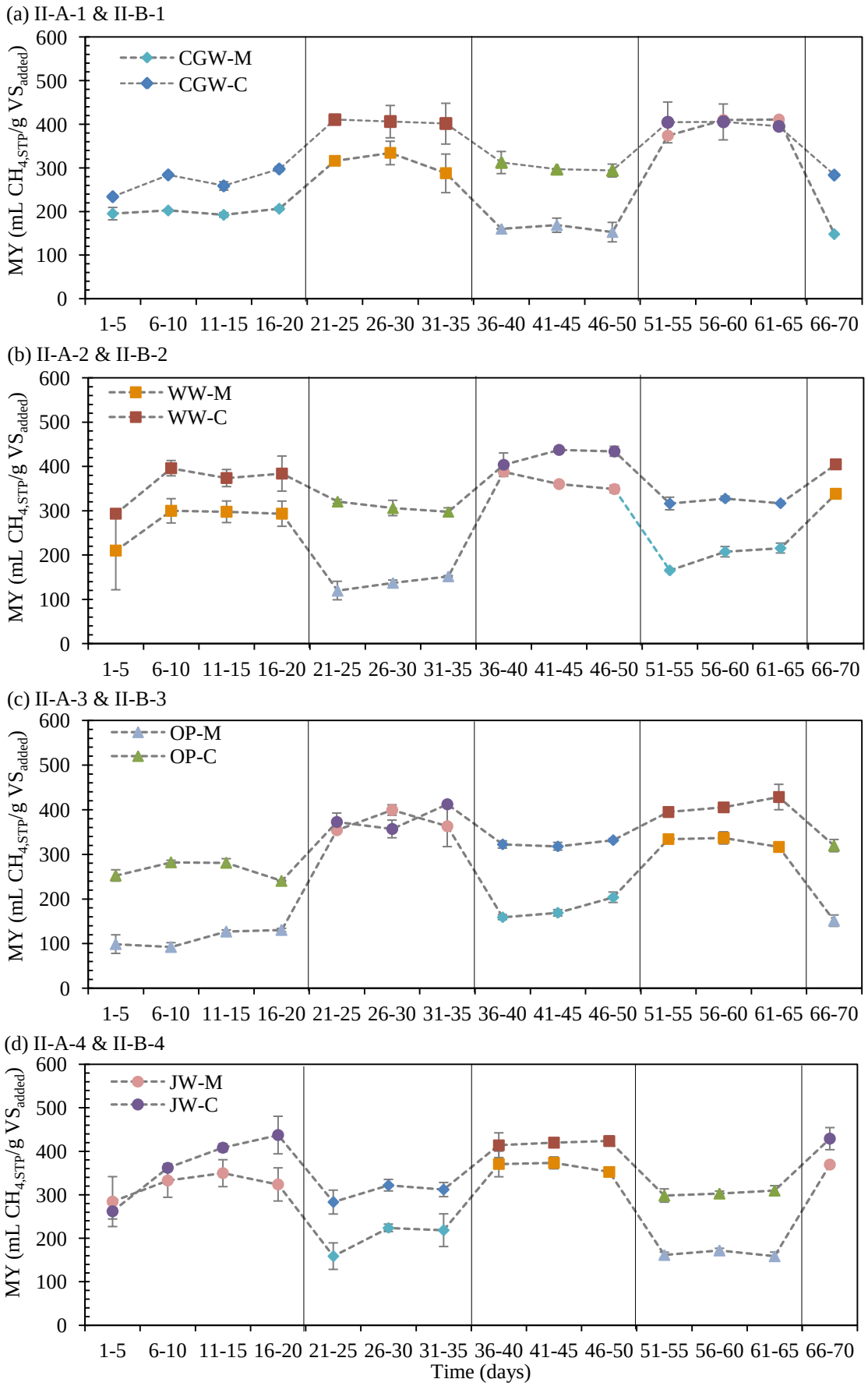


Figure 6-5: Methane Yields (MY) variation during semi-continuous assays for reactors of Group II [CGW-M (- ♦ -), CGW-C (- ♦ -), WW-M (- ■ -), WW-C (- ■ -), OP-M (- ▲ -), OP-C (- ▲ -), JW-M (- ● -), JW-C (- ● -)]

Table 6-6: Methane yields data reported in literature

Type of digestion	Substrates	Operational parameters	Value	Unit	Reference
Co-digestion	sugar beet byproduct + pig manure	37 °C HRT=12 d OLR=7.4 gVS/L/d	362.2	mL CH_4/g VS_{fed}	Aboudi et al., 2015
Mono-digestion	sugar beet byproduct	35 °C HRT=20 d OLR=3.26 gVS/L/d	225.71	mL CH_4/g VS_{added}	Aboudi et al., 2016
Co-digestion	sugar beet byproduct + cow manure	35 °C HRT=15 d OLR=4.97 gVS/L/d	313.98	mL CH_4/g VS_{added}	Aboudi et al., 2016
Mono-digestion	process liquid pretreated cotton stalks	35 °C OLR=0.9-1.6 gVS/L/d	0.184	Nm^3 CH_4/kg VS_{added}	Adl et al., 2012
Co-digestion	slaughterhouse waste + fruit-vegetable waste + manure	35 °C HRT=30 d OLR=0.3-1.3 kgVS/ m^3/d	0.3	m^3 CH_4/kg VS_{added}	Alvarez and Lidén, 2008
Mono-digestion	food processing waste	55 °C HRT=7 d OLR=3 gVS/L/d	0.44	m^3 CH_4/kg VS	Aslanzadeh et al., 2014
Mono-digestion	organic fraction of municipal solid waste	55 °C HRT=10 d OLR=2 gVS/L/d	0.33	m^3 CH_4/kg VS	Aslanzadeh et al., 2014
Mono-digestion	municipal sludge cake	35 °C HRT=20 d LR=2.73 gTCOD/L/d	0.23	L CH_4/g VS/d	Barrantes Leiva et al., 2014
Co-digestion	municipal sludge cake + thickened waste activated sludge + landfill leachate	35 °C HRT=20 d LR=2.84 gTCOD/L/d	0.24	L CH_4/g VS/d	Barrantes Leiva et al., 2014
Co-digestion	municipal sludge cake + thickened waste activated sludge + landfill leachate + screen cake	35 °C HRT=20 d LR=2.97 gTCOD/L/d	0.24	L CH_4/g VS/d	Barrantes Leiva et al., 2014

Co-digestion	olive pomace + olive mill wastewater + whey	38 °C HRT=40 d OLR=4.5 gCOD/L/d	336	L CH_4/kg VS	Battista et al., 2013
Co-digestion	olive pomace + whey	38 °C HRT=40 d OLR=3.4 gCOD/L/d	311	L CH_4/kg VS	Battista et al., 2013
Mono-digestion	rendering waste	55 °C HRT=50 d OLR=1.5 kgVS/m^3/d	450	dm^3 CH_4/kg VS_{fed}	Bayr et al., 2014
Co-digestion	rendering waste + potato pulp	55 °C HRT=50 d OLR=1.5 kgVS/m^3/d	500-680	dm^3 CH_4/kg VS_{fed}	Bayr et al., 2014
Co-digestion	fruit and vegetable waste + cattle slurry	35 °C HRT=21 d OLR=5.01 kgVS/m^3/d	0.45	m^3 CH_4/kg VS_{added}	Callaghan et al., 2002
Co-digestion	winery waste + waste activated sludge	37 °C HRT=21 d OLR=2.8 $kgCOD_{fed}/m^3$/d	0.38 (65% methane)	Nm^3 biogas/kg COD	Da Ros et al., 2014
Co-digestion	winery wastewater sludge + wine lees	37 °C HRT=23 d OLR=3.2 kgCOD/m^3/d	0.386	m^3 biogas/kg COD	Da Ros et al., 2016a
Mono-digestion	fermented grape marcs	55 °C HRT=40 d OLR=1 kgVS/m^3/d	0.29 (61% methane)	Nm^3 biogas/kg VS_{fed}	Da Ros et al., 2016b
Co-digestion	olive mill wastewater + olive mill solid waste	37 °C HRT=36 d OLR=5.54 gCOD/L/d (acidifier) OLR=2.28 gCOD/L/d (methanizer)	0.534	m^3 CH_4/kg $TCOD_{in}$	Fezzani and Ben Cheikh, 2010
Co-digestion	food waste + green waste	55 °C HRT=15, 20, 30 d OLR=0.62-5.04gVS/L/d	425 (75:25 mixture) 385 (50:50 mixture)	NmL CH_4/g VS	Fitamo et al., 2016

Mono-digestion	industrial orange waste	55 °C HRT=40 d OLR=2.8 kgVS/m^3/d		m^3 CH_4/kg VS_{added}	Kaparaju and Rintala, 2006
Mono-digestion	orange peels	55 °C HRT=25 d OLR=1.0 gVS/L/d	0.627	m^3 CH_4/kg VS	Koppar and Pullammanappallil, 2013
Co-digestion	fruit and vegetable waste + food waste	35°C OLR=3 kgVS/m^3/d	0.49	m^3 CH_4/kg VS	Lin et al., 2011
Mono-digestion	orange peel waste	55 °C HRT=25 d OLR=1.20-3.67 kgCOD/m^3/d	0.27-0.29	L_{STP} CH_4/g COD_{added}	Martín et al., 2010
Co-digestion	orange peel waste + residual glycerol	35°C HRT=8.5-30 d OLR=1.91 kgVS/m^3/d	330	mL_{STP} CH_4/g VS_{added}	Martín et al., 2013
Co-digestion	vegetable processing waste + swine manure	37°C HRT=25 & 15 d OLR=0.48 & 0.59 gVS/L/d	277 & 285	mL CH_4/g VS_{added}	Molinuevo-Salces et al., 2012

6.4.4 pH, VA and TA

The variation in pH, VA, TA and VA/TA values for the assays of Group I and Group II is presented in Fig. 6-6 and Fig. 6-7, respectively. Parameters such as pH and the ratio between VA and TA are very important in anaerobic digestion, since they are excellent indicators of the equilibrium and the stability of the process (Battista et al., 2013; Cuetos et al., 2010). In particular, methane production can take place within a pH range between 6.5 and 8.5, with the optimum values ranging from 7.0 to 8.0 (Weiland, 2010), while the stability of a digester can be determined according to three critical VA/TA values. Specifically, when VA/TA is < 0.4 the digester can be considered stable, when it is in the range 0.4-0.8 some instability will be occurring, whereas values ≥ 0.8 are indicative of significant instability (Callaghan et al., 2002).

For the majority of the assays of Group I, pH (Fig. 6-6a) is maintained quite constant between 7 and 7.5 throughout the duration of the experiment, indicating that the assays were operating under optimum conditions (Aboudi et al., 2015). The only two exceptions are observed for assays I-A-3 and I-A-4. In the former case, a small difference is found in the initial period of the experiment (until day 25), where, starting from an initial value of 7.75, the pH gradually decreased until reaching the level of the previously mentioned range. On the other hand, in the latter case, the difference is much more significant. Specifically, pH values dropped below 7 since day 30 and after a period of stability that lasted until the end of Phase 3 (day 55), they suffered further reduction until gradually reaching a value as low as 5.29 on the last day of the experiment (day 70). VA profiles (Fig. 6-6b) present some intense differentiations among assays during the first 30 days of experiment, while in the subsequent phases, it would seem that there are no such significant differences. The only case where an odd behavior is noticed is that of assay I-A-4. In fact, in this assay, which was being fed with JW-M, VA values show a continuous increase starting from day 30 and until the end of the experiment. This variation in VA levels coincides with the already mentioned pH decrease. These results corroborate the earlier suppositions regarding system overloading. More specifically, after the increase in OLR during Phase 2, the microbial community apparently did not have enough time to degrade the excess organic matter that was receiving, resulting in its accumulation. This resulted in an imbalance in the operation of the system, which in turn caused an increased production of volatile acids and a drop in pH, thus leading to acidification and to reduced methane production (Aboudi et al., 2015; Ahring et al., 1995). Regarding TA profiles (Fig. 6-6c) during the first part of the experiment (until day 30), they are characterized by relatively stable values for all assays, with a slightly increasing trend, particularly for the assays being fed with mixed-substrates (B-assays), and especially after the beginning of Phase 2, when the OLR was increased. Between days 30 and 40 an intense decrease is observed, due to the temporary interruption in feeding, while after the beginning of the second part of the experiment (on day 40) the change in operational conditions leads to once again raised values. Specifically, the decrease in HRT caused the new values to be evidently more elevated, compared with those of the first part in the case of mixed-substrates, while for single-substrates the respective values are found on similar levels. It is also observed that generally, the assays being fed with single-substrates are associated to lower values in comparison with the assays corresponding to mixed-substrates. VA/TA profiles (Fig. 6-6d) follow similar patterns as those observed for VA concentrations, with their values being maintained below 0.43 for the whole duration of the experiment, while specifically after day 25 they are found below 0.2 and following a constant behavior. Such a relation between VA and TA values is indicative of stable conditions inside the reactors as well as of a good

buffering capacity (Ganesh et al., 2013; Montañés et al., 2013). These observations are true for all assays, except for I-A-4, in which the conditions are found quite unstable, due to the acidification phenomena being developed, with VA/TA levels confirming this fact and reaching values greater than unity and specifically above 2. Similar results were obtained in the study conducted by Aboudi et al. (2015), in which an acidification phenomenon was also observed, resulting in an acidity/alkalinity ratio of 2.47.

By observing the data referring to Group II, a similar behavior as in Group I was noticed for pH (Fig. 6-7a) during the first 20 days, with subsequent levels being even more stable in a range of 7-7.5, until the end of the experiment. Regarding the variation in VA concentrations (Fig. 6-7b), a general decreasing trend can be observed for the first 30 days of experiment, after which all values tend to stabilize around similar levels. Moreover, an increasing trend of TA concentrations (Fig. 6-7c) is visible with time. Consequently, the change in feeding material seems to have a similar effect for both types of assays, i.e. those being fed with single- (A-assays) and mixed-substrates (B-assays). Nevertheless, absolute values corresponding to A-assays tend to be lower than those corresponding to B-assays, as was described earlier in the case of Group I. Interestingly, it can be seen that while VA values of different assays are found closer to each other after day 35, the corresponding TA values are more distant from each other in the same period. Similarly to before, VA/TA patterns (Fig. 6-7d) resemble those of VA profiles. Moreover, in this case as well, the stability of the systems is evident, since all values are found below 0.44 during the whole experiment, with a more evident stabilization trend after day 35, when values are well below 0.2.

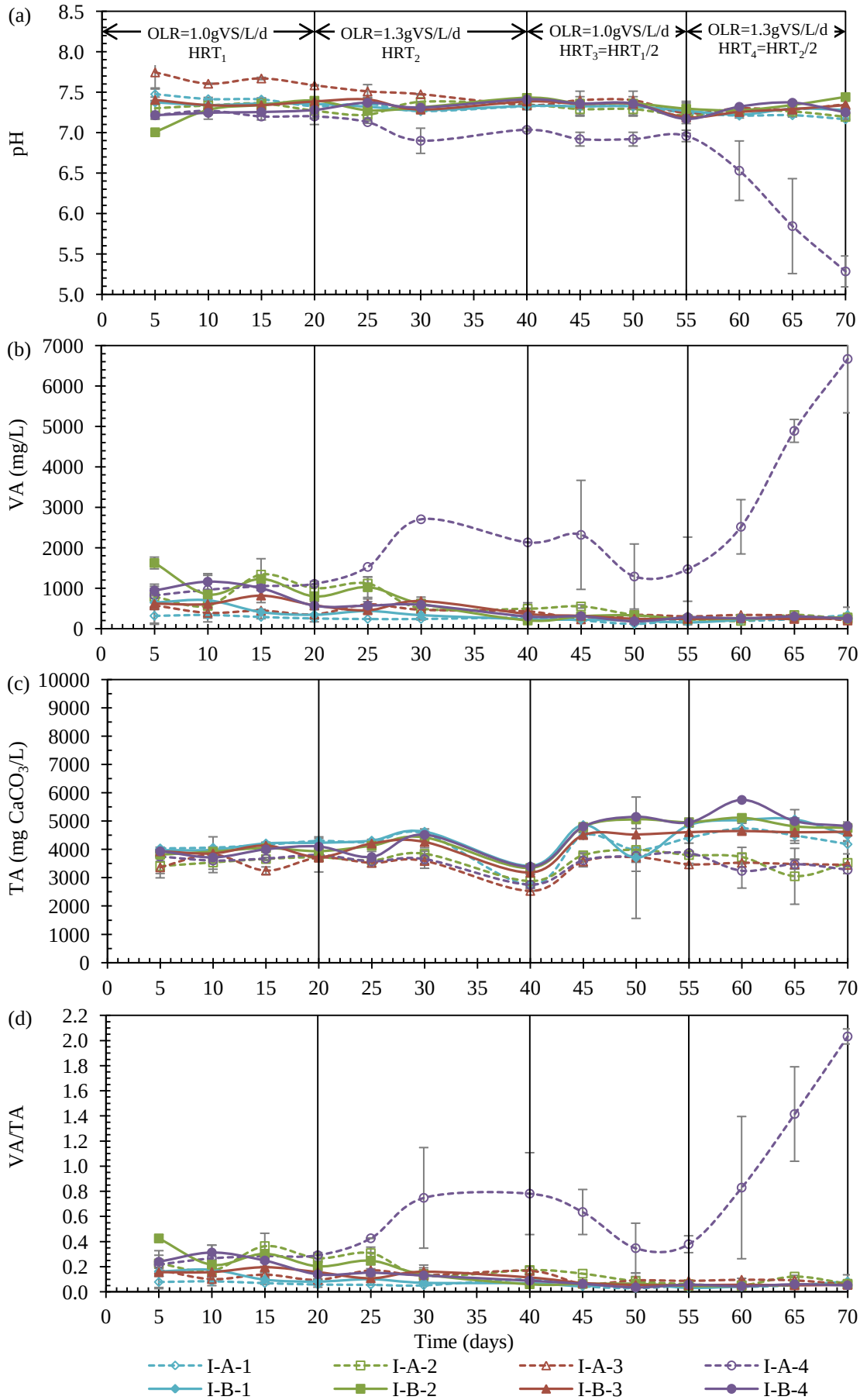


Figure 6-6: pH, VA, TA and VA/TA variation during semi-continuous assays for reactors of Group I [I-A-1 (- ◊ -), I-B-1 (—◆—), I-A-2 (- □ -), I-B-2 (—■—), I-A-3 (- Δ -), I-B-3 (—▲—), I-A-4 (- ○ -), I-B-4 (—●—)]

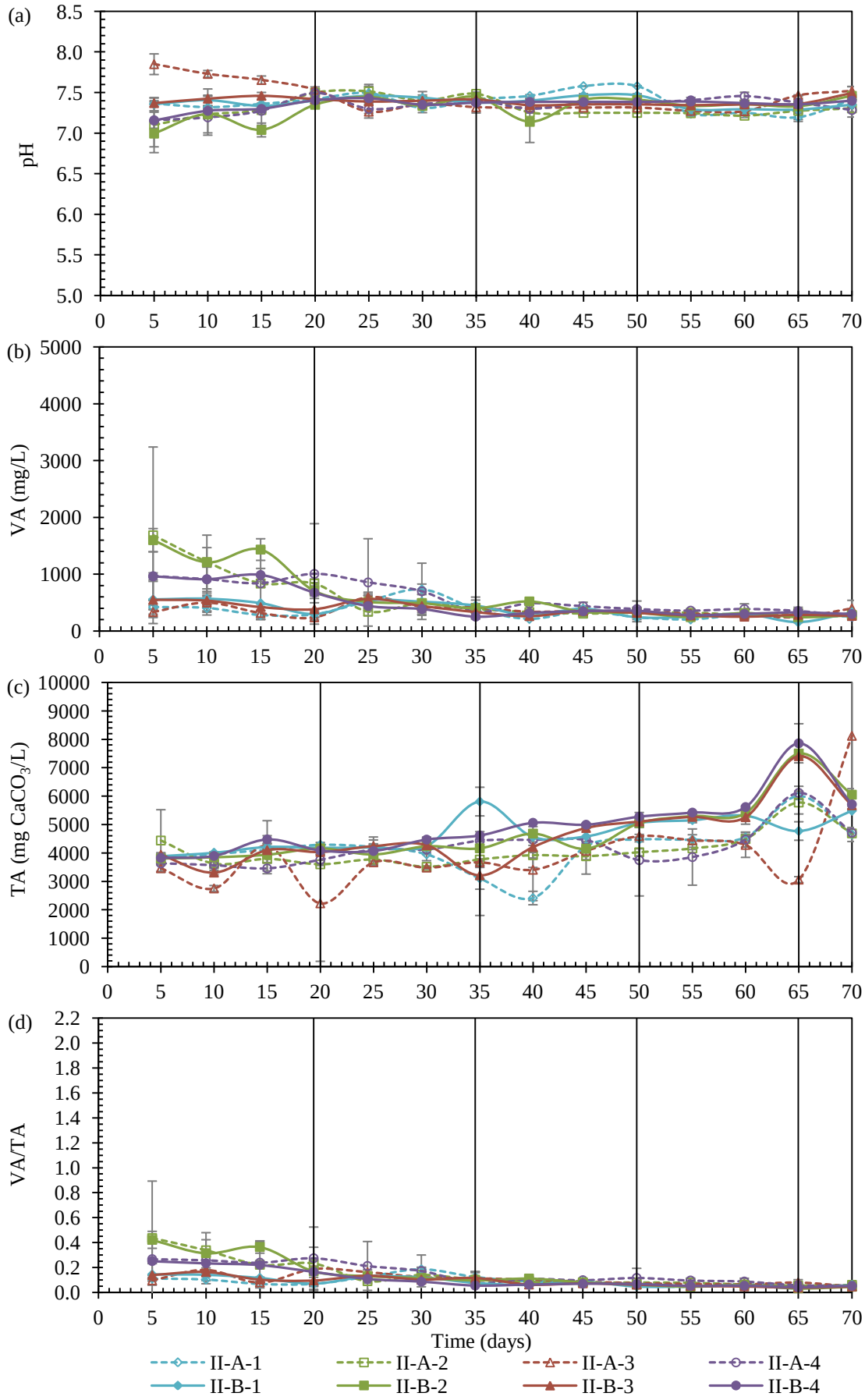


Figure 6-7: pH, VA, TA and VA/TA variation during semi-continuous assays for reactors of Group II [II-A-1 (- ◊ -), II-B-1 (—◆—), II-A-2 (- □ -), II-B-2 (—■—), II-A-3 (- Δ -), II-B-3 (—▲—), II-A-4 (- ○ -), II-B-4 (—●—)]

6.4.5 TAN and FAN

Fig. 6-8 and Fig. 6-9 depict the TAN and FAN profiles obtained for assays of Group I and Group II, respectively. TAN values were determined only for certain slurry samples, which corresponded to different sampling days for each Group of assays, according to the occurrence of a Phase change. More specifically, the samples being analyzed regarding this parameter were those taken on the day in which a Phase change occurred and those taken on the immediately next sampling day. The corresponding FAN values were then calculated by combining these data with the pH of the samples.

As it can be seen in Fig. 6-8a, TAN concentrations determined for the different assays on day 5, i.e. after the first feeding operation, are all very close to each other, while as the experiment develops, the differences among assays are made more evident. In fact, it can be noticed that the TAN levels encountered in the assays being fed with single-substrates (A-assays) are much lower than those of the assays being fed with mixed-substrates (B-assays). TAN release has been linked to the degradation of organic matter, mainly of protein compounds (Aslanzadeh et al., 2014; Fitamo et al., 2016). Due to the composition of the SOF sample, which includes cooked meat, mixed-substrates would have a higher protein content than single-substrates, thus possibly explaining higher TAN concentrations in the digestion slurries. Moreover, considering that SOF is a more easily biodegradable substrate compared with AW, its presence would result in a fasted degradation of nitrogen compounds. Lin et al. (2011) came to a similar conclusion after obtaining higher ammonium concentrations when increasing the food waste proportion during their co-digestion with fruit and vegetable waste. Furthermore, Cuetos et al. (2010) observed a similar pattern for mono-digestion of slaughterhouse waste and their co-digestion with the organic fraction of municipal solid waste. As far as the effect of the changes in operational conditions is concerned, the values determined at the end of Phase 1, were found increased for all B-assays, while for all A-assays except I-A-3, they were decreased. Moving on, although the initiation of Phase 2 and thus the increase in OLR, further reduced TAN concentrations in all assays, a renewed increase was observed by the end of this phase for B-assays, while in A-assays, values kept decreasing. In the beginning of the second part of the experiment (beginning of Phase 3) the decrease of both OLR and HRT resulted in lower TAN concentrations inside the reactors, in all cases, while the patterns followed during the rest of this phase were similar to those seen in Phase 1. Further increase in OLR and decrease in HRT led to the development of a continuous decreasing trend until the end of the assays. Decreasing TAN concentrations could be related to an eventual washout of microbial populations, resulting from these variations in operational parameters, as suggested by Aslanzadeh et al. (2014). As it is clearly shown by the results, this phenomenon would have been more intense for the single-substrates assays, since their already lower TAN amounts, compared with mixed-substrates assays, would have suffered an even more pronounced reduction as a result of washout. It is worth mentioning that the variations made during the second part of the experiment exerted a more significant impact on A-assays, regarding TAN levels. FAN concentrations inside the reactors of Group I (Fig. 6-8b) are found at much lower levels compared with their respective TAN concentrations. This was expected since the corresponding pH values, in each case, were kept within a close range without extreme variations, indicating the absence of high quantities of such a compound in the reactors. Nevertheless, the variation patterns observed for FAN resemble those noticed for TAN in certain cases.

The data depicted in Fig. 6-9a and referring to mixed-substrates, show some fluctuations among different phases, according to the changes in feeding materials. However, they are

maintained at relatively stable levels for the whole duration of the experiment, with the values corresponding to different assays being very close to each other. On the other hand, the data referring to single-substrates follow a general decreasing trend with some increased values being noticed at the end of Phase 2 and again at the end of Phase 3, with the curves corresponding to different reactors, getting closer with time. It is noteworthy that, also for this Group of assays, B-assays are associated with higher TAN concentrations compared with A-assays. As far as FAN concentrations (Fig. 6-9b) are concerned, all values are kept at very low levels, similar to those observed in Fig. 6-8b, without there being significant distinctions between assays.

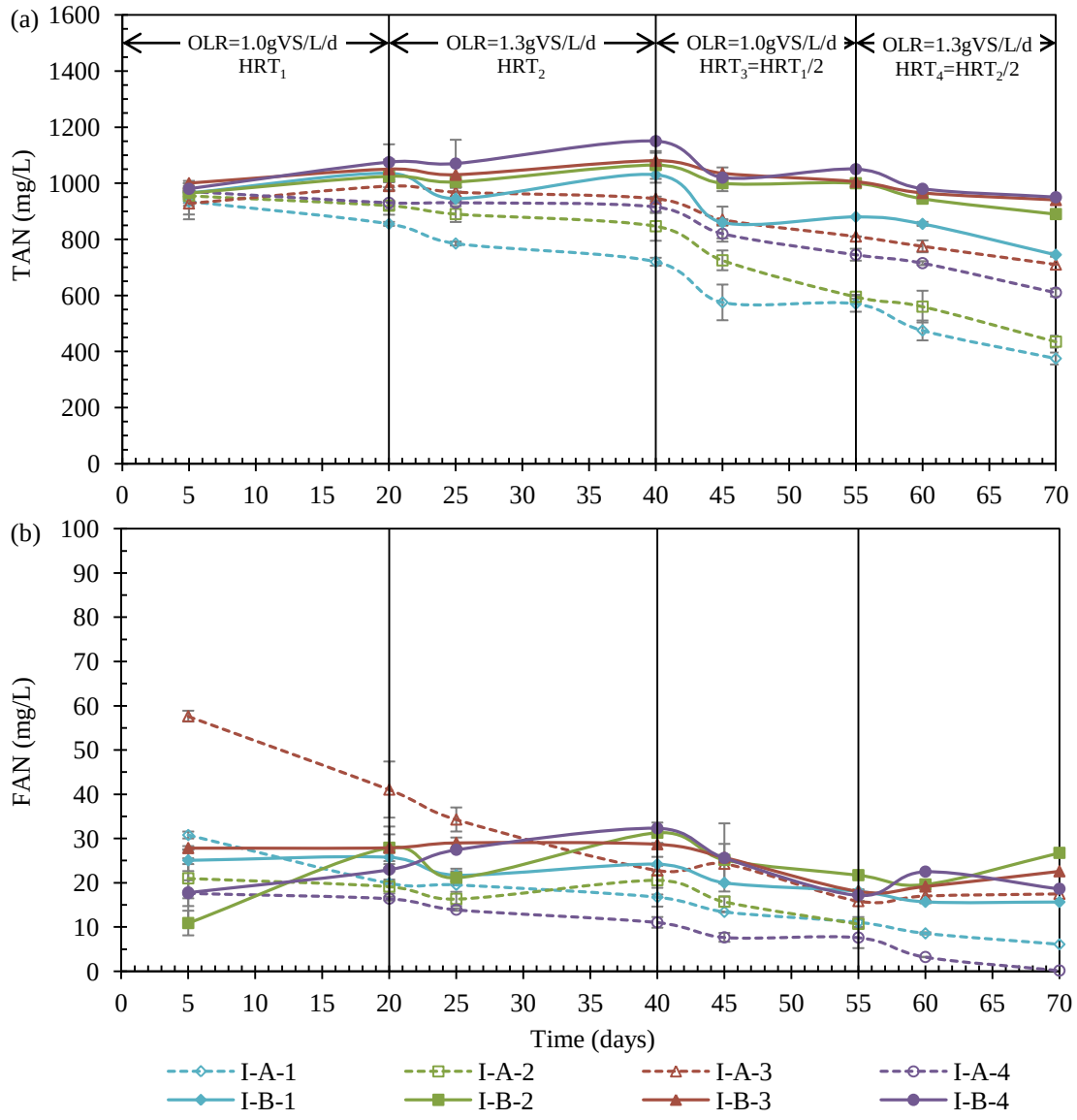


Figure 6-8: TAN and FAN variation during semi-continuous assays for reactors of Group I [I-A-1 (- ◊ -), I-B-1 (—◆—), I-A-2 (- □ -), I-B-2 (—■—), I-A-3 (- Δ -), I-B-3 (—▲—), I-A-4 (- ○ -), I-B-4 (—●—)]

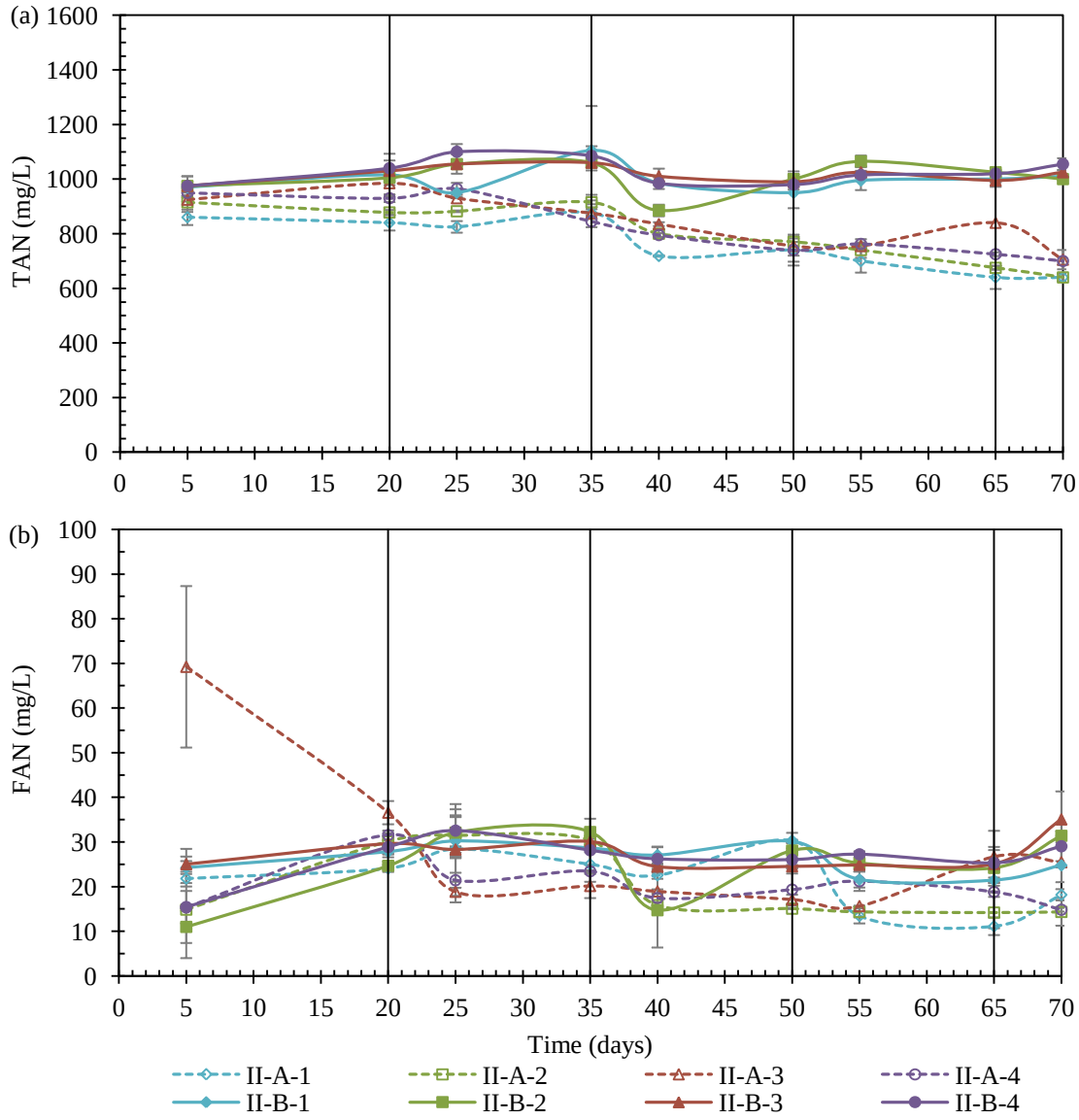


Figure 6-9: TAN and FAN variation during semi-continuous assays for reactors of Group II [II-A-1 (- ◊ -), II-B-1 (—◆—), II-A-2 (- □ -), II-B-2 (—■—), II-A-3 (- Δ -), II-B-3 (—▲—), II-A-4 (- ○ -), II-B-4 (—●—)]

6.4.6 sCOD

sCOD concentrations determined for the assays of both Groups I and II (Fig. 6-10a and Fig. 6-11a, respectively) generally seem to not have been subjected to significant variations. Nevertheless, some slight fluctuations are visible during the experiment, in both cases.

Regarding the assays of Group I (Fig. 6-10a), a decreasing trend is noticed for assays I-B-2, I-B-4 and I-A-2, while the value for assays I-A-1 and I-A-3 are found to be increasing, with these variations being more evident and pronounced during the first part of the experiment (Phases 1 and 2). Specifically, these behaviors may be a result at first, of the initial adaptation of the microbial community to the substrates (Phase 1) and subsequently, of the variation in OLR (Phase 2). On the other hand, the effects caused by the variations made in the second part of the experiment were not so intense. Moreover, constant sCOD levels are observed for assays I-B-1 and I-B-3 throughout the experiment. Anjum et al. (2016) observed that during the co-digestion of catering waste and lignocellulosic crop residues, sCOD

concentrations increased in the initial stage of the experiment and subsequently decreased. These variations were attributed to the progressive degradation of organic matter in the reactors. On the other hand, Aslanzadeh et al. (2014) noticed that the reduction in HRT from 10 to 7 d was associated with a sCOD decrease, while further decrease to 5 d led to an increase. Nevertheless, the obtained values were found to fluctuate within a relatively narrow range, similarly to what was observed in the present study. The only exception to the above observations is the assay I-A-4, for which sCOD values gradually increase with time until reaching a maximum of 11644 mg O_2/L on day 70. This differentiation for this specific assay coincides with the variations observed earlier regarding pH and VA profiles, indicating the existence of a relation between all these parameters. In fact, high sCOD concentrations at higher OLR and lower HRT are often the result of a decreased organic matter degradation rate, which in turn leads to the loss of balance in the anaerobic digestion system, with the latter usually being associated with VA accumulation and low pH values. Such a relation has already been mentioned in several studies (Bayr et al., 2014; Da Ros et al., 2016a; Lin et al., 2011; Montañés et al., 2013). Furthermore, similarly to before, there is a level difference in the values referring to A- and B- assays, with the former being lower than the latter.

As far as the assays of Group II are concerned, Fig. 6-11a presents curves with more significant differences between them during the first 30 days of the experiment, with those corresponding to II-A-1, II-B-1, IIA-3 and II-B-3 being characterized by a slightly increasing trend and those corresponding to II-A-2, II-B-2, IIA-4 and II-B-4 showing a slightly decreasing trend. On the other hand, after day 35, the differences between values tend to be minimized, with no particular trend being noticed for any of the assays.

6.4.7 TPH

TPH profiles show a constant behavior for the assays of Group I (Fig. 6-10b), without particularly intense fluctuations with time. Nevertheless, slightly higher values are observed at higher OLR and subsequently at lower HRT, for the assays being fed with JW- and OP-substrates. Moreover, these assays are also those characterized by more elevated TPH concentrations in comparison with the remaining assays, with the values corresponding to JW-assays being particularly pronounced. Specifically, for I-A-4, TPH concentration reaches a maximum of 353 mg/L at the end of the experiment. The different TPH levels being observed for different types of feeding materials are most likely related to the TPH content of the original substrates, i.e. CGW, WW, OP and JW. In fact, although phenolic compounds are found in all four plants from which these substrates originate (Anagnostopoulou et al., 2006; Kalderis and Diamadopoulos, 2010; Kouakou et al., 2007; Pattara et al., 2010; Spigno and De Faveri, 2007; Vatai et al., 2009), what is important, is which part of the plant has the highest content in such compounds. For instance, although the olive fruit contains various phenolic compounds in significant amounts, the actual quantity that is retained in the portion that ultimately comprises olive pomace is quite lower. This is attributed to the processes used for olive oil production (Cardoso et al., 2005). Similarly, in the case of grapes, phenolic compounds are mainly found in skins and seeds rather than marc and stalks, however winery waste often contain all of these materials (Negro et al., 2003; Pala et al., 2014; Rodríguez Montealegre et al., 2006). On the other hand, TPH contents in citrus fruits, such as oranges, are usually found higher in peels rather than tissues (Anagnostopoulou et al., 2006; Ghasemi et al., 2009). This could very well explain the higher TPH levels observed in Fig. 6-10b for JW-substrates, since the latter are mainly composed of orange peels.

Fig. 6-11b presents a rather different picture for the assays of Group II, with a generally stable behavior for the whole duration of the experiment, with a slightly increasing trend, especially for the assays being fed with mixed-substrates. On the other hand, in the case of the assays corresponding to single-substrates, it is interesting to notice that among different assays, similar patterns are developed during the phases in which JW-M is fed to each reactor. As mentioned and explained before, JW-substrates are probable to contain higher amounts of phenolic compounds, due to their composition. Therefore, after first feeding the reactors with such material, TPH concentrations were raised to significantly higher levels, similar to those showed in Fig. 6-10b, due the repeated addition of high quantities of phenolic compounds to the reactors. Moreover, as soon as the feeding material was changed at the beginning of the next phase, the values started to follow a decreasing trend until reaching the previous levels, as a result of the progressive removal of digestion slurry and the addition of fresh feeding material, which contained lower TPH amounts than the previous.

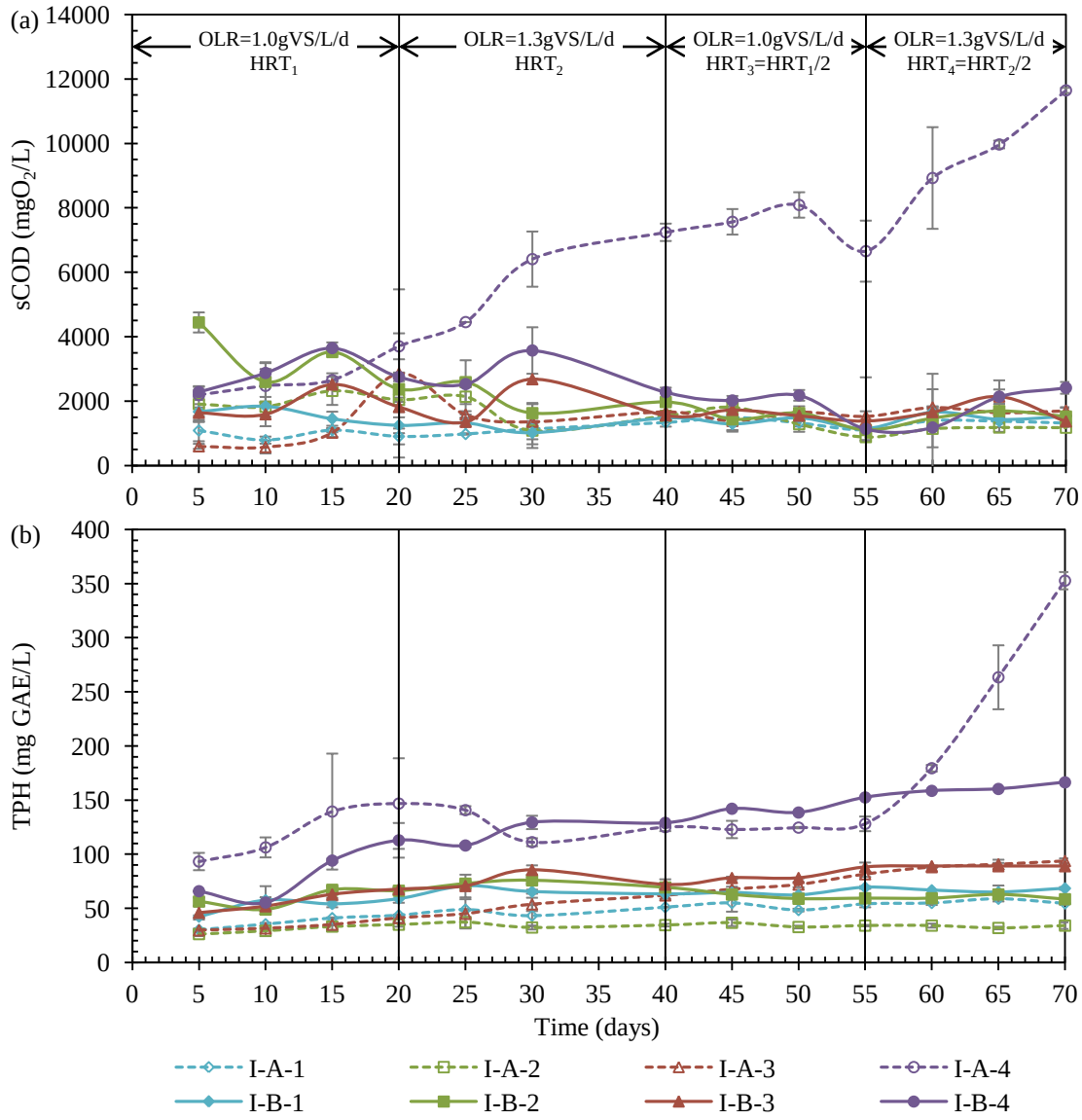


Figure 6-10: sCOD and TPH variation during semi-continuous assays for reactors of Group I [I-A-1 (- ◊ -), I-B-1 (—◆—), I-A-2 (- □ -), I-B-2 (—■—), I-A-3 (- Δ -), I-B-3 (—▲—), I-A-4 (- ○ -), I-B-4 (—●—)]

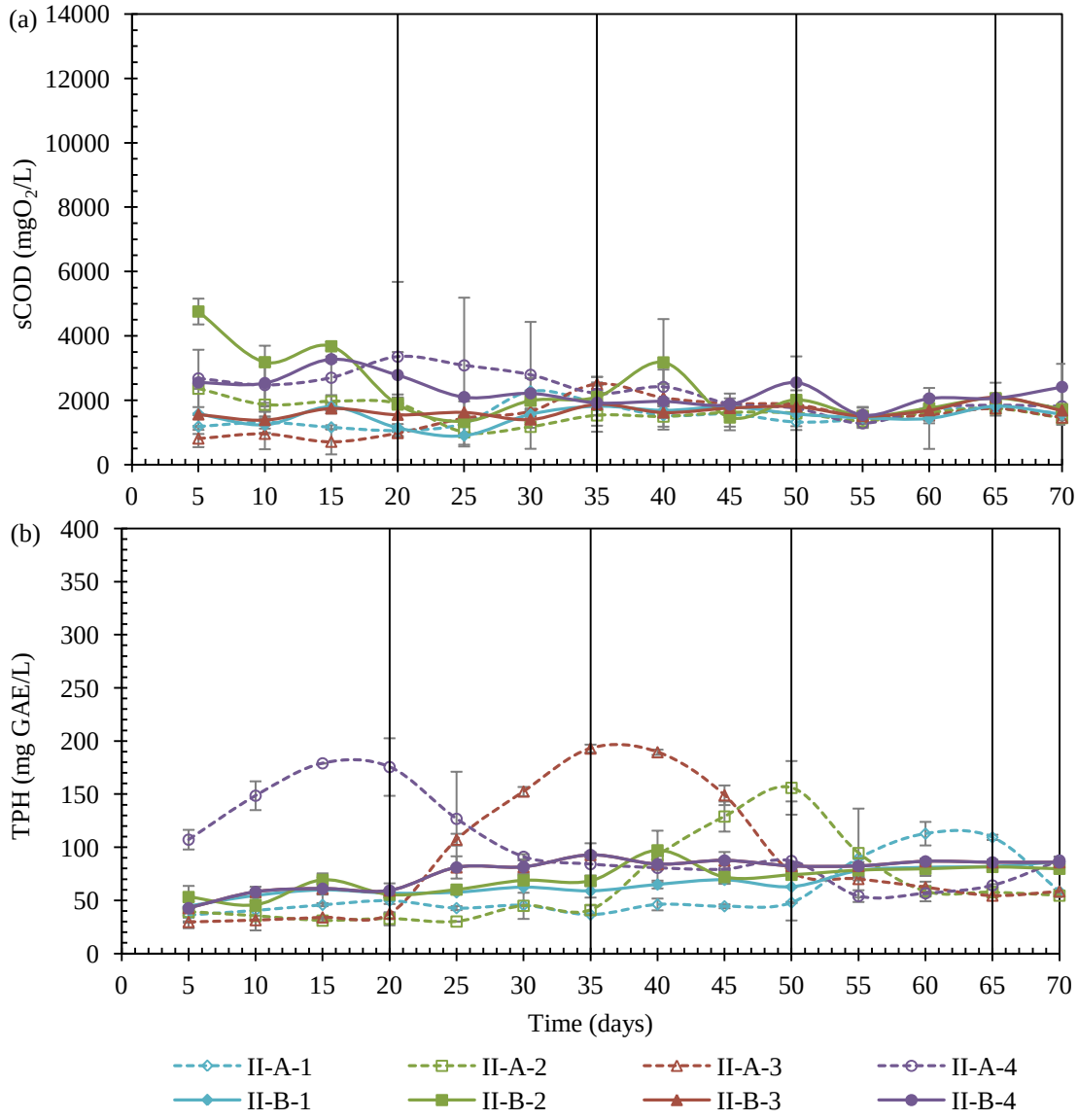


Figure 6-11: sCOD and TPH variation during semi-continuous assays for reactors of Group II [II-A-1 (- ◊ -), II-B-1 (—◆—), II-A-2 (- □ -), II-B-2 (—■—), II-A-3 (- Δ -), II-B-3 (—▲—), II-A-4 (- ○ -), II-B-4 (—●—)]

6.4.8 Kinetic modeling

In order to better interpret the results referring to methane production during the experiment, it is important to be able to estimate the variations in the kinetics of the anaerobic digestion process. In an attempt to serving this purpose, the data obtained for the different feeding periods (every five days) were fitted to a pseudo-first-order exponential model, aiming at calculating an apparent rate constants (k') for each of them. The results of this procedure can be seen in Fig. 6-12 and Fig. 6-13.

The model was found to describe the experimental data at a good level for all assays with a value for the coefficient of determination R^2>0.90, while for most assays R^2 was above 0.99. As it can be seen in the figures, the variation in k' values seems to reflect the patterns followed by the methane production and methane yields data, for both Groups of assays.

For the assays of Group I (Fig. 6-12), despite some slight fluctuations in k' values, especially at the beginning of each phase, the changes in operational parameters appear not to have caused significant effects on the kinetics of the process for assays I-A-1, I-B-1, I-A-3, I-B-3 and I-B-4. On the contrary, more intense fluctuations are observed for assays I-A-2, I-B-2 and I-A-4. For assays I-A-2 and I-B-2, it seems that the increase in OLR and the decrease in HRT during Phase 2 and Phase 4, respectively, retarded the anaerobic degradation for these substrates. These data coincide with the corresponding results observed for MY, corroborating the suppositions regarding overloading phenomena causing inhibition of the process. On the other hand, the pattern noticed for I-A-4, does not represent an accurate description of the particular phenomena actually occurring in this assay, despite being a result of the mathematical modeling of the data. Therefore, it would not be possible to use them for interpreting the data.

As far as Group II is concerned, as it can be seen in Fig. 6-13 the degradation rate of the assays being fed with mixed-substrates appears to be maintained on a rather constant level, despite the change in feeding material, confirming the earlier assumptions concerning the stable operation of these assays. On the other hand, this is not the case for the assays being fed with single-substrates, since some more intense fluctuations can be observed, especially in the phases during which CGW-M was fed to the reactors. Lower k' values during these periods indicate the difficulty of the microbial populations in consuming this material and are consistent with the results observed in the previous paragraphs. On the basis of the results obtained in the present study, OP-substrates could also be characterized as a less easily degradable substrate. Nevertheless, the data of Fig. 6-13 show that feeding these substrates to the reactors after more easily degradable substrates (JW-M and JW-C) leads to improved corresponding k' values, while this does not happen for CGW-substrates.

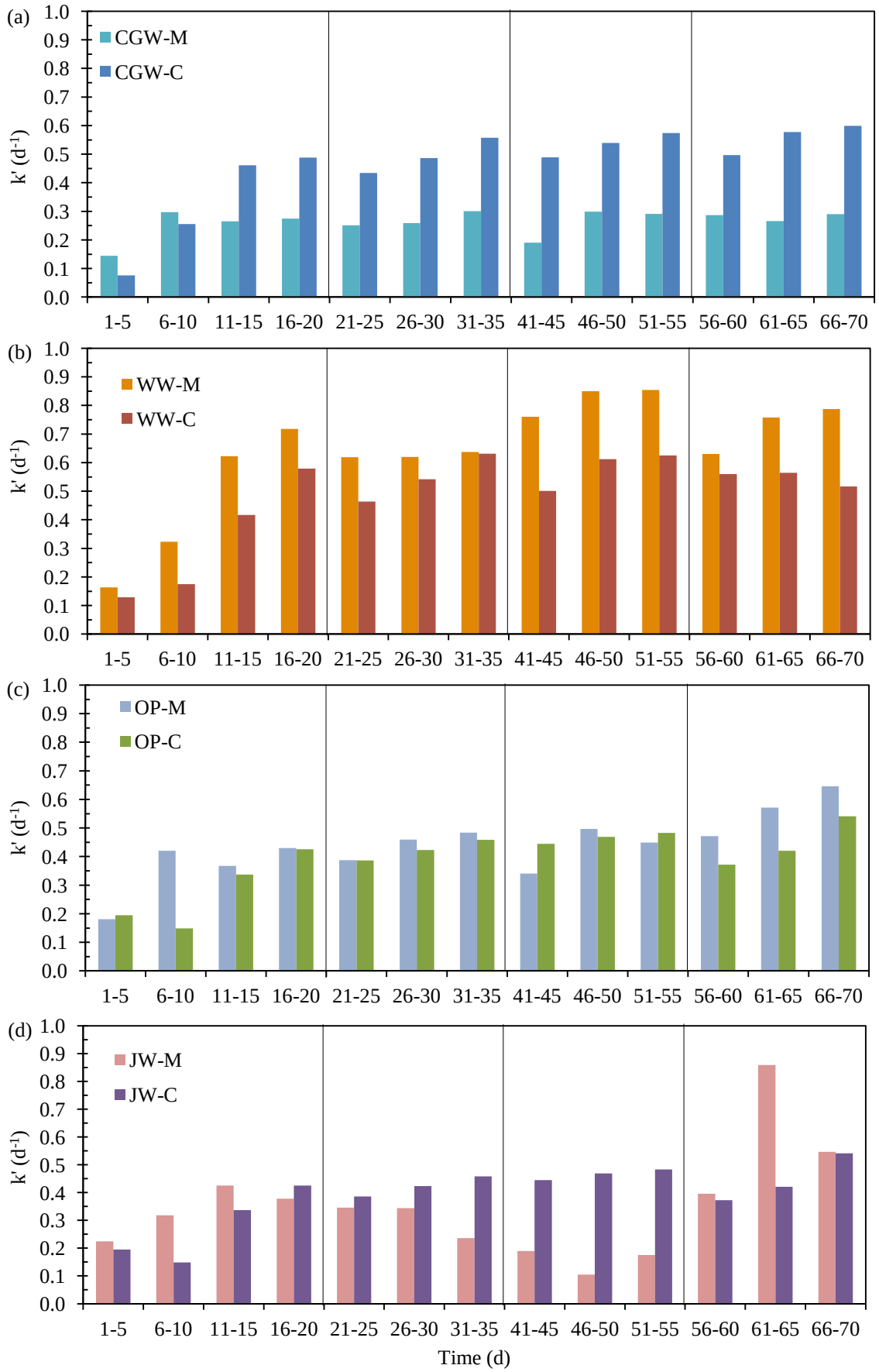


Figure 6-12: Apparent rate constants (*k'*) calculated for different feeding periods during semi-continuous assays for reactors of Group I [(a) I-A-1 & I-B-1, (b) I-A-2 & I-B-2, (c) I-A-3 & I-B-3, (d) I-A-4 & I-B-4] [CGW-M (■), CGW-C (■), WW-M (■), WW-C (■), OP-M (■), OP-C (■), JW-M (■), JW-C (■)]

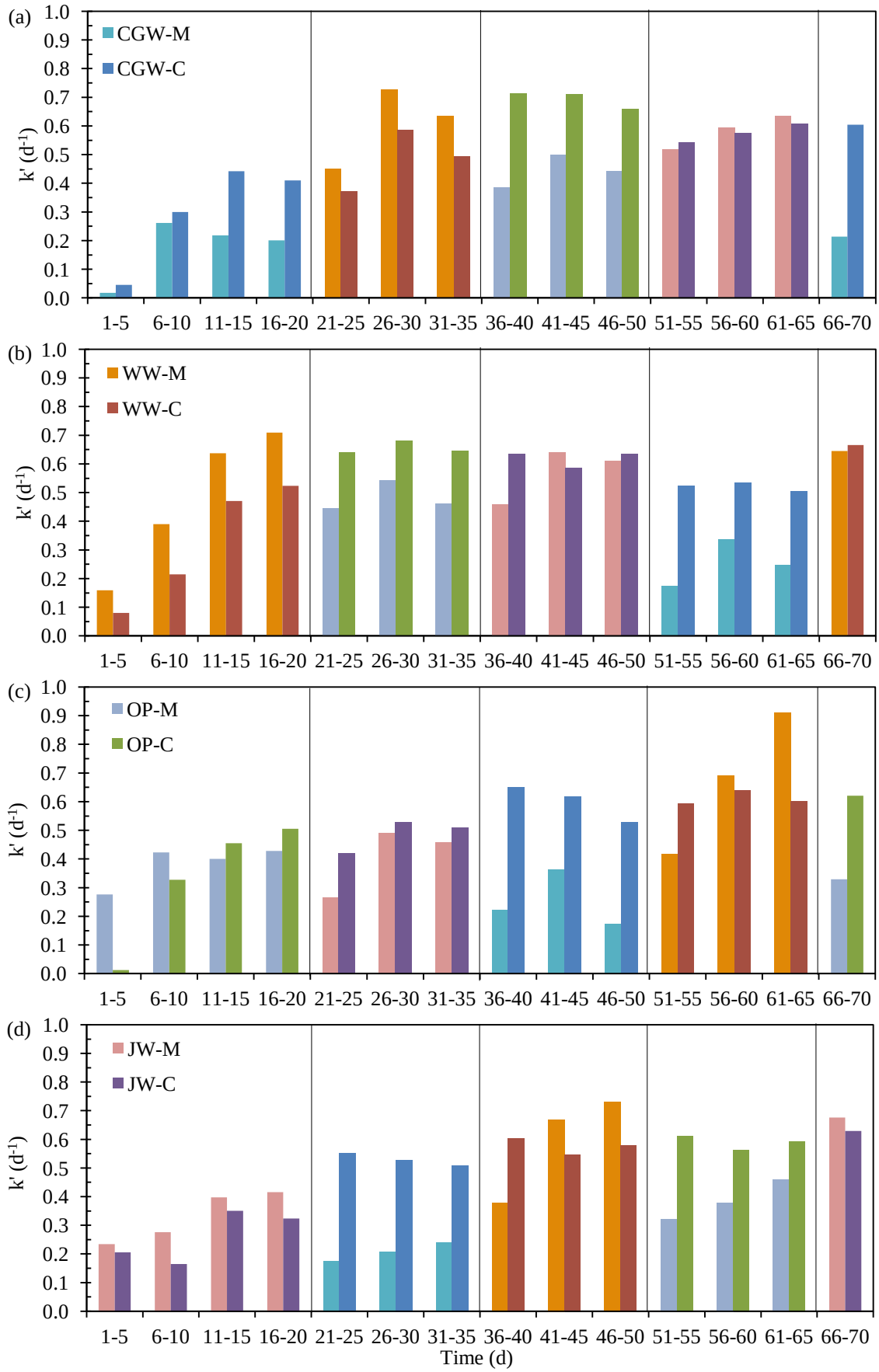


Figure 6-13: Apparent rate constants (*k'*) calculated for different feeding periods during semi-continuous assays for reactors of Group II [(a) II-A-1 & II-B-1, (b) II-A-2 & II-B-2, (c) II-A-3 & II-B-3, (d) II-A-4 & II-B-4] [CGW-M (■), CGW-C (■), WW-M (■), WW-C (■), OP-M (■), OP-C (■), JW-M (■), JW-C (■)]

6.4.9 Energy potential and mass requirements

Considering a lower heating value of 35800 kJ/m^3 CH_4, specific energy values were calculated for the assays of both Group I (Table 6-7) and Group II (Table 6-8), expressed in relation to both the quantity of VS and the quantity of wet mass corresponding to each separate feeding material. More specifically, the values corresponding to Group I were calculated using the maximum MY among those obtained during the different phases of the experiment (Fig. 6-4), i.e. during Phase 3. On the other hand, for Group II, average MY values corresponding to each feeding material were calculated by taking into consideration all the average MY that were obtained from all assays of this Group, during the phases in which each material was fed to the reactors.

According to both Table 6-7 and Table 6-8, there is a notable difference between the amounts of energy corresponding to different single-substrates, when referring to energy per kg of VS mass, with WW- and JW-substrates providing higher values compared with CGW- and OP-substrates. However, when expressing specific energy in relation to the wet mass of the materials, these differences are significantly reduced. Interestingly, in the latter case, the highest value is provided by the OP-single-substrate. On the other hand, specific energy values corresponding to mixed-substrates are found ranging on much higher levels, compared with their respective single-substrates, while not presenting intense differences between each other.

Daily mass requirements per m^3 of working volume of anaerobic digestion reactor, for both Groups of assays were calculated by taking into account both the OLR (1.0 gVS/L/d) and the composition (AW:SOF on a wet basis) of each feeding material.

Clearly, the amounts of substrates required for assays of both Group I and Group II are the same, since the same OLR was considered. The required amounts of wet mass of WW and JW are higher compared with the amounts of CGW and OP. This is related to the high moisture and VS contents of the two former materials. On the other hand, a relatively constant requirement in SOF can be noticed among different feeding materials.

Table 6-7: Energy potential and mass requirement calculations for assays of Group I

Feeding material	Specific Energy (kJ/kgVS)	Specific Energy (kJ/kgWet)	Mass requirements (kg/m^3 reactor/d) [a]	
			AW	SOF
CGW-M	6619	1030	1.84	-
WW-M	12433	2420	3.42	-
OP-M	5518	2438	1.51	-
JW-M	11323	1774	4.26	-
CGW-C	11696	1926	0.70	3.62
WW-C	14735	2585	1.41	3.58
OP-C	10382	2385	0.66	3.37
JW-C	15434	2522	1.75	3.50

[a] the values refer to working volume of reactor

Table 6-8: Energy potential and mass requirement calculations for assays of Group II

Feeding material	Specific Energy (kJ/kgVS)	Specific Energy (kJ/kgWet)	Mass requirements (kg/m^3 reactor/d) [a]	
			AW	SOF
CGW-M	6812	1060	1.84	-
WW-M	11764	2290	3.42	-
OP-M	5246	2318	1.51	-
JW-M	13244	2075	4.26	-
CGW-C	11016	1814	0.70	3.62
WW-C	14537	2550	1.41	3.58
OP-C	10685	2454	0.66	3.37
JW-C	14466	2363	1.75	3.50

[a] the values refer to working volume of reactor

Considering an anaerobic digester with a working volume of 2 m^3, annual mass requirements, methane volumes produced and energy potentials, were calculated for the data of both Groups of assays and the results are presented in Table 6-9 (Group I) and Table 6-10 (Group II), respectively.

Table 6-9 presents the annual data, considering the use of each feeding material for the entire duration of a year. On the other hand, the data of Table 6-10 was calculated assuming that the four single-substrates and the four mixed-substrates were to be used sequentially during a year, for a duration of three months each.

Table 6-9: Annual mass requirements, methane production and energy potential for a 2 m^3 (working volume) anaerobic digester (calculations based on Group I data)

Feeding material	Mass requirements (kg)		Methane production (m^3 CH_4)	Energy potential (MJ)
	AW	SOF		
CGW-M	1340	-	0.135	4832
WW-M	2500	-	0.254	9076
OP-M	1102	-	0.113	4028
JW-M	3106	-	0.231	8265
CGW-C	511	2645	0.238	8538
WW-C	1033	2613	0.300	10757
OP-C	480	2457	0.212	7579
JW-C	1274	2556	0.315	11267

Table 6-10: annual mass requirements, methane production and energy potential for a 2 m^3 (working volume) anaerobic digester (calculations based on Group II data)

Feeding material	Mass requirements (kg)		Methane production (m^3 CH_4)	Energy potential (MJ)
	AW	SOF		
CGW-M	335	-	0.0347	1243
WW-M	625	-	0.0600	2147
OP-M	275	-	0.0267	957
JW-M	777	-	0.0675	2417
Total (year)	-	-	0.1890	6765
CGW-C	128	661	0.0562	2010
WW-C	258	653	0.0741	2653
OP-C	120	614	0.0545	1950
JW-C	319	639	0.0737	2640
Total (year)	-	2568	0.2585	9253

In the case of Group I (Table 6-9), the quantities of AW required in a year range from 1102 to 3106 kg, if used in mono-digestion systems, and from 480 to 1274 kg, if used in co-digestion systems. Moreover, if the latter systems were to be used, additional amounts of SOF ranging from 2457 to 2645 kg would be required. Such amounts result in a methane production in the ranges 0.11-0.25 m^3 and 0.21-0.31 m^3, respectively and in energy potentials between 4.0 and 9.1 GJ, and between 7.6 and 11.3 GJ, respectively.

On the other hand, in the case of Group II (Table 6-10), the required quantities of AW corresponding to a 3-month period are between 275 and 777 kg, for mono-digestion systems, and between 120 and 319 kg, for co-digestion systems. The corresponding total amount of SOF for the annual operation of a co-digestion system is of 2568 kg. Sequentially feeding the anaerobic digester with the different feeding materials, would result in methane productions of 0.19 and 0.26 m^3 for mono- and co-digestion systems, respectively, and in energy potentials of 6.8 and 9.3 GJ, respectively.

6.4.10 Characteristics of digested materials

Table 6-11, Table 6-12, Table 6-13 and Table 6-14 present the basic characteristics of the digested materials, determined after the end of the experiment for both Groups of assays. More specifically, Table 6-11 and Table 6-13 provide the TS and VS analysis of the whole digested materials, as well as the elemental composition of the solid fraction and the chemical characteristics of the liquid fraction, for assays of Group I and Group II, respectively. On the other hand, the other two tables (Table 6-12 and Table 6-14) present the total metal concentrations of the solid fractions, for assays of Group I and Group II, respectively.

The results of Table 6-11 and Table 6-13 showed that digested materials obtained from the assays containing single-substrates (A-assays) are characterized by higher TS and VS values compared with those of mixed-substrates (B-assays), in both Groups. Morever, elemental analysis of the solids revealed that A-digested materials have higher C, H and N contents, but lower C/N compared with B-digested materials. Moreover, from the data presented in Table 6-12 and Table 6-14, it is obvious that among tested metals, Ca is the one with the highest concentration for all digested materials, with K and Mg following. In addition, it can be observed that the estimated concentrations for Cd, Cu, Hg, Ni, Pb and Zn are all in accordance with the limit values established by the Council Directive 86/278/EEC for sludge which is intended for agricultural use.

The highest TS and VS values for Group I (Table 6-11) were observed for the materials obtained from assays I-A-3 and I-B-3, which were being fed with OP-substrates. This could be attributed to the fact that these specific feeding materials (OP-M and OP-C) initially contained higher amounts of solids, as it can be seen in Table 6-4. Furthermore, in Group I, the highest C contents were noticed for the materials of the assays I-A-2 and I-B-2, with the latter also having the highest values for H and N contents among B-materials. Interestingly, among A-materials, the one corresponding to the assay I-A-4 was characterized by the highest H and N contents. Nevertheless the respective values for I-A-2 were highly comparable. The values obtained for C/N showed similarities between materials generated by CGW- and OP-substrates, as well as by WW- and JW-substrates, for both A- and B-assays. As far as the liquid fractions are concerned, all values seem to be ranging around similar levels, indicating the achievement of resembling stable conditions inside the reactors. The only exception is observed for assay I-A-4, with these final results corroborating all the earlier observations and suppositions regarding instability issues. Nevertheless, it is evident that B-materials are generally characterized by higher values. As far as metals are concerned (Table 6-12), it is noticed that all the concentrations referring to B-materials are higher than those referring to A-materials. This can probably be attributed to the presence of the SOF sample in the co-digestion reactors. The SOF sample contains a variety of materials, mainly food waste, therefore it most likely contributed to the increased concentrations of the tested elements. There is only one possible exception, that of Co for assays I-A-1 and I-B-1, where the concentration for the former is higher than that for the latter. However, in this case, the values are low enough for the difference to be considered within the measurement error.

Regarding the assays of Group II (Table 6-12 and Table 6-14), all A-materials, as well as all B-materials present similar characteristics, with all values being highly comparable to each other. This phenomenon was expected, since by the end of the experiment, all reactors would have been fed with all four feeding materials, thus resulting in residues of similar compositions. Nevertheless, small differences were also expected, due to the fact that for each assay, the number of feedings corresponding to the first feeding material (5 feedings), which differed in each case, was higher than the number of feedings for each of the other three

materials (3 feedings). Moreover, in the case of total metal concentrations in particular, although for most elements B-materials present higher concentrations than A-materials (as it was noticed for Group I), there are certain elements, namely Al, Co, Cr, Mg, Mn and Ni, for which the opposite is observed. It is possible, that the combination of co-digestion and of sequential feeding of the assays with four different materials, created an environment which was favorable for the increased consumption of these specific elements by microbial populations, resulting in lower concentrations being found in the digestates.

For both Groups of assays, the characteristics of digested materials are generally found quite comparable to previously published data (Alburquerque et al., 2012; Barrantes Leiva et al., 2014; Da Ros et al., 2016a; Möller and Müller, 2012; Mumme et al., 2011; Sheets et al., 2015; Tambone et al., 2010; Trzcinski and Stuckey, 2011). Moreover, most digested materials (except that produced in I-A-4) have characteristics, such as organic matter content (i.e. VS/TS), pH, C/N and metals concentrations, that are in accordance to those proposed for land application of such materials, according to the information reported by Teglia et al. (2011) and to Council Directive 86/278/EEC (1986).

Table 6-11: Characteristics of digested materials at the end of the experiment for reactors of Group I

Properties	Assays: Group I							
	I-A-1	I-A-2	I-A-3	I-A-4	I-B-1	I-B-2	I-B-3	I-B-4
TS (%) [a]	6.14	4.54	7.77	2.47	3.73	3.42	4.58	2.10
VS (%) [a]	3.68	3.95	7.21	2.09	2.69	2.84	4.03	1.62
VS/TS	0.60	0.87	0.93	0.85	0.72	0.83	0.88	0.77
Elemental composition of solid fraction [b]								
C (%)	24.9	51.4	46.8	45.3	25.8	48.0	43.6	38.0
H (%)	3.85	7.24	6.94	7.52	4.60	7.38	7.04	6.07
O (%)	29.9	25.1	37.3	28.8	40.0	23.8	34.9	29.9
N (%)	1.18	3.21	1.90	3.30	1.64	3.70	2.56	3.42
S (%)	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL
C/N	21.1	16.0	24.7	13.7	15.8	13.0	17.0	11.1
Empirical formula	$C_{24.6}H_{45.6}O_{22.1}N$	$C_{18.7}H_{31.6}O_{6.8}N$	$C_{28.8}H_{51.2}O_{17.2}N$	$C_{16.0}H_{31.9}O_{7.6}N$	$C_{18.4}H_{39.3}O_{21.3}N$	$C_{15.1}H_{27.9}O_{5.6}N$	$C_{19.8}H_{38.5}O_{11.9}N$	$C_{12.9}H_{24.8}O_{7.6}N$
Chemical characteristics of liquid fraction								
pH	7.17	7.20	7.35	5.29	7.28	7.44	7.34	7.25
VA (mg/L)	336.1	225.9	200.0	6668	245.0	275.0	255.0	250.0
TA (mg $CaCO_3$/L)	4185	3528	3456	3283	4494	4760	4627	4824
VA/TA	0.082	0.064	0.058	2.032	0.055	0.058	0.055	0.052
TAN (mg/L)	375	435	710	610	745	890	940	950
sCOD (mg O_2/L)	1317	1175	1698	11644	1524	1519	1367	2410
TPH (mg GAE/L)	54.60	34.14	93.78	352.8	68.53	58.51	88.99	166.5

DL: Detection Limit , [a] wb: wet basis , [b] db: dry basis

Table 6-12: Metals concentrations (mg/kg) of digested materials at the end of the experiment for reactors of Group I

Metals	Limit values of 86/278/EEC	Assays: Group I							
		I-A-1	I-A-2	I-A-3	I-A-4	I-B-1	I-B-2	I-B-3	I-B-4
Al	-	2246	< DL	< DL	1211	3374	2768	< DL	2683
As	-	< DL	< DL	< DL	< DL	1.926	< DL	< DL	< DL
Ba	-	77.09	114.4	79.10	201.5	147.9	231.4	112.4	225.7
Ca	-	41010	22704	13296	33580	53233	71732	23892	57167
Cd	20-40	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL
Co	-	2.579	< DL	< DL	< DL	2.054	< DL	< DL	< DL
Cr	-	15.77	4.760	0.983	7.557	17.16	12.14	3.846	11.10
Cu	1000-1750	36.97	90.78	49.78	122.5	97.33	185.3	77.82	175.0
Hg	16-25	20.15	1.393	0.604	10.47	5.645	5.240	10.67	21.74
K	-	2454	7236	977.9	3878	4358	9355	2544	6041
Mg	-	6356	3090	306.6	2708	6365	7207	1375	4500
Mn	-	252.6	44.84	23.03	41.70	222.2	109.0	45.50	87.46
Mo	-	3.053	3.432	2.632	5.086	15.33	8.380	5.184	7.923
Ni	300-400	36.43	19.56	1.604	7.881	31.10	108.9	7.911	26.32
Pb	750-1200	17.21	34.17	24.14	62.69	42.17	72.72	34.38	70.34
Se	-	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL
Zn	2500-4000	162.2	336.1	225.1	530.0	403.6	637.9	349.3	772.6

DL: Detection Limit , all values are expressed as mean concentrations on a dry basis

Table 6-13: Characteristics of digested materials at the end of the experiment for reactors of Group II

Properties	Assays: Group II							
	II-A-1	II-A-2	II-A-3	II-A-4	II-B-1	II-B-2	II-B-3	II-B-4
TS (%) [a]	5.75	5.45	5.37	5.20	3.97	4.48	4.24	3.69
VS (%) [a]	4.17	4.24	4.24	4.06	3.11	3.53	3.41	2.90
VS/TS	0.73	0.78	0.79	0.78	0.78	0.79	0.80	0.79
Elemental composition of solid fraction [b]								
C (%)	33.5	36.5	34.5	37.1	37.2	37.2	40.1	41.5
H (%)	4.82	5.15	5.00	5.31	5.65	5.43	5.90	6.28
O (%)	32.2	34.2	37.5	34.1	32.7	33.5	31.3	27.9
N (%)	1.97	1.85	1.94	1.52	2.68	2.76	2.96	2.93
S (%)	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL
C/N	17.0	19.8	17.8	24.3	13.9	13.5	13.6	14.1
Empirical formula	$C_{19.8}H_{34.2}O_{14.3}N$	$C_{23.0}H_{39.0}O_{16.2}N$	$C_{20.7}H_{36.0}O_{16.9}N$	$C_{28.4}H_{48.8}O_{19.6}N$	$C_{16.2}H_{29.5}O_{10.7}N$	$C_{15.7}H_{27.6}O_{10.6}N$	$C_{15.8}H_{27.9}O_{9.3}N$	$C_{16.5}H_{30.0}O_{8.3}N$
Chemical characteristics of liquid fraction								
pH	7.42	7.31	7.52	7.29	7.35	7.46	7.50	7.40
VA (mg/L)	255.6	284.4	400.0	260.0	328.0	275.0	267.5	300.0
TA (mg $CaCO_3$/L)	4650	4687	8121	4709	5484	6050	5676	5711
VA/TA	0.055	0.061	0.049	0.055	0.060	0.045	0.047	0.053
TAN (mg/L)	640	640	705	700	1010	1000	1025	1055
sCOD (mg O_2/L)	1587	1444	1462	1810	1560	1722	1683	2413
TPH (mg GAE/L)	58.51	54.60	58.51	87.68	86.38	79.85	85.94	95.52

DL: Detection Limit , [a] wb: wet basis , [b] db: dry basis

Table 6-14: Metals concentrations (mg/kg) of digested materials at the end of the experiment for reactors of Group II

Metals	Limit values of 86/278/EEC	Assays: Group II							
		II-A-1	II-A-2	II-A-3	II-A-4	II-B-1	II-B-2	II-B-3	II-B-4
Al	-	1942	1768	1426	1815	2154	1987	1080	1253
As	-	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL
Ba	-	122.5	125.4	125.1	135.0	161.9	180.5	156.2	162.5
Ca	-	43622	42673	32580	40311	59872	55770	43293	49762
Cd	20-40	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL
Co	-	1.309	0.865	0.444	0.613	< DL	< DL	< DL	< DL
Cr	-	12.45	12.03	8.622	9.65	9.463	14.93	7.788	8.037
Cu	1000-1750	79.05	78.88	96.22	88.12	104.3	126.1	109.3	112.0
Hg	16-25	2.076	< DL	2.929	3.855	10.01	8.582	4.628	1.416
K	-	3646	3873	3539	3983	4737	5897	5094	5769
Mg	-	5711	5427	4494	5030	5651	5310	3881	4417
Mn	-	157.3	141.9	113.0	124.4	119.9	108.8	85.99	86.63
Mo	-	3.395	3.270	3.329	3.456	4.403	5.031	4.685	4.570
Ni	300-400	19.61	16.45	15.22	16.45	20.94	12.35	12.42	9.260
Pb	750-1200	37.11	46.07	46.22	51.37	53.62	60.78	85.48	56.10
Se	-	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL
Zn	2500-4000	396.8	332.3	360.3	360.1	458.3	510.7	489.5	481.3

DL: Detection Limit , all values are expressed as mean concentrations on a dry basis

6.5 Conclusions

The results of the present study showed higher performance for the assays in which SOF was added to AW as a co-substrate. Both in mono-digestion and co-digestion assays, maximum methane yields were achieved by keeping the OLR at 1.0 gVS/L/d and halving the HRT corresponding to each feeding material, with WW- and JW-substrates presenting the overall highest methane yields. Lower HRT and higher OLR led to reduced methane yields, mainly attributed to system overloading, particularly for WW- and JW-assays. Specifically in the case of the JW-single-substrate assay, severe instability and ultimately failure was recorded. On the contrary, CGW- and OP-assays appeared to be able to eventually withstand further variations in operational parameters, since nor inhibition, or instability was indicated by their results. Sequential feeding was proved to have a positive effect on the performance of the reactors, since it led to a more equilibrated system operation. In fact, the immediate response of the systems to the change in feeding materials, with no signs of inhibition and stable chemical parameters, reveals a good level of adaptation of the microbial populations to the investigated substrates. This latter phenomenon was more pronounced in the case of mixed-substrates, due to their similar composition, which includes SOF. Despite the operational stability of the reactors, fluctuations in methane yields were observed between substrates, especially single-substrates. Therefore, in order to maintain steady methane production levels when sequentially using different substrates, the OLR and HRT being adopted should be set accordingly, so as to eliminate fluctuations. For instance, instead of using the same conditions for all substrates, those with a lower degradability, such as CGW and OP, are recommended to be fed to the system at a higher OLR, compared with those that are more easily degradable, such as WW and JW. Moreover, in order to maximize the performance of such a system, the feeding material used at operation startup should be selected carefully. More specifically, it is recommended that a more easily degradable substrate was the first to be fed to the system, in order to facilitate the microbial community. However, the use of a JW-substrate should be avoided, especially if the system was to be operated with single-substrates, since acidification phenomena are more probable to occur in that case. Instead, a WW-substrate would be a better choice. On the other hand, if mixed-substrates were to be used, WW- and JW-substrates would both represent a good choice for startup, since the presence of SOF in them would prevent inhibition phenomena from developing. Moreover, the use of CGW- and OP-substrates would also be acceptable, since, here as well, the presence of SOF would facilitate the adaptation process. Nevertheless, the use of the latter substrates would probably lead to a slightly reduced performance. The characteristics of the obtained digested materials suggest that they would be worth considering for nutrient recovery and/or land application, although additional test would have to be conducted.

6.6 References

Aboudi, K., Álvarez-Gallego, C.J., Romero-García, L.I. (2015). Semi-continuous anaerobic co-digestion of sugar beet byproduct and pig manure: Effect of the organic loading rate (OLR) on process performance. *Bioresource Technology*, *194*, 283–290.

Aboudi, K., Álvarez-Gallego, C.J., Romero-García, L.I. (2016). Biomethanization of sugar beet byproduct by semi-continuous single digestion and co-digestion with cow manure. *Bioresource Technology*, *200*, 311–9.

Adl, M., Sheng, K., Gharibi, A. (2012). Technical assessment of bioenergy recovery from cotton stalks through anaerobic digestion process and the effects of inexpensive pre-treatments. *Applied Energy*, *93*, 251–260.

Ahring, B.K., Sandberg, M., Angelidaki, I. (1995). Volatile fatty acids as indicators of process imbalance in anaerobic digestors. *Applied Microbiology and Biotechnology*, *43*(3), 559–565.

Alburquerque, J.A., de la Fuente, C., Ferrer-Costa, A., Carrasco, L., Cegarra, J., Abad, M., Bernal, M.P. (2012). Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. *Biomass and Bioenergy*, *40*, 181–189.

Alibardi, L., Cossu, R. (2015). Composition variability of the organic fraction of municipal solid waste and effects on hydrogen and methane production potentials. *Waste Management*, *36*, 147–155.

Altaş, L. (2009). Inhibitory effect of heavy metals on methane-producing anaerobic granular sludge. *Journal of Hazardous Materials*, *162*(2–3), 1551–1556.

Alvarez, R., Lidén, G. (2008). Semi-continuous co-digestion of solid slaughterhouse waste, manure, and fruit and vegetable waste. *Renewable Energy*, *33*(4), 726–734.

Anagnostopoulou, M.A., Kefalas, P., Papageorgiou, V.P., Assimopoulou, A.N., Boskou, D. (2006). Radical scavenging activity of various extracts and fractions of sweet orange peel (Citrus sinensis). *Food Chemistry*, *94*(1), 19–25.

Anjum, M., Khalid, A., Mahmood, T., Aziz, I. (2016). Anaerobic co-digestion of catering waste with partially pretreated lignocellulosic crop residues. *Journal of Cleaner Production*, *117*, 56–63.

Aslanzadeh, S., Rajendran, K., Taherzadeh, M. J. (2014). A comparative study between single- and two-stage anaerobic digestion processes: Effects of organic loading rate and hydraulic retention time. *International Biodeterioration & Biodegradation*, *95*, 181–188.

Astals, S., Batstone, D.J., Mata-Alvarez, J., Jensen, P.D. (2014). Identification of synergistic impacts during anaerobic co-digestion of organic wastes. *Bioresource Technology*, *169*, 421–7.

Banks, C.J., Salter, A.M., Heaven, S., Riley, K. (2011). Energetic and environmental benefits of co-digestion of food waste and cattle slurry: A preliminary assessment. *Resources, Conservation and Recycling*, *56*(1), 71–79.

Barrantes Leiva, M., Koupaie, E.H., Eskicioglu, C. (2014). Anaerobic co-digestion of wine/fruit-juice production waste with landfill leachate diluted municipal sludge cake under semi-continuous flow operation. *Waste Management*, *34*(10), 1860–1870.

Battista, F., Ruggeri, B., Fino, D., Erriquens, F., Rutigliano, L., Mescia, D. (2013). Toward the scale-up of agro-food feed mixture for biogas production. *Journal of Environmental Chemical Engineering*, *1*(4), 1223–1230.

Bayr, S., Ojanperä, M., Kaparaju, P., Rintala, J. (2014). Long-term thermophilic mono-digestion of rendering wastes and co-digestion with potato pulp. *Waste Management*, *34*(10), 1853–1859.

Bouallagui, H., Rachdi, B., Gannoun, H., Hamdi, M. (2009). Mesophilic and thermophilic anaerobic co-digestion of abattoir wastewater and fruit and vegetable waste in anaerobic sequencing batch reactors. *Biodegradation*, *20*(3), 401–409.

Boukroufa, M., Boutekedjiret, C., Petigny, L., Rakotomanomana, N., Chemat, F. (2015). Bio-refinery of orange peels waste: A new concept based on integrated green and solvent free extraction processes using ultrasound and microwave techniques to obtain essential oil, polyphenols and pectin. *Ultrasonics Sonochemistry*, *24*, 72–79.

Cadavid-Rodriguez, L.S., Horan, N. (2012). Reducing the environmental footprint of wastewater screenings through anaerobic digestion with resource recovery. *Water and Environment Journal*, *26*(3), 301–307.

Callaghan, F.J., Wase, D.A.J., Thayanithy, K., Forster, C.F. (2002). Continuous co-digestion of cattle slurry with fruit and vegetable wastes and chicken manure. *Biomass and Bioenergy*, *22*(1), 71–77.

Cardoso, S.M., Guyot, S., Marnet, N., Lopes-da-Silva, J.A., Renard, C.M.G.C., Coimbra, M.A. (2005). Characterisation of phenolic extracts from olive pulp and olive pomace by electrospray mass spectrometry. *Journal of the Science of Food and Agriculture*, *85*(1), 21–32.

Carlini, M., Castellucci, S., Moneti, M. (2015). Anaerobic Co-digestion of Olive-mill Solid Waste with Cattle Manure and Cattle Slurry: Analysis of Bio-methane Potential. *Energy Procedia*, *81*, 354–367.

Cesaro, A., Belgiorno, V. (2014). Pretreatment methods to improve anaerobic biodegradability of organic municipal solid waste fractions. *Chemical Engineering Journal*, *240*, 24–37.

Chen, G., Liu, G., Yan, B., Shan, R., Wang, J., Li, T., Xu, W. (2016). Experimental study of co-digestion of food waste and tall fescue for bio-gas production. *Renewable Energy*, *88*, 273–279.

Chen, X., Yan, W., Sheng, K., Sanati, M. (2014). Comparison of high-solids to liquid anaerobic co-digestion of food waste and green waste. *Bioresource Technology*, *154*, 215–221.

Cotton Australia (cottonaustralia.com.au) (accessed on 2016)

Council Directive (EU) 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture [1986] OJ L181/6-12.

Cuetos, M.J., Gómez, X., Otero, M., Morán, A. (2010). Anaerobic digestion and co-digestion of slaughterhouse waste (SHW): Influence of heat and pressure pre-treatment in biogas yield. *Waste Management*, *30*(10), 1780–1789.

Da Ros, C., Cavinato, C., Pavan, P., Bolzonella, D. (2014). Winery waste recycling through anaerobic co-digestion with waste activated sludge. *Waste Management*, *34*(11), 2028–2035.

Da Ros, C., Cavinato, C., Pavan, P., Bolzonella, D. (2016a). Mesophilic and thermophilic anaerobic co-digestion of winery wastewater sludge and wine lees: An integrated approach for sustainable wine production. *Journal of Environmental Management.*

Da Ros, C., Cavinato, C., Bolzonella, D., Pavan, P. (2016b). Renewable energy from thermophilic anaerobic digestion of winery residue: Preliminary evidence from batch and continuous lab-scale trials. *Biomass and Bioenergy*, *91*, 150–159.

Díaz, A.B., Alvarado, O., de Ory, I., Caro, I., Blandino, A. (2013). Valorization of grape pomace and orange peels: Improved production of hydrolytic enzymes for the clarification of orange juice. *Food and Bioproducts Processing, 91*, 580–586.

DIRECTIVE 2009/28/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/E [2009] OJ L140/16-62.

El Achkar, J.H., Lendormi, T., Hobaika, Z., Salameh, D., Louka, N., Maroun, R.G., Lanoisellé, J.-L. (2016). Anaerobic digestion of grape pomace: Biochemical characterization of the fractions and methane production in batch and continuous digesters. *Waste Management, 50*, 275–282.

Esposito, G., Frunzo, L., Giordano, A., Liotta, F., Panico, A., Pirozzi, F. (2012). Anaerobic co-digestion of organic wastes. *Reviews in Environmental Science and Bio/Technology*, *11*(4), 325–341.

Fezzani, B., Ben Cheikh, R. (2010). Two-phase anaerobic co-digestion of olive mill wastes in semi-continuous digesters at mesophilic temperature. *Bioresource Technology*, *101*(6), 1628–1634.

Fitamo, T., Boldrin, A., Boe, K., Angelidaki, I., Scheutz, C. (2016). Co-digestion of food and garden waste with mixed sludge from wastewater treatment in continuously stirred tank reactors. *Bioresource Technology, 206*, 245–254.

Fonoll, X., Astals, S., Dosta, J., Mata-Alvarez, J. (2015). Anaerobic co-digestion of sewage sludge and fruit wastes: Evaluation of the transitory states when the co-substrate is changed. *Chemical Engineering Journal*, *262*, 1268–1274.

Fotidis, I.A., Karakashev, D., Kotsopoulos, T.A., Martzopoulos, G.G., Angelidaki, I. (2013). Effect of ammonium and acetate on methanogenic pathway and methanogenic community composition. *FEMS Microbiology Ecology, 83*(1), 38–48.

Fountoulakis, M.S., Drakopoulou, S., Terzakis, S., Georgaki, E., Manios, T. (2008). Potential for methane production from typical Mediterranean agro-industrial by-products. *Biomass and Bioenergy, 32*(2), 155–161.

Fountoulakis, M.S., Manios, T. (2009). Enhanced methane and hydrogen production from municipal solid waste and agro-industrial by-products co-digested with crude glycerol. *Bioresource Technology*, *100*(12), 3043–3047.

Ganesh, R., Torrijos, M., Sousbie, P., Steyer, J.P., Lugardon, A., Delgenes, J.P. (2013). Anaerobic co-digestion of solid waste: Effect of increasing organic loading rates and characterization of the solubilised organic matter. *Bioresource Technology*, *130*, 559–569.

Ghasemi, K., Ghasemi, Y., Mohammad, A., Ebrahimzadeh, A. (2009). Antioxidant Activity, Phenol and Flavonoid Contents of 13 Citrus Species Peels and Tissues. *Pak. J. Pharm. Sci*, *22*(3), 277–281.

Gómez, X., Cuetos, M.J., Cara, J., Morán, A., García, A.I. (2006). Anaerobic co-digestion of primary sludge and the fruit and vegetable fraction of the municipal solid wastes. Conditions for mixing and evaluation of the organic loading rate. *Renewable Energy*, *31*(12), 2017–2024.

Gou, C., Yang, Z., Huang, J., Wang, H., Xu, H., Wang, L. (2014). Effects of temperature and organic loading rate on the performance and microbial community of anaerobic co-digestion of waste activated sludge and food waste. *Chemosphere*, *105*, 146–151.

Hamawand, I., Sandell, G., Pittaway, P., Chakrabarty, S., Yusaf, T., Chen, G., Seneweera, S., Al-Lwayzy, S., Bennett, J., Hopf, J. (2016). Bioenergy from Cotton Industry Wastes: A review and potential. *Renewable and Sustainable Energy Reviews*, *66*, 435–448.

Kalderis, D., Diamadopoulos, E. (2010). Valorization of Solid Waste Residues from Olive Oil Mills: A Review. *Terrestrial and Aquatic Environmental Toxicology*, *4*(Special Issue 1), 7–20.

Kaparaju, P.L.N., Rintala, J.A. (2006). Thermophilic Anaerobic Digestion of Industrial Orange Waste. *Environmental Technology*, *27*(6), 623–633.

Kayhanian, M. (1999). Ammonia Inhibition in High-Solids Biogasification: An Overview and Practical Solutions. *Environmental Technology*, *20*(4), 355–365.

Kim, D.-H., Oh, S.-E. (2011). Continuous high-solids anaerobic co-digestion of organic solid wastes under mesophilic conditions. *Waste Management*, *31*(9–10), 1943–1948.

Koppar, A., Pullammanappallil, P. (2013). Anaerobic digestion of peel waste and wastewater for on site energy generation in a citrus processing facility. *Energy*, *60*, 62–68.

Kouakou, T.H., Waffo-Téguo, P., Kouadio, Y.J., Valls, J., Richard, T., Decendit, A., Mérillon, J.-M. (2007). Phenolic compounds and somatic embryogenesis in cotton (*Gossypium hirsutum* L.). *Plant Cell, Tissue and Organ Culture*, *90*(1), 25–29.

Lesteur, M., Bellon-Maurel, V., Gonzalez, C., Latrille, E., Roger, J.M., Junqua, G., Steyer, J. P. (2010). Alternative methods for determining anaerobic biodegradability: a review. *Process Biochemistry*, *45*(4), 431-440.

Li, Y., Park, S.Y., Zhu, J. (2011). Solid-state anaerobic digestion for methane production from organic waste. *Renewable and Sustainable Energy Reviews*, *15*(1), 821–826.

Lin, J., Zuo, J., Gan, L., Li, P., Liu, F., Wang, K., Chen, L., Gan, H. (2011). Effects of mixture ratio on anaerobic co-digestion with fruit and vegetable waste and food waste of China. *Journal of Environmental Sciences*, *23*(8), 1403–1408.

Martín, M.A., Siles, J.A., Chica, A.F., Martín, A. (2010). Biomethanization of orange peel waste. *Bioresource Technology*, *101*(23), 8993–8999.

Martín, M.A., Fernández, R., Serrano, A., Siles, J. A. (2013). Semi-continuous anaerobic co-digestion of orange peel waste and residual glycerol derived from biodiesel manufacturing. *Waste Management*, *33*(7), 1633–1639.

Mata-Alvarez, J., Dosta, J., Romero-Güiza, M. S., Fonoll, X., Peces, M., Astals, S. (2014). A critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renewable and Sustainable Energy Reviews*, *36*, 412–427.

Molinuevo-Salces, B., González-Fernández, C., Gómez, X., García-González, M.C., Morán, A. (2012). Vegetable processing wastes addition to improve swine manure anaerobic digestion: Evaluation in terms of methane yield and SEM characterization. *Applied Energy*, *91*(1), 36–42.

Möller, K., Müller, T. (2012). Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Engineering in Life Sciences*, *12*(3), 242–257.

Montalvo, S., Gonzalez, P., Mena, C., Guerrero, L., Borja, R. (2012). Influence of the food to microorganisms (F/M) ratio and temperature on batch anaerobic digestion processes with and without zeolite addition. *Journal of Environmental Science and Health, Part A*, *47*(12), 1785–1794.

Montañés, R., Pérez, M., Solera, R. (2013). Mesophilic anaerobic co-digestion of sewage sludge and a lixiviation of sugar beet pulp: optimisation of the semi-continuous process. *Bioresource Technology*, *142*, 655–62.

Moraes, B.S., Triolo, J.M., Lecona, V.P., Zaiat, M., Sommer, S.G. (2015). Biogas production within the bioethanol production chain: Use of co-substrates for anaerobic digestion of sugar beet vinasse. *Bioresource Technology*, *190*, 227–234.

Mumme, J., Eckervogt, L., Pielert, J., Diakité, M., Rupp, F., Kern, J. (2011). Hydrothermal carbonization of anaerobically digested maize silage. *Bioresource Technology*, *102*(19), 9255–9260.

Nain, M. Z., Jawed, M. (2006). Performance of anaerobic reactors at low organic load subjected to sudden change in feed substrate types. *Journal of Chemical Technology & Biotechnology*, *81*, 958–965.

Negro, C., Tommasi, L., Miceli, A. (2003). Phenolic compounds and antioxidant activity from red grape marc extracts. *Bioresource Technology*, *87*(1), 41–44.

Negro, V., Ruggeri, B., Mancini, G., Fino, D. (2016). Recovery of D-limonene through moderate temperature extraction and pyrolytic products from orange peels. *Journal of Chemical Technology & Biotechnology*.

Nogales, R., Cifuentes, C., Benitez, E. (2005). Vermicomposting of Winery Waste: A Laboratory Study. *Journal of Environmental Science and Health , Part B : Pesticides , Food Contaminants , and Agricultural Wastes*, *40*(4), 659–673.

Pala, M., Kantarli, I.C., Buyukisik, H.B., Yanik, J. (2014). Hydrothermal carbonization and torrefaction of grape pomace: A comparative evaluation. *Bioresource Technology*, *161*, 255–262.

Panichnumsin, P., Nopharatana, A., Ahring, B., Chaiprasert, P. (2010). Production of methane by co-digestion of cassava pulp with various concentrations of pig manure. *Biomass and Bioenergy*, *34*(8), 1117–1124.

Passos, F., García, J., Ferrer, I. (2013). Impact of low temperature pretreatment on the anaerobic digestion of microalgal biomass. *Bioresource Technology*, *138*, 79–86.

Pattara, C., Cappelletti, G.M., Cichelli, A. (2010). Recovery and use of olive stones: Commodity, environmental and economic assessment. *Renewable and Sustainable Energy Reviews*, *14*(5), 1484–1489.

Pellera, F.-M., Gidarakos, E. (2016). Effect of substrate to inoculum ratio and inoculum type on the biochemical methane potential of solid agroindustrial waste. *Journal of Environmental Chemical Engineering*, *4*(3), 3217–3229.

Placido, J., Capareda, S. (2013). Evaluation of ligninolytic enzymes, ultrasonication and liquid hot water as pretreatments for bioethanol production from cotton gin trash, in: *ASABE Annual International Meeting*. pp. 203–208.

Raposo, F., Fernández-Cegrí, V., De la Rubia, M.A., Borja, R., Béline, F., Cavinato, C., Demirer, G., Fernández, B., Fernández-Polanco, M., Frigon, J.C., Ganesh, R., Kaparaju, P., Koubova, J., Méndez, R., Menin, G., Peene, A., Scherer, P., Torrijos, M.. Uellendahl, H.. Wierinck, I., de Wilde, V. (2011). Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. *Journal of Chemical Technology & Biotechnology*, *86*, 1088-1098.

Rodríguez Montealegre, R., Romero Peces, R., Chacón Vozmediano, J.L., Martínez Gascueña, J., García Romero, E. (2006). Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate. *Journal of Food Composition and Analysis*, *19*(6), 687–693.

Sheets, J.P., Yang, L., Ge, X., Wang, Z., Li, Y. (2015). Beyond land application: Emerging technologies for the treatment and reuse of anaerobically digested agricultural and food waste. *Waste Management*, *44*, 94–115.

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, *299*, 152–178.

Spigno, G., De Faveri, D.M. (2007). Antioxidants from grape stalks and marc: Influence of extraction procedure on yield, purity and antioxidant power of the extracts. *Journal of Food Engineering*, *78*(3), 793–801.

Tambone, F., Scaglia, B., D'Imporzano, G., Schievano, A., Orzi, V., Salati, S., Adani, F. (2010). Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. *Chemosphere*, *81*(5), 577–583.

Teglia, C., Tremier, A., Martel, J.-L. (2011). Characterization of Solid Digestates: Part 1, Review of Existing Indicators to Assess Solid Digestates Agricultural Use. *Waste and Biomass Valorization*, *2*(1), 43–58.

Trzcinski, A.P., Stuckey, D.C. (2011). Parameters affecting the stability of the digestate from a two-stage anaerobic process treating the organic fraction of municipal solid waste. *Waste Management*, *31*(7), 1480–1487.

Vatai, T., Škerget, M., Knez, Ž. (2009). Extraction of phenolic compounds from elder berry and different grape marc varieties using organic solvents and/or supercritical carbon dioxide. *Journal of Food Engineering*, *90*(2), 246–254.

Ward, A.J., Hobbs, P.J., Holliman, P.J., Jones, D.L. (2008). Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technology*, *99*(17), 7928–7940.

Weiland, P. (2010). Biogas production: current state and perspectives. *Applied Microbiology and Biotechnology*, *85*, 849.860.

Zhang, C., Su, H., Tan, T. (2013). Batch and semi-continuous anaerobic digestion of food waste in a dual solid–liquid system. *Bioresource Technology*, *145*, 10–16.

Ziganshin, A.M., Schmidt, T., Lv, Z., Liebetrau, J., Richnow, H.H., Kleinsteuber, S., Nikolausz, M. (2016). Reduction of the hydraulic retention time at constant high organic loading rate to reach the microbial limits of anaerobic digestion in various reactor systems. *Bioresource Technology*.

CHAPTER 7

Discussion and Further research

This chapter presents an overview of the results obtained in the previous Chapters (3, 4, 5 and 6). More specifically, the results are discussed in terms of substrate solubilization, methane production, as well as energy estimations. Moreover, a conclusive discussion is made and further research topics are evaluated.

7.1 Discussion

7.1.1 Comparison of pretreatments

In Chapters 4 and 5 two different pretreatment methods were adopted prior to anaerobic digestion of four agroindustrial waste, namely winery waste (WW), cotton gin waste (CGW), olive pomace (OP) and juice industry waste (JW). Specifically, these methods were microwave pretreatment at five different temperatures (Chapter 4) and chemical pretreatment using a total of eight reagents, at three condition sets, including different reagent dosages, temperatures and process durations (Chapter 5).

Both pretreatments resulted in similar COD solubilization degrees (Table 7-1) for the investigated substrates, with the highest values being achieved after chemical pretreatment for WW and CGW and after microwave pretreatment for OP and JW. As far as the effect of the different pretreatments on degradability is concerned, the data referring to BMP assays of pretreated substrates showed that both methods generated materials with a lower methane potential compared with the untreated (raw) substrates. This was attributed to the fact that during both pretreatments, a portion of the organic matter of the substrates was removed from their solid matrix and transferred to the liquid fraction, as a result of the disruption of their structure. Nevertheless, by comparing the data corresponding to the microwave and chemically pretreated materials having the best performance in terms of methane production (Table 7-2), it is observed that chemical pretreatment provided better results in three out of four cases, namely for WW, CGW and JW. In fact, for these substrates higher SMY (mL $CH_{4,\ STP}$/g VS) were obtained after chemical pretreatment, while also shorter periods were required in order to achieve at least 80% of the total methane production (t_{80}). On the other hand, in the case of OP, microwave pretreatment showed a better methane efficiency than chemical pretreatment. It is noted that in Table 7-2, only SMY expressed in relation to the mass (in g VS) of raw substrate are presented, since they provide a better comparison between pretreatments. Interestingly, regarding the BI for the two pretreatments, it seems that for WW, OP and JW, the samples produced via microwave pretreatment had a slightly higher biodegradability degree than those obtained through chemical pretreatment. In the case of CGW however, higher biodegradability coincided with a higher methane potential., i.e. for the chemically pretreated sample.

In order to compare the two pretreatment methods in terms of energy consumption and production, it would be necessary to calculate the energy parameters presented in Chapters 4 and 5, considering that the amount of substrate being treated is the same for both treatments. Due to the difference in solid to liquid ratio for the two methods, the values of Table 4-7 and Table 5-8 are not directly comparable. Among the two pretreatments, the microwave method being adopted had the most limitations, since it provided for the use of four vessels, with each one containing a specific amount of substrate. Therefore, new calculations were made only for chemical pretreatment, considering that the amount of substrate being treated was the same as that corresponding to the four microwave vessels. By observing the results of this procedure (Table 7-3), it is obvious that the energy consumption for chemical pretreatment is much higher than that for microwave pretreatment. This depends not only on the operating power of the two heating systems, i.e. 600 W for microwave and 888 W for conventional heating, but also on the longer process duration required for chemical pretreatment (4 and 8 h, compared with 10.2–22.7 min). A similar observation was also made by Kuglarz et al. (2013) when comparing the energy efficiencies of microwave and thermal pretreatments.

Table 7-1: Comparison of COD solubilization (%) for the four substrates, after microwave and chemical pretreatment

Substrates	Microwave pretreatment		Chemical pretreatment	
	Range (%)	Conditions for max	Range (%)	Conditions for max
WW	43.5-49.7	175 °C – 5 min	40.4-57.7	H_3Cit – 90 °C – 4 h
CGW	7.50-22.1	200 °C – 5 min	5.47-22.7	H_3Cit – 90 °C – 4 h
OP	3.01-26.2	200 °C – 5 min	2.24-12.06	NaOH – 90 °C – 4 h (second highest H_3Cit – 90 °C – 4 h, 10.8%)
JW	35.5-71.4	200 °C – 5 min	39.42-67.5	H_3Cit – 90 °C – 4 h

Table 7-2: Comparison of maximum specific methane yields (SMY) ($mLCH_{4,\ STP}/gVS_{Raw}$) and t_{80} (d) for the four substrates, after microwave and chemical pretreatment

Substrates	Microwave pretreatment				Chemical pretreatment			
	SMY_{Raw} ($mLCH_{4,\ STP}/gVS_{Raw}$)	t_{80} (d)	BI (%)	Conditions	SMY_{Raw} ($mLCH_{4,\ STP}/gVS_{Raw}$)	t_{80} (d)	BI (%)	Conditions
WW	154.0	23	37.1	125 °C – 5 min	190.7	17	34.7	EtOH – 60 °C – 8 h
CGW	171.6	19	41.3	150 °C – 5 min	204.7	19	57.4	EtOH – 90 °C – 4 h
OP	268.8	19	30.4	150 °C – 5 min	217.3	21	27.3	H_3Cit – 90 °C – 4 h
JW	166.0	11	82.0	150 °C – 5 min	275.0	7	75.9	H_2O_2 – 60 °C – 8 h

Table 7-3: Comparison of energy parameters E_C, E_M, E_Q, E_T (kJ/kgVS_{Raw}) and E_i/E_o, for the four substrates, after microwave and chemical pretreatment

Substrates	E_C	E_M	E_Q	E_T	E_i/ E_o	Conditions
Microwave pretreatment						
WW	211711	5513	30347	-175850	5.9	125 °C – 5 min
CGW	85007	6145	13125	-65737	4.4	150 °C – 5 min
OP	121929	9624	19054	-93251	4.3	150 °C – 5 min
JW	405401	5943	63960	-335498	5.8	150 °C – 5 min
Chemical pretreatment						
WW	9893385	6828	43175	-9843383	197.9	EtOH – 60 °C – 8 h
CGW	1705900	7329	93437	-1605134	16.9	EtOH – 90 °C – 4 h
OP	2446843	7779	93456	-2345608	24.2	H_3Cit – 90 °C – 4 h
JW	12481406	9844	43534	-12428027	233.8	H_2O_2 – 60 °C – 8 h

Table 7-4: Comparison of TPH release for the four substrates, after microwave and chemical pretreatment

Substrates	Microwave pretreatment			Chemical pretreatment		
	Range		Value for max-SMY sample	Range		Value for max-SMY sample
	mg GAE/gVS	mg GAE/L	mg GAE/L	mg GAE/gVS	mg GAE/L	mg GAE/L
WW	6.31-63.2	81.6-818	219 ± 61	3.05-17.4	30.5-171	60.4 ± 4.4
CGW	3.44-18.9	129-710	275 ± 20	4.27-10.2	42.8-102	80.1 ± 4.8
OP	2.75-51.3	72.0-1341	318 ± 29	1.89-11.8	18.9-118	48.2 ± 1.3
JW	15.1-77.2	119-606	204 ± 34	8.00-19.0	80.0-191	88.4 ± 4.7

As already mentioned in earlier chapters, the pretreatment processes provided negative energy balances, under the conditions applied, despite considering the use of the liquid fractions for covering the heating needs of downstream anaerobic digestion. Reducing exposute time and operating power, as well as increasing solid to liquid ratio were proposed as means of reducing energy expenditure. However, another possible option for improving the efficiency of the pretreatment process could be the valorization of the liquid fractions obtained after pretreatment. More specifically, added-value chemical compounds (e.g. phenolic compounds) being released in the liquid phase during pretreatment, could be recovered for further use, thus providing an extra economical benefit from the pretreatment process.

Recent research has shown that recovery of added-value components from waste originating from food production processes is a very promising management and valorization option. Such components include phenolic compounds, carotenoids, essential oils, pectin and water insoluble fibers (Boukroufa et al., 2015; Galanakis, 2012). Among the above mentioned compounds, phenols are of particular interest, since they have been found to possess antimicrobial and antioxidant properties, as well as free radical scavenging abilities. These characteristics make them very attractive for food, pharmaceutical and cosmetic industries (Boukroufa et al., 2015; Fontana et al., 2013; Proestos and Komaitis, 2008). Another possible application for phenols is the replacement of petroleum-based phenol in phenolic resins (Bu et al., 2012). Although extraction of phenols and other added-value compounds from biomass materials can be carried out through the use of various methods (e.g. normal stirring, Soxhlet extraxtion and solid-liquid extraction using acids, alkali, solvents, water and supercritical fluids, enzyme-assisted, microwave-assisted and ultrasound-assisted extraction, hydrodistillation and steam diffusion, pulsed electric field extraction, pervaporation, high voltage electrical discharge and laser ablation) (Boukroufa et al., 2015; Galanakis, 2012; Goula et al., 2016), solid-liquid extraction and microwave-assisted extraction are the most popular (Bu et al., 2012; Li et al., 2011; Meneses et al., 2013; Proestos and Komaitis, 2008). Considering the TPH release levels obtained after microwave and chemical pretreatment (Table 7-4), it seems that the liquid fraction generated through microwave pretreatment would be the most suitable for phenols recovery, due to their higher TPH content. Nevertheless, the use of liquid fractions obtained through chemical pretreatment should not be completely excluded, since these solutions may contain different added-value compounds, other than phenols, that could potentially be recovered. Numerous relevant studies, investigating the extraction of phenols from materials obtained through processing of olives (Ahmad-Qasem et al., 2013; Japón-Luján et al., 2006; Mylonaki et al., 2008; Obied et al., 2005; Rafiee et al., 2011; Romero-García et al., 2014), grapes (Casazza et al., 2010; Fontana et al., 2013; Goula et al., 2016; Krishnaswamy et al., 2013; Li et al., 2011; Moschona et al., 2016) and citrus (Anagnostopoulou et al., 2006; Boukroufa et al., 2015; Hayat et al., 2009; Li et al., 2006), can be found in literature, with their majority focusing on microwave-assisted extraction. Adsorption and membrabe processes are the most common methods used to recover phenolic compounds, once extracted (Castro-Muñoz et al., 2016; Galanakis, 2012; González-Muñoz et al., 2003; Moschona et al., 2016; Soto et al., 2011). Among these methods, adsorption would appear as a more convenient option, due to the wide variety of potential adsorbents, including low-cost adsorbents such as natural materials, bioadsorbents and agricultural and industrial waste in their raw form or/and after their conversion to activated carbons (Ahmaruzzaman, 2008; Moschona et al., 2016; Soto et al., 2011).

Another possible option for valorizing the liquid fractions obtained after the pretreatment process could be the recovery of the chemical reagents used for pretreatment, i.e. ethanol,

citric acid and hydrogen peroxide. Through this practice, operational costs could be reduced, by avoiding the frequent purchase of reagents. To this regard, necessary reagents could also be recovered from the wastewater of the same agro-industries producing the solid waste of interest. In fact, both orange juice manufacturing and winery wastewater, have been found to contain a variety of compounds, including organic acids, such as citric acid and acetic acid, sugars, ethanol, phenolic compounds, etc. (Conradie et al., 2014; Mosse et al., 2011; Viuda-Martos et al., 2011).

7.1.2 Anaerobic digestion in semi-continuous mode

Chapter 6 focused on investigating the anaerobic digestion of WW, CGW, OP and JW in semi-continuous mode. Both mono-digestion and co-digestion assays were conducted, by using SOF as a co-substrate, while the assays were divided into two groups, depending on the conditions being applied. Specifically, the first group of assays (Group I) focused on investigating the variation in OLR and HRT, while the second group (Group II) had the purpose of evaluating the performance of anaerobic digestion systems being fed with different substrates in a sequential order, based on seasonality.

The data obtained from semi-continuous assays clearly demonstrated that the operation of a co-digestion system would result in an improved and more stable performance, compared with a mono-digestion system. This is true both when the substrates are fed to the system separately, and in sequential order. Sequential feeding would encompass multiple benefits, considering that it would provide a sustainable management and utilization option of more than one regional and seasonal waste materials, while it would also allow the operation and exploitation of such a system during longer time periods, or even continuously. Nevertheless, the eventual application of either one of the two feeding modalities, namely each material separately or in sequential order, using two, three or four materials, would depend on the needs and the availability of substrates of a specific area. For example, each Mediterranean country produces different quantities of grapes, olives, oranges and seed cotton, which would result in different waste amounts ultimately being generated. Table 7-5 and Table 7-6 provide rankings for the production of these four commodities and their processing products in twenty Mediterranean countries. These rankings were calculated considering the productions of each country for the year 2013. Specifically, for each country, the ranking of a specific commodity was calculated by dividing the production of that commodity by the highest production value among all four of them. According to these data, Greece would probably benefit from the use of all four agroindustrial waste, and in particular from those generated through wine and olive oil production, with the latter observation being true also for Spain. On the other hand, countries such as Albania, Bosnia and Herzegovina, Croatia, France, Italy, Malta, and Montenegro would certainly benefit from the use of grapes processing waste as a substrate, while the use of olive pomace would be a more viable option for Libya, Morocco, the Syrian Arab Republic and Tunisia. On the other hand, Cyprus would take the most advantage from both grapes and oranges processing waste, while this would also be the case for Algeria, Israel and Lebanon, with the addition of olive oil production waste. In the case of Egypt, the use of oranges and cotton processing waste could potentially offer considerable benefit, while as far as Turkey is concerned, all waste materials would be good feedstock options for anaerobic digestion, except probably grapes processing waste. In fact, similarly to Egypt, Turkey as well, has a low wine production despite the high grapes production.

Table 7-5: Rankings of the production of grapes, olives, oranges and seed cotton for Mediterranean countries

Countries	Grapes	Olives	Oranges	Seed cotton
Albania	100	49.8	4.00	0.44
Algeria	64.1	65.0	100	0.01
Bosnia and Herzegovina	100	0.48	0.41	0.00
Croatia	100	18.9	0.09	0.00
Cyprus	66.6	34.5	100	0.00
Egypt	48.1	18.8	100	15.1
France	100	0.49	0.07	0.00
Greece	49.9	100	42.0	45.4
Israel	94.4	85.3	100	31.7
Italy	100	36.7	21.3	0.00
Lebanon	70.2	78.1	100	0.00
Libya	24.0	100	36.4	0.00
Malta	100	0.12	18.9	0.00
Montenegro	100	7.25	22.8	0.00
Morocco	36.9	100	64.3	0.02
Slovenia	100	2.16	0.00	0.00
Spain	80.9	100	36.7	1.57
Syrian Arab Republic	36.4	100	94.1	20.1
Tunisia	12.0	100	11.8	0.18
Turkey	100	41.8	44.4	56.1

Table 7-6: Rankings of the production of wine, olive oil, orange juice and cotton lint for Mediterranean countries

Countries	Wine	Olive oil	Orange juice	Cotton lint
Albania	100	4.44	0.00	1.28
Algeria	77.0	100	36.9	0.04
Bosnia and Herzegovina	100	0.00	0.00	0.00
Croatia	100	2.17	0.00	0.00
Cyprus	100	15.0	68.9	0.00
Egypt	4.25	5.66	0.00	100
France	100	0.11	0.02	0.00
Greece	100	98.2	14.0	89.9
Israel	22.6	53.5	100	60.9
Italy	100	10.8	0.67	0.00
Lebanon	93.8	100	11.7	0.00
Libya	0.00	100	0.00	0.00
Malta	100	0.16	6.12	0.00
Montenegro	100	1.13	0.00	0.00
Morocco	30.2	100	85.9	0.06
Slovenia	100	1.60	0.00	0.00
Spain	100	34.7	4.41	1.78
Syrian Arab Republic	0.05	100	0.00	62.0
Tunisia	14.9	100	0.60	0.34
Turkey	3.60	22.6	0.20	100

7.2 Further research

Further research regarding substrate pretreatment should focus on improving the efficiency of the processes, in terms of energy and cost. More specifically, the determination of additional parameters for the liquid fractions obtained after pretreatment, would offer a more comprehensive picture of the composition of such solutions, i.e. of the types of compounds present in them and therefore, it would be possible to propose and test more targeted recovery and reuse options, aiming towards a biorefinery concept. These compounds could include not only substrate hydrolysis products resulting from both pretreatments, but also residual amounts of the reagents used for chemical pretreatment. Investigating the possible inhibitory effects of such compounds, on the eventual anaerobic digestion of these liquid fractions, would allow the evaluation of their actual usability as substrates for methane production.

As far as anaerobic digestion in semi-continuous mode is concerned, further research should involve upscaling the whole process, by using larger digester volumes, both working and nominal, which would also allow the operation of the system for longer time periods, resulting in a more accurate estimation of the performance of a pilot- or full-scale unit. Such a scale-up would also make the determination of suitable conditions for constantly stable reactor operation, not only in terms of chemical parameters, but also in terms of methane production, easier. This would involve the investigation of different combinations of OLR and HRT, for both feeding modalities, i.e. when feeding each material separately, or all the materials in a sequential order. Such an investigation would aim at avoiding instability phenomena inside the digester and at maintaining a constant methane production without significant fluctuations. For example, the operation of a system with different OLR for materials of different degradability degrees, i.e. higher OLR for less degradable materials and lower OLR for more degradable materials, would provide useful information, in order to achieve a more equilibrated daily methane production.

Further investigations regarding land application of digestates should involve pot and plot trials, aiming at determining possible phytotoxic effects, due to the eventual presence of potentially toxic substances or pathogens, while different types of ecotoxicity test could also be performed. Moreover, alternative options for digestate use, such as composting and biochar production (Inyang et al., 2010; Inyang et al., 2011; Inyang et al., 2012; Sun et al., 2013; Troy et al., 2013; Yao et al., 2011) could also be evaluated.

7.3 References

Ahmad-Qasem, M.H., Cánovas, J., Barrajón-Catalán, E., Micol, V., Cárcel, J.A., García-Pérez, J.V. (2013). Kinetic and compositional study of phenolic extraction from olive leaves (var. Serrana) by using power ultrasound. *Innovative Food Science & Emerging Technologies*, *17*, 120–129.

Ahmaruzzaman, M. (2008). Adsorption of phenolic compounds on low-cost adsorbents: A review. *Advances in Colloid and Interface Science*, *143*(1), 48–67.

Anagnostopoulou, M.A., Kefalas, P., Papageorgiou, V.P., Assimopoulou, A.N., Boskou, D. (2006). Radical scavenging activity of various extracts and fractions of sweet orange peel (Citrus sinensis). *Food Chemistry*, *94*(1), 19–25.

Boukroufa, M., Boutekedjiret, C., Petigny, L., Rakotomanomana, N., Chemat, F. (2015). Bio-refinery of orange peels waste: A new concept based on integrated green and solvent free extraction processes using ultrasound and microwave techniques to obtain essential oil, polyphenols and pectin. *Ultrasonics Sonochemistry*, *24*, 72–79.

Bu, Q., Lei, H., Ren, S., Wang, L., Zhang, Q., Tang, J., Ruan, R. (2012). Production of phenols and biofuels by catalytic microwave pyrolysis of lignocellulosic biomass. *Bioresource Technology*, *108*, 274–279.

Casazza, A.A., Aliakbarian, B., Mantegna, S., Cravotto, G., Perego, P. (2010). Extraction of phenolics from *Vitis vinifera* wastes using non-conventional techniques. *Journal of Food Engineering*, *100*(1), 50–55.

Castro-Muñoz, R., Yáñez-Fernández, J., Fíla, V. (2016). Phenolic compounds recovered from agro-food by-products using membrane technologies: An overview. *Food Chemistry*, *213*, 753–762.

Conradie, A., Sigge, G.O., Cloete, T.E. (2014). Influence of winemaking practices on the characteristics of winery wastewater and water usage of wineries. *South African Journal of Enology and Viticulture*, *35*(1), 10–19.

Fontana, A.R., Antoniolli, A., Bottini, R. (2013). Grape Pomace as a Sustainable Source of Bioactive Compounds: Extraction, Characterization, and Biotechnological Applications of Phenolics. *Journal of Agricultural and Food Chemistry*, *61*(38), 8987–9003.

Galanakis, C.M. (2012). Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. *Trends in Food Science & Technology*, *26*(2), 68–87.

González-Muñoz, M. ., Luque, S., Álvarez, J. ., Coca, J. (2003). Recovery of phenol from aqueous solutions using hollow fibre contactors. *Journal of Membrane Science*, *213*(1), 181–193.

Goula, A.M., Thymiatis, K., Kaderides, K. (2016). Valorization of grape pomace: Drying behavior and ultrasound extraction of phenolics. *Food and Bioproducts Processing*, *100*, 132–144.

Hayat, K., Hussain, S., Abbas, S., Farooq, U., Ding, B., Xia, S., Jia, C., Zhang, X., Xia, W. (2009). Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity in vitro. *Separation and Purification Technology*, *70*(1), 63–70.

Inyang, M., Gao, B., Ding, W., Pullammanappallil, P., Zimmerman, A.R., Cao, X. (2011). Enhanced Lead Sorption by Biochar Derived from Anaerobically Digested Sugarcane Bagasse. *Separation Science and Technology*, *46*(12), 1950–1956.

Inyang, M., Gao, B., Pullammanappallil, P., Ding, W., Zimmerman, A.R. (2010). Biochar from anaerobically digested sugarcane bagasse. *Bioresource Technology*, *101*(22), 8868–8872.

Inyang, M., Gao, B., Yao, Y., Xue, Y., Zimmerman, A.R., Pullammanappallil, P., Cao, X., 2012. Removal of heavy metals from aqueous solution by biochars derived from anaerobically digested biomass. *Bioresource Technology 110*, 50–56.

Japón-Luján, R., Luque-Rodríguez, J.M., Luque de Castro, M.D. (2006). Multivariate optimisation of the microwave-assisted extraction of oleuropein and related biophenols from olive leaves. *Analytical and Bioanalytical Chemistry, 385*(4), 753–759.

Krishnaswamy, K., Orsat, V., Gariépy, Y., Thangavel, K. (2013). Optimization of Microwave-Assisted Extraction of Phenolic Antioxidants from Grape Seeds (*Vitis vinifera*). *Food and Bioprocess Technology, 6*(2), 441–455.

Kuglarz, M., Karakashev, D., Angelidaki, I. (2013). Microwave and thermal pretreatment as methods for increasing the biogas potential of secondary sludge from municipal wastewater treatment plants. *Bioresource Technology, 134*, 290–297.

Li, B.B., Smith, B., Hossain, M.M. (2006). Extraction of phenolics from citrus peels: II. Enzyme-assisted extraction method. *Separation and Purification Technology, 48*(2), 189–196.

Li, Y., Skouroumounis, G.K., Elsey, G.M., Taylor, D.K. (2011). Microwave-assistance provides very rapid and efficient extraction of grape seed polyphenols. *Food Chemistry, 129*(2), 570–576.

Meneses, N.G.T., Martins, S., Teixeira, J.A., Mussatto, S.I. (2013). Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains. *Separation and Purification Technology, 108*, 152–158.

Moschona, A., Ziagova, M.G., Iliadou, A., Liakopoulou-Kyriakides, M. (2016). Optimization Studies for Selective Recovery of Phenolics from Wine Wastes. *Agricultural Sciences, 7*(4), 187–195.

Mosse, K.P.M., Patti, A.F., Christen, E.W., Cavagnaro, T.R. (2011). Review: Winery wastewater quality and treatment options in Australia. *Australian Journal of Grape and Wine Research, 17*(2), 111–122.

Mylonaki, S., Kiassos, E., Makris, D.P., Kefalas, P. (2008). Optimisation of the extraction of olive (Olea europaea) leaf phenolics using water/ethanol-based solvent systems and response surface methodology. *Analytical and Bioanalytical Chemistry, 392*(5), 977–985.

Obied, H.K., Allen, M.S., Bedgood, D.R., Prenzler, P.D., Robards, K., Stockmann, R. (2005). Bioactivity and Analysis of Biophenols Recovered from Olive Mill Waste. *Journal of Agricultural and Food Chemistry, 53*(4), 823–837.

Proestos, C., Komaitis, M. (2008). Application of microwave-assisted extraction to the fast extraction of plant phenolic compounds. *LWT - Food Science and Technology, 41*(4), 652–659.

Rafiee, Z., Jafari, S.M., Alami, M., Khomeiri, M. (2011). Microwave-assisted extraction of phenolic compounds from olive leaves ; a comparison with maceration. *Journal of Animal & Plant Sciences, 21*(4), 738–745.

Romero-García, J.M., Niño, L., Martínez-Patiño, C., Álvarez, C., Castro, E., Negro, M.J. (2014). Biorefinery based on olive biomass. State of the art and future trends. *Bioresource Technology, 159*, 421–432.

Soto, M.L., Moure, A., Domínguez, H., Parajó, J.C. (2011). Recovery, concentration and purification of phenolic compounds by adsorption: A review. *Journal of Food Engineering, 105*(1), 1–27.

Sun, L., Wan, S., Luo, W. (2013). Biochars prepared from anaerobic digestion residue, palm bark, and eucalyptus for adsorption of cationic methylene blue dye: Characterization, equilibrium, and kinetic studies. *Bioresource Technology, 140*, 406–413.

Troy, S.M., Nolan, T., Leahy, J.J., Lawlor, P.G., Healy, M.G., Kwapinski, W. (2013). Effect of sawdust addition and composting of feedstock on renewable energy and biochar production from pyrolysis of anaerobically digested pig manure. *Biomass and Bioenergy, 49*, 1–9.

Viuda-Martos, M., Fernandez-Lopez, J., Sayas-Barbera, E., Sendra, E., Perez-Alvarez, J.A. (2011). Physicochemical characterization of the orange juice waste water of a citrus by-product. *Journal of Food Processing and Preservation, 35*(2), 264–271.

Yao, Y., Gao, B., Inyang, M., Zimmerman, A.R., Cao, X., Pullammanappallil, P., Yang, L. (2011). Biochar derived from anaerobically digested sugar beet tailings: Characterization and phosphate removal potential. *Bioresource Technology 102*(10), 6273–6278.

APPENDICES

Appendix A: Multiple-stage-modeling for BMP assays of untreated (raw) substrates (Chapter 3)

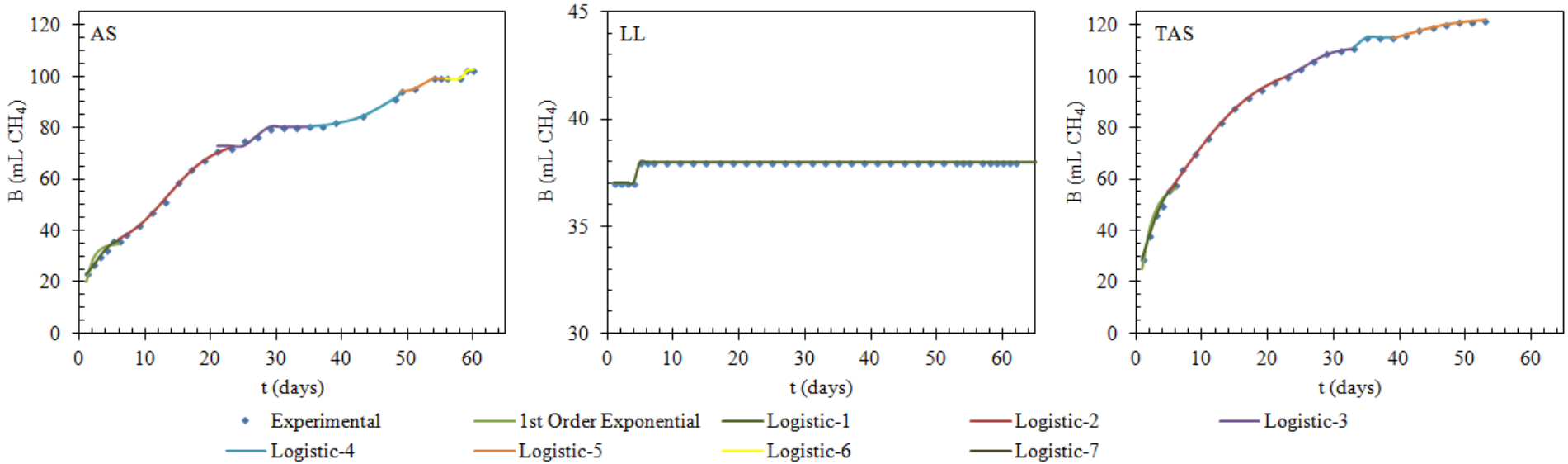


Figure A-1: Fitting for combination of 1st Order Exponential and Logistic models, blank trials (inocula only, AS, LL and TAS)

Table A-1: Modeling parameters for multiple-stage-modeling approach, trials with different SIR

Varying parameter	Time period (days)	Model applied	Model parameters				Goodness of fit	
WW								
SIR=0.25	1 - 7	1st Order	B_{max}	K			RSS	R^2
		Exponential	240.3	0.2866			81	0.9956
	6 - 46	Logistic	B_0	P	R_m	λ	RSS	R^2
			93.73	162.3	4.49	0.00	58	0.9909
SIR=0.5	1 - 6	1st Order	B_{max}	K			RSS	R^2
		Exponential	167.8	0.4517			311	0.9449
	5 - 17	Logistic	B_0	P	R_m	λ	RSS	R^2
			119.7	273.2	29.8	9.84	3	1.000
	17 - 60	Logistic	B_0	P	R_m	λ	RSS	R^2
			26.60	450.0	9.12	0.00	96	0.9938
SIR=1	1 - 6	1st Order	B_{max}	K			RSS	R^2
		Exponential	12.32	0.6999			0	0.9811
	6 - 23	Logistic	B_0	P	R_m	λ	RSS	R^2
			13.29	40.54	8.20	14.8	7	0.9978
	21 - 35	Logistic	B_0	P	R_m	λ	RSS	R^2
			53.34	96.25	16.5	29.8	3	0.9997
	35 - 60	Logistic	B_0	P	R_m	λ	RSS	R^2
			136.9	96.61	6.40	42.7	13	0.9989
SIR=2	1 - 3	1st Order	B_{max}	K			RSS	R^2
		Exponential	21.20	0.5122			3	0.9213
	2 - 56	Logistic	B_0	P	R_m	λ	RSS	R^2
			15.96	38.45	16.1	6.08	9	0.9980
	56 - 60	Logistic	B_0	P	R_m	λ	RSS	R^2
			53.94	10.89	1.20	63.9	0	0.9975
CGW								
SIR=0.25	1 - 21	1st Order	B_{max}	K			RSS	R^2
		Exponential	189.01	0.1409			145	0.9954
	19 - 46	Logistic	B_0	P	R_m	λ	RSS	R^2
			54.50	147.0	5.66	8.45	4	0.9951
SIR=0.5	1 - 21	1st Order	B_{max}	K			RSS	R^2
		Exponential	302.7	0.0560			191	0.9963
	21 - 60	Logistic	B_0	P	R_m	λ	RSS	R^2
			78.52	231.4	4.16	17.7	11	0.9994
SIR=1	1 - 4	1st Order	B_{max}	K			RSS	R^2
		Exponential	156.7	0.2241			12	0.9939
	4 - 19	Logistic	B_0	P	R_m	λ	RSS	R^2
			0.000	349.3	28.3	7.04	76	0.9991
	19 - 60	Logistic	B_0	P	R_m	λ	RSS	R^2
			287.6	200.2	5.38	27.6	87	0.9981
SIR=2	1 - 5	1st Order	B_{max}	K			RSS	R^2
		Exponential	327.9	0.1216			11	0.9985
	5 - 21	Logistic	B_0	P	R_m	λ	RSS	R^2
			0.000	451.7	31.0	7.60	106	0.9990
	21 - 60	Logistic	B_0	P	R_m	λ	RSS	R^2
			234.8	455.5	6.65	22.7	53	0.9994
OP								
SIR=0.25	1 - 7	1st Order	B_{max}	K			RSS	R^2
		Exponential	160.47	0.1190			58	0.9845
	7 - 31	Logistic	B_0	P	R_m	λ	RSS	R^2
			0.000	183.6	6.18	6.35	29	0.9967
	31 - 39	Logistic	B_0	P	R_m	λ	RSS	R^2
			178.0	11.43	3.07	34.2	0	0.9966

	39 - 46	Logistic	B_0	P	R_m	λ	RSS	R^2
			185.4	11.87	2.24	41.9	0	0.9969
SIR=0.5	1 - 2	1st Order	B_{max}	K			RSS	R^2
		Exponential	36.89	1.368			0	1.0000
	3 - 23	Logistic	B_0	P	R_m	λ	RSS	R^2
			30.50	202.8	15.1	12.1	54	0.9991
	23 - 60	Logistic	B_0	P	R_m	λ	RSS	R^2
			34.87	250.7	4.69	6.75	7	0.9987
SIR=1	1 - 3	1st Order	B_{max}	K			RSS	R^2
		Exponential	58.36	0.5936			7	0.9708
	4 - 23	Logistic	B_0	P	R_m	λ	RSS	R^2
			41.77	297.6	24.1	12.35	66	0.9995
	21 - 60	Logistic	B_0	P	R_m	λ	RSS	R^2
			0.000	421.3	7.95	5.13	35	0.9979
SIR=2	1 - 6	1st Order	B_{max}	K			RSS	R^2
		Exponential	132.2	0.1928			3	0.9991
	6 - 23	Logistic	B_0	P	R_m	λ	RSS	R^2
			70.04	350.5	30.0	14.08	37	0.9997
	21 - 60	Logistic	B_0	P	R_m	λ	RSS	R^2
			0.000	519.6	12.4	9.25	81	0.9972
JW								
SIR=0.25	1 - 9	1st Order	B_{max}	K			RSS	R^2
		Exponential	205.8	0.3874			180	0.9891
	7 - 31	Logistic	B_0	P	R_m	λ	RSS	R^2
			180.3	41.46	2.92	11.1	2	0.9984
	29 - 41	Logistic	B_0	P	R_m	λ	RSS	R^2
			222.0	4.035	3.69	35.4	0	0.9805
SIR=0.5	1 - 11	1st Order	B_{max}	K			RSS	R^2
		Exponential	414.3	0.2936			158	0.9980
	9 - 25	Logistic	B_0	P	R_m	λ	RSS	R^2
			314.1	136.0	5.83	8.47	5	0.9986
	25 - 41	Logistic	B_0	P	R_m	λ	RSS	R^2
			423.6	45.72	1.62	27.8	1	0.9988
SIR=1	1 - 2	1st Order	B_{max}	K			RSS	R^2
		Exponential	130.9	2.106			0	1.0000
	3 - 9	Logistic	B_0	P	R_m	λ	RSS	R^2
			44.41	167.7	25.3	2.33	4	0.9997
	9 - 23	Logistic	B_0	P	R_m	λ	RSS	R^2
			189.3	122.6	11.8	13.9	94	0.9993
	21 - 41	Logistic	B_0	P	R_m	λ	RSS	R^2
			306.0	288.6	31.3	32.1	320	0.9976
SIR=2	1 - 7	1st Order	B_{max}	K			RSS	R^2
		Exponential	130.9	1.040			207	0.8671
	1 - 7	Logistic	B_0	P	R_m	λ	RSS	R^2
			10.89	127.3	18.4	0.00	9	0.9943
	7 - 33	Logistic	B_0	P	R_m	λ	RSS	R^2
			135.1	65.96	6.12	17.2	6	0.9993
	33 - 41	Logistic	B_0	P	R_m	λ	RSS	R^2
			3.469	201.3	20.6	23.0	0	0.9645

Table A-2: Modeling parameters for multiple-stage-modeling approach, trials with different inocula

Varying parameter	Time period (days)	Model applied	Model parameters				Goodness of fit	
Blanks								
AS	1 - 6	1st Order	B_{max}	K			RSS	R^2
		Exponential	34.65	0.8641			26	0.8111
	1 - 6	Logistic	B_0	P	R_m	λ	RSS	R^2
			19.06	18.62	4.02	2.63	1	0.9907
	6 - 23	Logistic	B_0	P	R_m	λ	RSS	R^2
			31.44	44.07	3.12	13.4	3	0.9981
	21 - 35	Logistic	B_0	P	R_m	λ	RSS	R^2
			72.67	7.333	29.3	27.0	9	0.9097
	35 - 49	Logistic	B_0	P	R_m	λ	RSS	R^2
			79.08	372.4	16.4	67.3	1	0.9965
	49 - 56	Logistic	B_0	P	R_m	λ	RSS	R^2
			94.00	5.000	20.1	51.1	0	1.000
	56 - 60	Logistic	B_0	P	R_m	λ	RSS	R^2
			98.99	3.506	10.4	58.8	0	1.000
LL	1 - 68	Logistic	B_0	P	R_m	λ	RSS	R^2
			37.00	1.000	10.5	4.48	0	1.000
TAS	1 - 6	1st Order	B_{max}	K			RSS	R^2
		Exponential	58.20	0.5690			28	0.9536
	1 - 6	Logistic	B_0	P	R_m	λ	RSS	R^2
			0.000	62.02	8.94	1.21	2	0.9959
	5 - 23	Logistic	B_0	P	R_m	λ	RSS	R^2
			15.68	90.60	3.65	6.49	4	0.9985
	23 - 33	Logistic	B_0	P	R_m	λ	RSS	R^2
			96.55	14.91	1.90	25.7	0	0.9999
	33 - 39	Logistic	B_0	P	R_m	λ	RSS	R^2
			109.2	5.838	7.33	33.2	0	1.000
	39 - 53	Logistic	B_0	P	R_m	λ	RSS	R^2
			110.2	12.21	0.84	41.0	0	0.9906
WW (SIR=0.5)								
AS	1 - 6	1st Order	B_{max}	K			RSS	R^2
		Exponential	167.8	0.4517			311	0.9449
	5 - 17	Logistic	B_0	P	R_m	λ	RSS	R^2
			119.7	273.2	29.8	9.84	3	1.000
	17 - 60	Logistic	B_0	P	R_m	λ	RSS	R^2
			26.60	450.0	9.12	0.00	96	0.9938
LL	1 - 7	1st Order	B_{max}	K			RSS	R^2
		Exponential	117.3	0.6968			10	0.9961
	9 - 47	Logistic	B_0	P	R_m	λ	RSS	R^2
			121.2	235.2	20.0	35.2	34	0.9998
	47 - 68	Logistic	B_0	P	R_m	λ	RSS	R^2
			305.7	77.38	2.01	42.2	3	0.9943
TAS	1 - 4	1st Order	B_{max}	K			RSS	R^2
		Exponential	446.2	0.1528			366	0.9713
	3 - 15	Logistic	B_0	P	R_m	λ	RSS	R^2
			109.0	286.0	24.5	6.34	46	0.9990
	15 - 31	Logistic	B_0	P	R_m	λ	RSS	R^2
			351.2	84.92	5.15	17.6	8	0.9972
	31 - 53	Logistic	B_0	P	R_m	λ	RSS	R^2
			134.4	327.2	8.51	8.02	8	0.9894
CGW (SIR=0.25)								
AS	1 - 21	1st Order	B_{max}	K			RSS	R^2
		Exponential	189.0	0.1409			145	0.9954
	19 - 46	Logistic	B_0	P	R_m	λ	RSS	R^2

			54.50	147.0	5.66	8.45	4	0.9951
LL	1 - 2	1st Order	B_{max}	K			*RSS*	R^2
		Exponential	45.40	1.959			0	1.0000
	2 - 68	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			41.15	21.84	5.21	3.99	0	0.9995
TAS	1 - 3	1st Order	B_{max}	K			*RSS*	R^2
		Exponential	115.8	0.3390			2	0.9979
	4 - 7	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			0.00	128.2	15.2	2.69	1	0.9975
	7 - 35	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			0.0	200.1	6.08	4.16	64	0.9930
	35 - 53	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			197.4	14.09	1.06	41.2	1	1.000
OP (SIR=0.25)								
AS	1 - 7	1st Order	B_{max}	K			*RSS*	R^2
		Exponential	160.5	0.1190			58	0.9845
	7 - 31	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			0.000	183.6	6.18	6.35	29	0.9967
	31 - 39	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			178.0	11.43	3.07	34.2	0.31	0.9966
	39 - 46	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			185.4	11.87	2.24	41.9	0.13	0.9969
LL	1 - 3	1st Order	B_{max}	K			*RSS*	R^2
		Exponential	45.69	2.500			0	0.9698
	3 - 47	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			43.02	16.95	3.22	5.50	1	0.9969
	45 - 53	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			60.00	3.0	9.72	48.0	0	1.0000
	51 - 68	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			53.43	341.0	6.90	95.2	5	0.9988
TAS	1 - 4	1st order	B_{max}	K			*RSS*	R^2
		exponential	105.1	0.3196			1	0.9995
	5 - 25	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			0.000	197.6	8.30	5.40	26	0.9980
	25 - 53	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			170.3	45.53	1.79	26.4	4	0.9955
JW (SIR=0.5)								
AS	1 - 11	1st Order	B_{max}	K			*RSS*	R^2
		Exponential	414.3	0.3			158	0.9980
	9 - 25	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			314.1	136.0	5.83	8.47	5	0.9986
	25 - 41	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			423.6	45.72	1.62	27.8	1	0.9988
LL	1 - 4	1st Order	B_{max}	K			*RSS*	R^2
		Exponential	125.9	0.7929			61	0.9580
	3 - 41	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			74.31	84.20	10.3	3.29	3	0.9993
	39 - 68	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			159.0	131.4	7.79	61.4	40	0.9983
TAS	1 - 4	1st Order	B_{max}	K			*RSS*	R^2
		Exponential	237.8	0.1309			5	0.9979
	3 - 11	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			57.81	117.1	24.3	4.86	49	0.9940
	9 - 21	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			146.3	115.4	6.06	15.9	10	0.9970
	19 - 39	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			74.66	241.5	9.40	16.4	45	0.9951
	37 - 53	Logistic	B_0	P	R_m	λ	*RSS*	R^2
			110.7	222.1	4.55	12.3	5	0.9839

Appendix B: Additional data from optimization procedure for microwave pretreatment (Chapter 4)

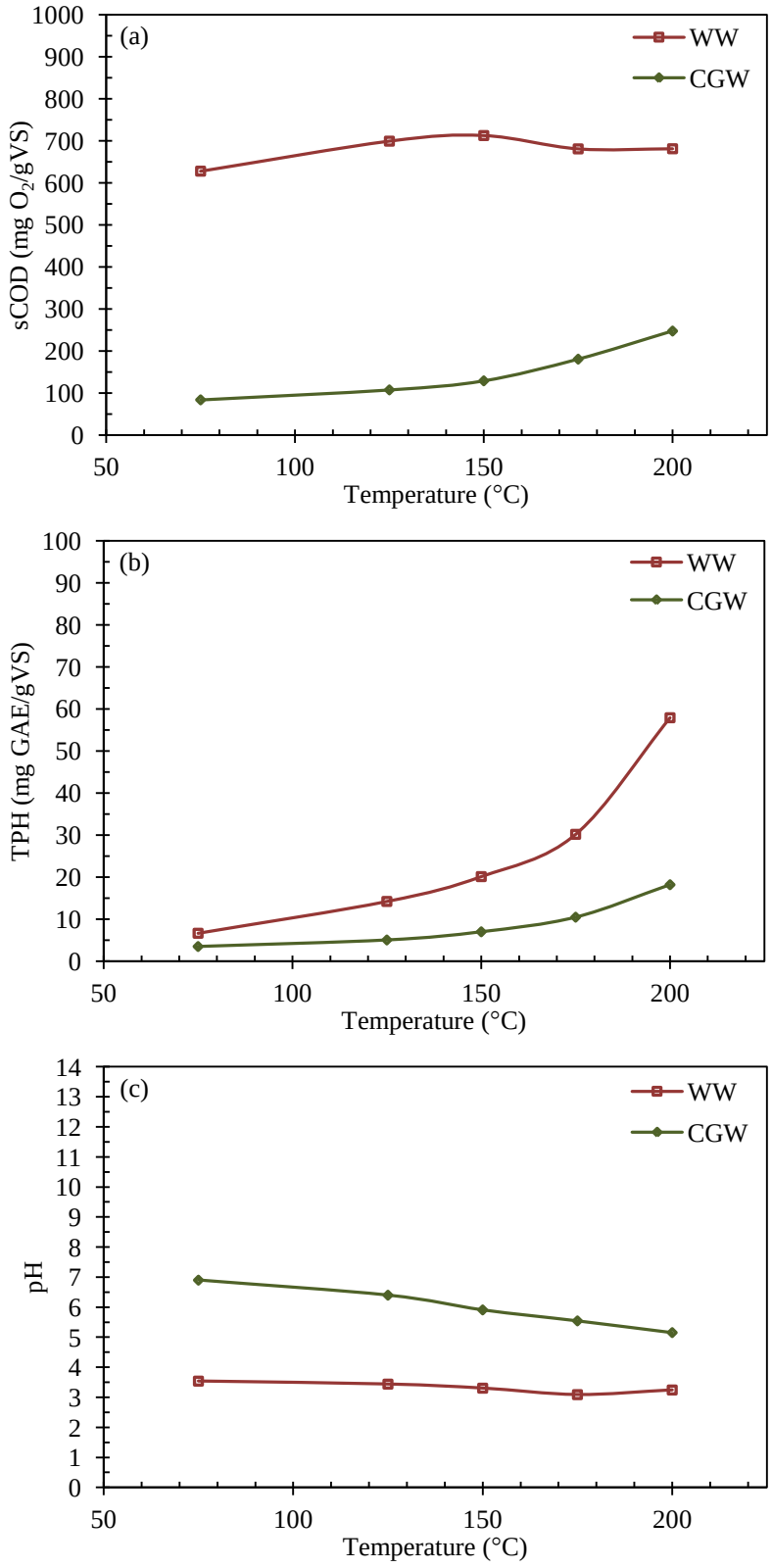


Figure B-1: Variation of (a) sCOD, (b) TPH and (c) pH, after microwave pretreatment as a function of temperature

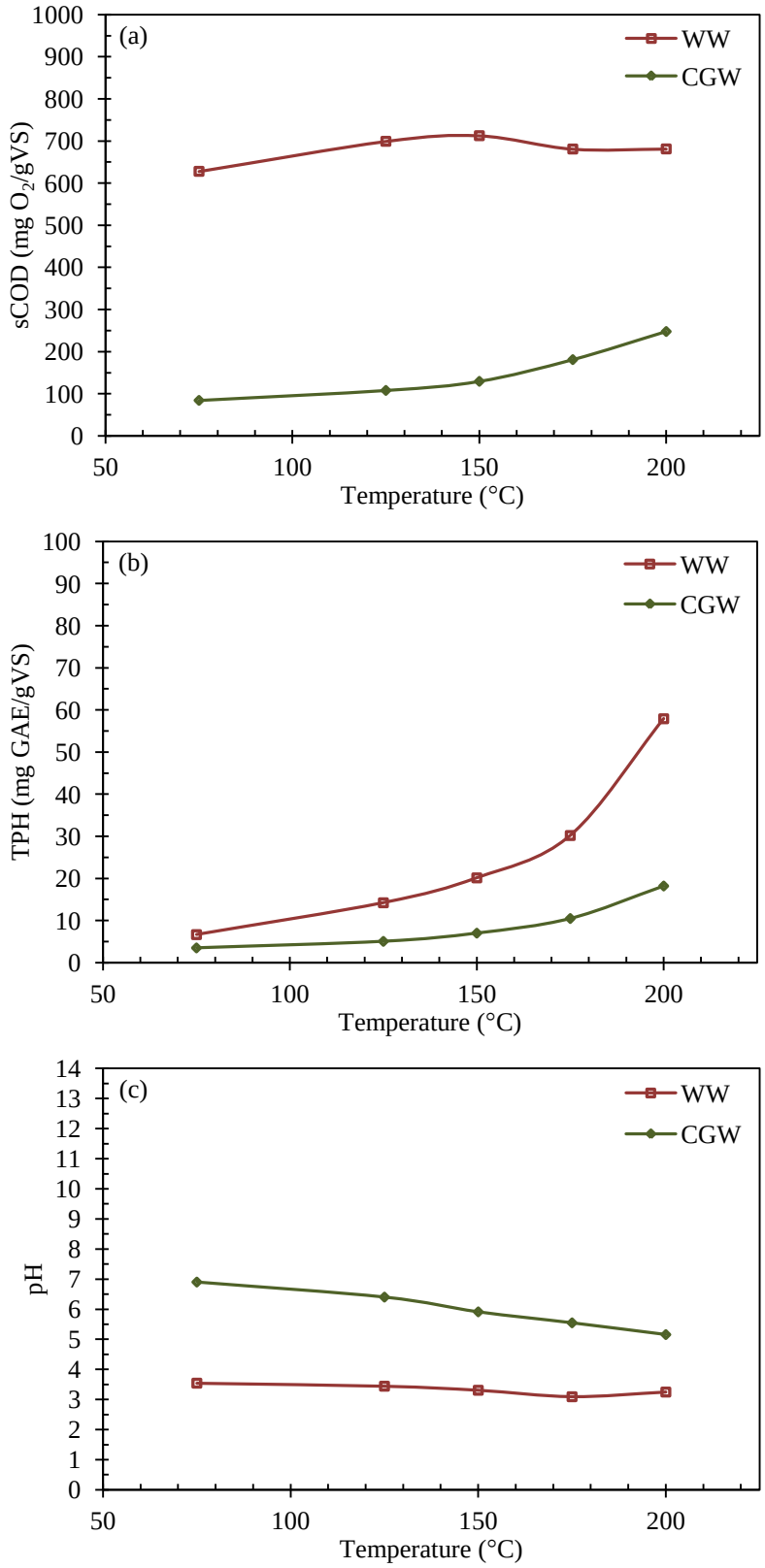


Figure B-2: Variation of (a) sCOD, (b) TPH and (c) pH, after microwave pretreatment as a function of temperature

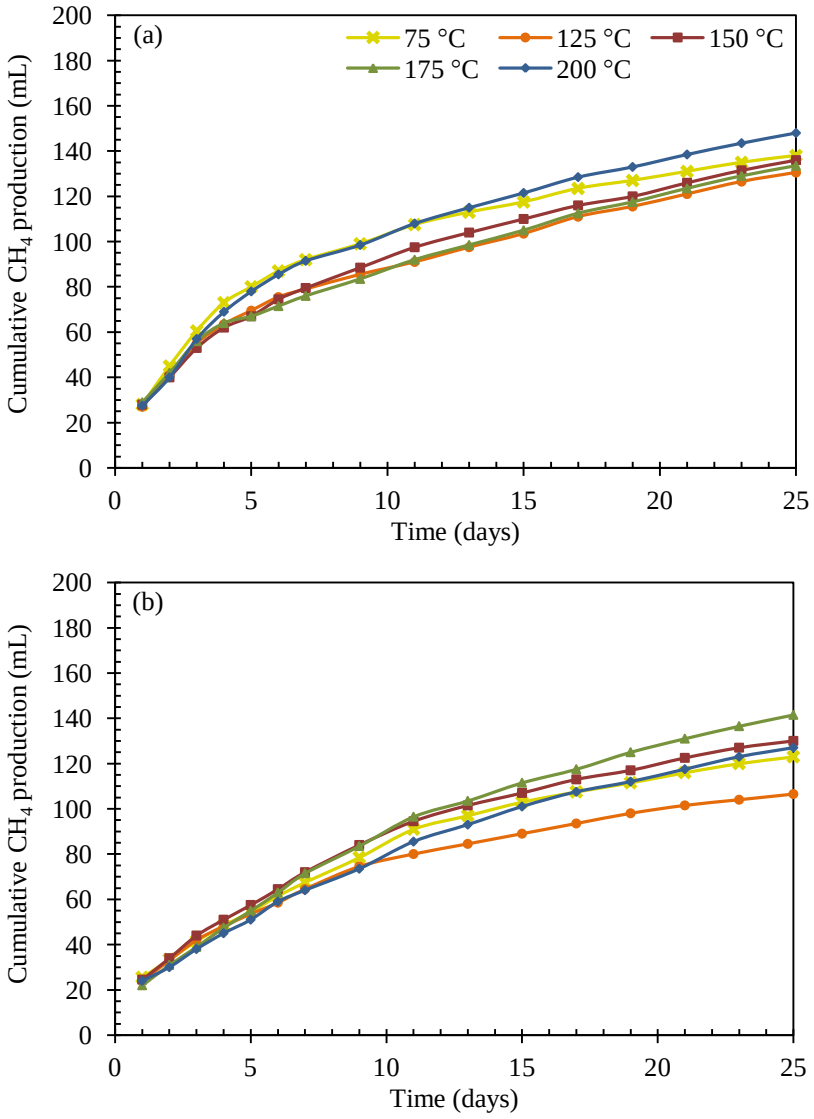
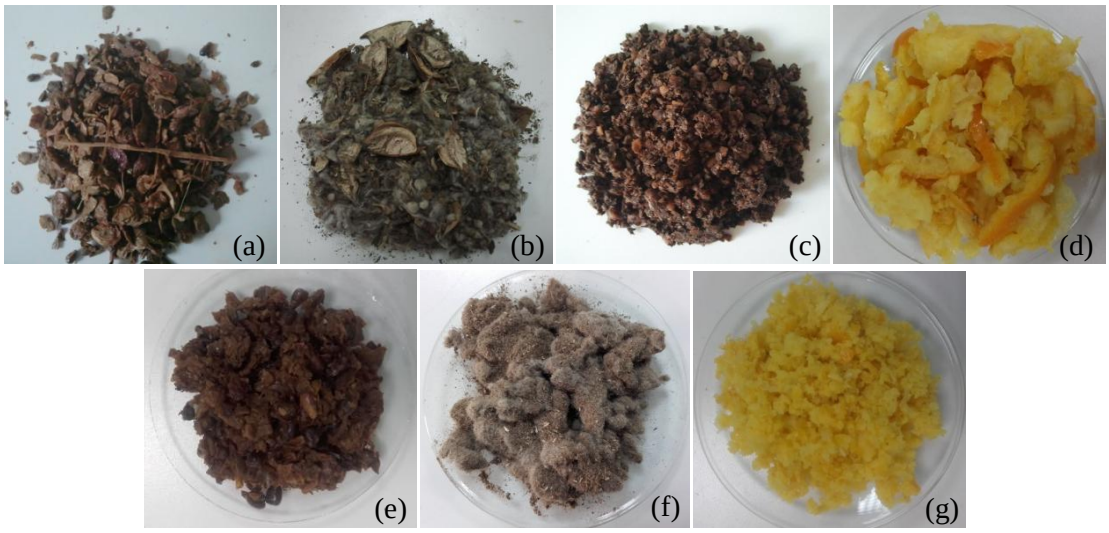
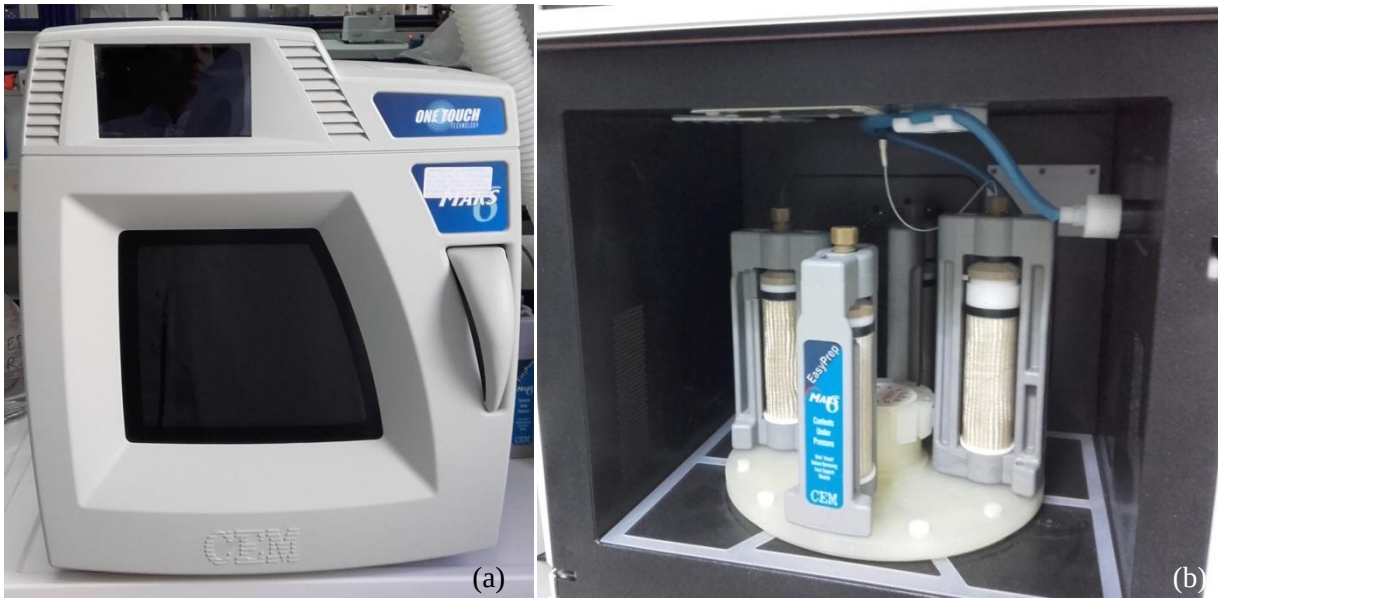


Figure B-3: Cumulative methane production for (a) WW and (b) CGW, pretreated with microwave heating at different temperatures

Appendix C: Photos



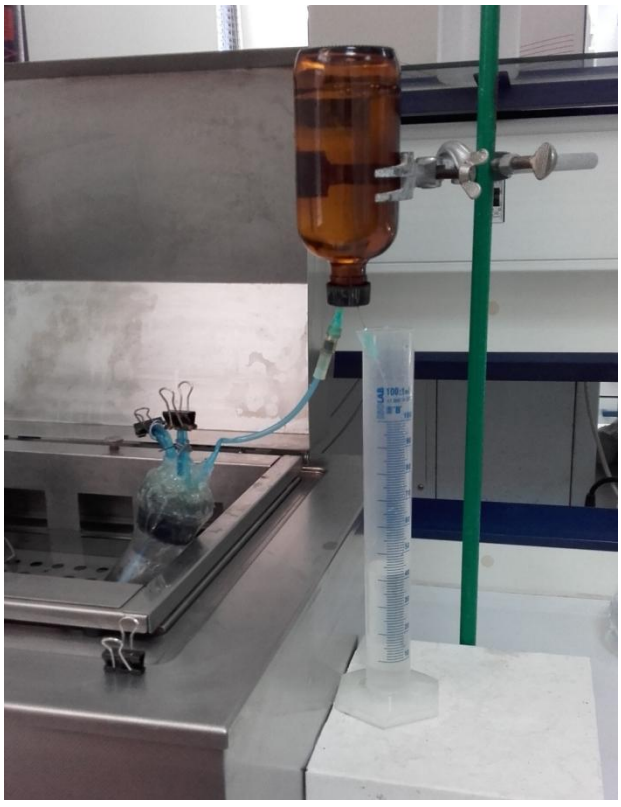
Picture 1: Agroindustrial waste as received and after size reduction: WW (a, e), CGW (b, f), OP (c) and JW (d, g)



Picture 2: Microwave pretreatment of agroindustrial waste: (a) microwave reaction system (MARS), (b) Teflon vessels inside the MARS

Picture 3: Chemical pretreatment of agroindustrial waste

Picture 4: Reactors inside the incubator during (a) BMP and (b) semi-continuous assays

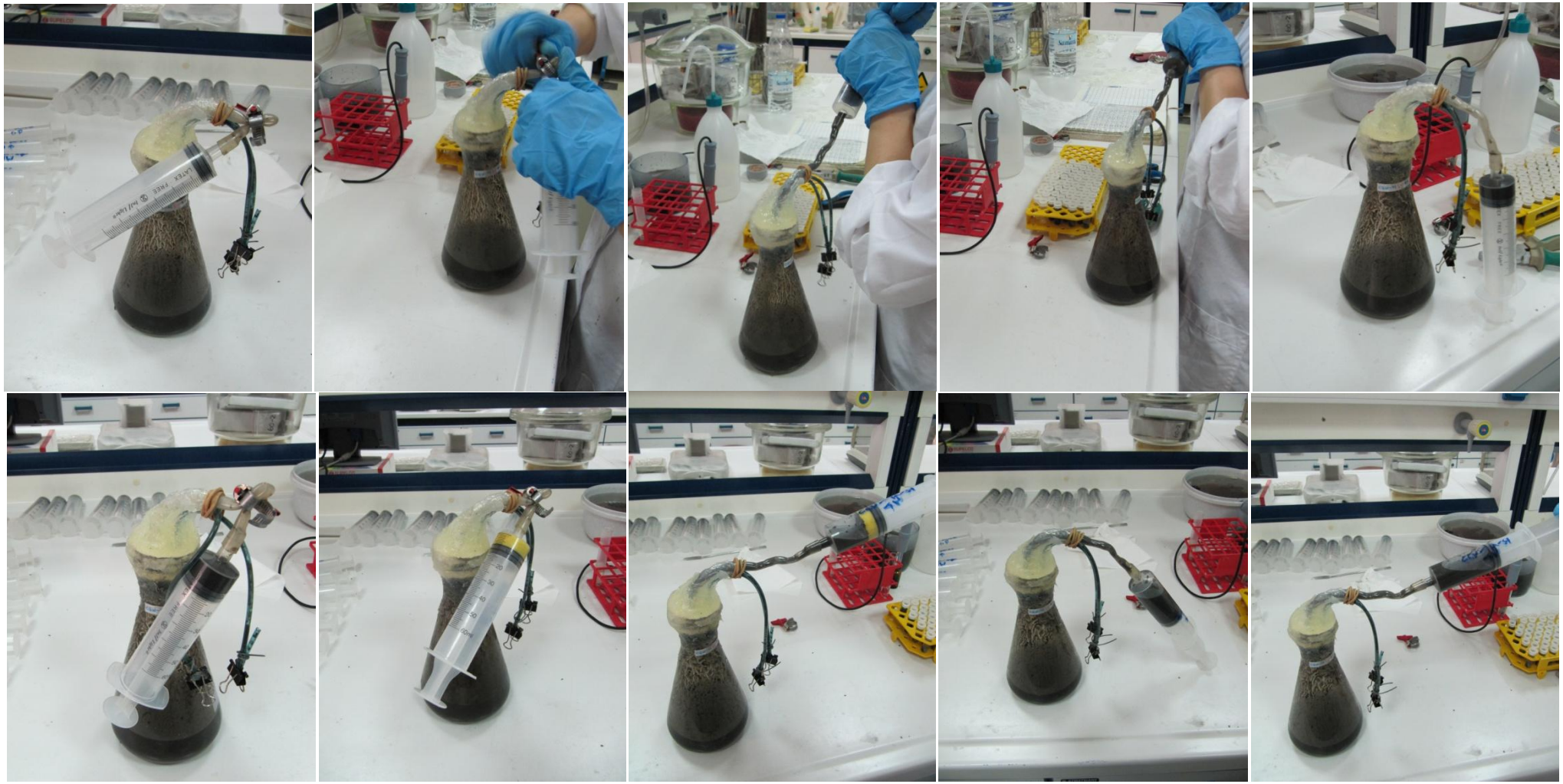


Picture 5: Apparatus for methane production measurement

Picture 6: Preparation of feeding materials for semi-cotinuous assays

Picture 7: Feeding materials used in semi-continuous assays: (a) single-substrates, (b) mixed-substrates

Picture 8: Preparation for feeding procedure



Picture 9: Procedure for sampling and feeding during semi-continuous assays