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STRATEGIES TO IMPROVE BIOENERGY AND BIOCHEMICAL PRODUCTION FROM ORGANIC SOLID WASTE

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Abstract

The development of the direct biomass utilization for diverse industrial applications is turning the organic solid waste into one of the most promising feedstocks for the production of wide spectrum of competitive bio-based products with great market significance. The bioconversion of organic waste into value added bio-chemicals supply chain as a waste management strategy should be a viable method towards the implementation of the circular economy principles. This approach is an important challenge from the technological, economic and environmental perspectives.

The organic fraction of municipal solid waste (OFMSW) is globally produced in large amounts and the low cost availability of this substance makes it attractive to reduce the negative environmental impact of the fossil resource utilization. One of the most relevant advantages in the consideration of the sustainable management of organic waste is the generation of valuable products from low-value streams that are generated continuously. The production of the multiple targets required a highly efficient conversion process of organic waste. Currently, the anaerobic digestion (AD) is one of the preferred treatments for the intensive biodegradation of OFMSW and it is considered as well-established waste to energy technology.

To date, the main product considered in AD processes is biogas, consisting of a mixture of methane and carbon dioxide. However, hydrogen gas (H_2) and soluble metabolites, which are intermediate products of this process have a higher added value than biogas, could potentially be extracted prior to their conversion into methane.

The fractionation of substrate composition (carbohydrates, proteins and lipids) by means of pretreatments is a necessary step to improve the efficiency of bioconversion. Suitable pretreatments for to the anaerobic processes can be used to overcome the process limitation of the substrate bioconversion and to promote the modification of the internal structure of the biomass.

The chemical pretreatments with organic solvents seems to be one of the most effective pretreatment to fractionate the organic substrates into its major components. These pretreatments have already been applied to the lignocellulosic fraction (LF) of organic solid waste to improve enzymatic accessibility and to fractionate lignocellulosic biomass in high purity cellulose, lignin and hemicellulose,

but their application to OFMSW is still limited. The OFMSW pretreatment through organic solvent could increase, via substrate fractionation, the highest valorisation of its potential to generate both bioenergy and value-added chemical, so as to ensure the recovery of the main components of OFMSW in a sustainable perspective. The organic solvent is indeed expected to improve the hydrolysis as well as to increase the available substrate-carbon.

The analysis of scientific literature highlighted that formic acid applications represent innovative chemical pretreatment options for OFMSW to be converted into high-quality intermediates by means anaerobic processes. Furthermore, research development has recently pointed out that the wide range of molecules accumulated during anaerobic processes represent new opportunity for recycling waste into added-value molecules, such as short chain carboxylic acids and alcohols.

The research project aimed to study the applicability of formic acid pretreatment in order to propose alternative and suitable uses of pretreated organic waste. The capacity of the operating conditions of the pretreatment to improve the quality of useful components of the substrate were studied in order to assess the technical and economic feasibility of the pretreatment for the anaerobic processes; the production of the building blocks of the value added chemicals was evaluated as well.

The experimental activity was divided in two main steps. In the first experimental phase, various combinations of the different operating conditions of the pretreatment were studied according to a factorial design and the relation between pretreatment effects and organic matter composition was assessed and statistically explored by means of an analysis of variance. This experimental phase was also characterized by the Biochemical Methane Potential (BMP) test of the different solid pretreated substrates as well as the estimation of the soluble metabolic products in order to evaluate the effectiveness of the pretreatment on both the bio-methane production and the conversion of the chemical structure of the soluble biomass.

In the last step of the experimental activity, the combination of the organic solvent pretreatments and the dark fermentation tests of the organic waste was performed in order to promote the simultaneous recovery of valuable streams of biochemicals.

The first phase of research activity were conducted at the Sanitary Environmental Engineering Division (SEED) of Salerno University (Italy). This part of the research was focused on the evaluation of the effect of the formic acid pretreatment on organic waste conversion using differently composed OFMSW substrates. Different operating conditions of the pretreatment were applied to the substrates according to a specific factorial design. The individual and combined effect of the operating parameters on the solubilisation, the biochemical properties and chemical conversion of the pretreated substrates were statistically quantified. Results showed that specific combinations of the operating conditions of the formic acid pretreatment induce the

disintegration of the biomass and the increase of the soluble compounds. These effects result in improved biodegradability and, consequently, in higher bio-methane production from the BMP tests of the pretreated substrates. Conversely, some pretreatment combinations induced a stronger disintegration of the biomass and the formation of by-products which are less biodegradable than the other pretretated substrate. However, these pretreatment combinations involved the production of liquid substrates rich in volatile fatty acid and other metabolites which could be used as building blocks for different kinds of bio-chemical products.

The second phase of the research activity was performed at the Laboratory of the Environmental Biotechnology (LBE) of the National Institute for Agricultural Research (INRA – France).

The aim of the research activity at LBE was to evaluate the feasibility of the combination of the formic acid pretreatment of OFMSW substrates with the dark fermentation tests in order to promote the recovery of carbon sources as well as hydrogen production. Detailed evaluation of the hydrogen production was carried out. The combination of formic acid pretreatments and dark fermentation tests promoted the simultaneous production of the hydrogen and the other biomolecules. Conversely, when the hydrogen production was inhibited by specific pretreatment conditions, the results showed a significant production of the metabolic products like lactate and ethanol which are characterized by a world market as larger as that of hydrogen.

The results of the experimental activity showed that the application of the formic acid pretreatment prior to the anaerobic processes to organic solid waste allow the simultaneous production of the energy and the value added biomolecules carrier.

The research activity proved that the formic acid pretreatment to the organic solid waste represents an innovative and promising method in recovering and adding value to waste, possibly through a multi-product approach of environmental biorefinery.

Sommario

L'utilizzo diretto delle biomasse per diverse applicazioni industriali sta trasformando i rifiuti solidi organici in una delle materie prime più promettenti per la produzione di prodotti biologici caratterizzati da grande rilevanza sul mercato. La bioconversione dei rifiuti organici in prodotti biochimici a valore aggiunto come strategia di gestione dei rifiuti potrebbe essere un metodo perseguibile per l'attuazione dei principi dell'economia circolare. Questo approccio è una sfida importante da un punto di vista tecnologico, economico e ambientale.
La frazione organica dei rifiuti solidi urbani (FORSU) è prodotta globalmente in grandi quantità e la sua disponibilità a basso costo la rende attraente per ridurre l'impatto ambientale derivante dell'utilizzo delle risorse fossili. Uno dei vantaggi più rilevanti nella considerazione della gestione sostenibile dei rifiuti organici è la generazione di prodotti a valore aggiunto da flussi di basso valore generati continuamente. La produzione di differenti prodotti richiede un processo di conversione altamente efficiente dei rifiuti organici. Attualmente, la digestione anaerobica (DA) è uno dei trattamenti maggiormente utilizzati per la biodegradazione intensiva della FORSU ed è considerata una tecnologia ormai consolidata per la produzione di energia.
Ad oggi, il principale prodotto derivante dai processi di DA è il biogas, costituito da una miscela di metano e anidride carbonica. Tuttavia, l'idrogeno gassoso (H_2) ed i metaboliti solubili, che sono prodotti intermedi di tale processo ed hanno un valore aggiunto più elevato del biogas, potrebbero potenzialmente essere estratti prima della loro conversione in metano.
Il frazionamento della composizione del substrato (carboidrati, proteine e lipidi) mediante pretrattamenti è un passo necessario per migliorare l'efficienza della bioconversione. Specifici pretrattamenti prima dei processi anaerobici possono essere utilizzati per superare le limitazioni del processo di bioconversione del substrato e per promuovere la modifica della struttura interna della biomassa.
I pretrattamenti chimici con solventi organici sembrano essere uno dei pretrattamenti più efficaci per frazionare i substrati organici nei suoi componenti principali. Questi pretrattamenti sono già stati applicati alla frazione lignocellulosica (FL) dei rifiuti solidi organici per migliorare l'accessibilità enzimatica e per frazionare la biomassa lignocellulosica in cellulosa di elevata purezza, lignina ed emicellulosa, ma la loro

applicazione alla FORSU è ancora limitata. Il pretrattamento della FORSU attraverso un solvente organico potrebbe aumentare, mediante il frazionamento del substrato, la massima valorizzazione del suo potenziale per generare sia bioenergia sia sostanze chimiche a valore aggiunto, in modo da garantire il recupero dei componenti principali del substrato in una prospettiva sostenibile. Il solvente organico dovrebbe migliorare l'idrolisi e aumentare il substrato a base di carbonio disponibile.

L'analisi della letteratura scientifica ha evidenziato che le applicazioni con acido formico rappresentano opzioni innovative di pretrattamento chimico della FORSU in grado di convertire il substrato in prodotti chimici intermedi di elevata qualità mediante processi anaerobici. Inoltre, lo sviluppo della ricerca ha recentemente sottolineato che l'ampia gamma di molecole accumulate durante i processi anaerobici rappresenta una nuova opportunità per riciclare i rifiuti in molecole a valore aggiunto, come gli acidi carbossilici a catena corta e gli alcooli.

Il progetto di ricerca ha avuto l'obiettivo di studiare l'applicabilità del pretrattamento di acido formico al fine di proporre usi alternativi e idonei dei rifiuti organici pretrattati. È stata studiata la capacità delle condizioni operative del pretrattamento di migliorare la qualità dei componenti utili del substrato, al fine di valutare la fattibilità tecnica ed economica del pretrattamento per i processi anaerobici. Inoltre, è stata valutata la produzione degli elementi costitutivi dei prodotti chimici a valore aggiunto.

L'attività sperimentale è stata suddivisa in due fasi principali. Nella prima fase sperimentale, sono state studiate, secondo un disegno fattoriale, differenti combinazioni delle diverse condizioni operative del pretrattamento ed è stata valutata e analizzata statisticamente mediante un'analisi della varianza la relazione tra gli effetti del pretrattamento e la composizione della materia organica. Questa fase sperimentale è stata anche caratterizzata dall'implementazione di test per valutare il potenziale metanigeno (BMP test) dei diversi substrati pretrattati nonché dalla stima dei prodotti metabolici solubili al fine di valutare l'efficacia del pretrattamento, in riferimento sia alla produzione di biometano sia alla conversione della struttura chimica della biomassa solubile.

Nell'ultima fase dell'attività sperimentale, è stata implementata la combinazione del pretrattamento con acido formico e test di fermentazione dei rifiuti organici, al fine di promuovere il simultaneo recupero di differenti flussi a valore aggiunto di sostanze biochimiche.

La prima fase dell'attività di ricerca è stata condotta presso il Laboratorio di Ingegneria Sanitaria Ambientale (SEED) dell'Università degli Studi di Salerno (Italia). Questa parte della ricerca si è concentrata sulla valutazione dell'effetto del pretrattamento dell'acido formico sulla conversione dei rifiuti organici utilizzando

substrati di FORSU di diversa composizione. Diverse condizioni operative del pretrattamento sono state applicate ai substrati secondo uno specifico disegno fattoriale. L'effetto individuale e combinato dei parametri operativi sulla solubilizzazione, le proprietà biochimiche e la conversione chimica dei substrati pretrattati sono stati quantificati statisticamente. I risultati hanno mostrato che combinazioni specifiche delle condizioni operative del pretrattamento con acido formico inducono la disintegrazione della biomassa e l'aumento dei composti solubili. Questi effetti si traducono in una migliore biodegradabilità e, di conseguenza, in una maggiore produzione di biometano dai BMP test dei substrati pretrattati. Al contrario, alcune combinazioni di pretrattamento hanno indotto una più forte disintegrazione della biomassa e la formazione di sottoprodotti che sono meno biodegradabili rispetto al substrato pretrattato. Tuttavia, queste combinazioni di pretrattamento prevedevano la produzione di substrati liquidi ricchi di acidi grassi volatili e di altri metaboliti che potrebbero essere utilizzati come base per la produzione per diversi tipi di prodotti biochimici.

La seconda fase dell'attività di ricerca è stata condotta presso il Laboratorio di Biotecnologia Ambientale (LBE) dell'Istituto Nazionale di Ricerca Agronomica (INRA - Francia).

Lo scopo dell'attività di ricerca presso l'LBE è stato quello di valutare la fattibilità della combinazione del pretrattamento con acido formico dei substrati di FORSU con i test di fermentazione al fine di promuovere il recupero di fonti di carbonio e la produzione di idrogeno. È stata, inoltre, effettuata una valutazione dettagliata della produzione di idrogeno. La combinazione del pretrattamento con acido formico ed i test di fermentazione ha promosso la produzione simultanea dell'idrogeno e delle altre biomolecole. Al contrario, quando la produzione di idrogeno è stata inibita da specifiche condizioni di pretrattamento, i risultati hanno mostrato una produzione significativa dei prodotti metabolici come l'acido lattico e l'etanolo, i quali sono caratterizzati da un mercato mondiale attuale più vasto rispetto di quello dell'idrogeno.

I risultati dell'attività sperimentale hanno mostrato che l'applicazione del pretrattamento con acido formico prima dei processi anaerobici ai rifiuti solidi organici consente la produzione simultanea di energia e biomolecole a valore aggiunto.

L'attività di ricerca ha dimostrato che il pretrattamento con acido formico ai rifiuti solidi organici rappresenta un metodo innovativo e promettente per recuperare e aggiungere valore ai rifiuti, possibilmente attraverso un approccio multiproduttivo di bioraffineria ambientale.

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This thesis has come to an end, even if I still do not believe it!
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About the author

Anna Conte got with honours her Master Degree in Environmental Engineering at University of Salerno in 2014, defending a research thesis entitled “Application of electrokinetic processes to membrane bioreactors (MBRs) for wastewater treatment”. In 2015 she was admitted to the PhD course in Environmental and Civil Engineering at the Department of Civil Engineering of Salerno University. Since then, her research activity has been mainly focusing on the study of the treatment strategies of organic solid waste in order to enhance its valorisation.
She spent 5 months at the Laboratory of the Environmental Biotechnology (LBE) of the National Institute for Agricultural Research (INRA, France) as visiting researcher. She is currently involved in the research and consulting activities promoted by the Sanitary Environmental Engineering Division (SEED) of Salerno University in the field of Environmental Engineering.

Anna Conte ha conseguito con lode la laurea in Ingegneria per l’Ambiente ed il Territorio presso l'Università degli Studi di Salerno nel 2014, discutendo una tesi sperimentale dal titolo "Applicazione dei processi elettrocinetici ai bioreattori a membrana (MBR) per il trattamento delle acque reflue". Nel 2015 è stata ammessa al corso di Dottorato di Ricerca in Ingegneria Civile e Ambientale presso il Dipartimento di Ingegneria Civile dell'Università degli Studi di Salerno. Da allora, la sua attività di ricerca è focalizzata principalmente sulle strategie di trattamento dei rifiuti solidi organici per migliorare la loro valorizzazione.
Ha trascorso 5 mesi presso il Laboratorio di Biotecnologia Ambientale (LBE) dell'Istituto Nazionale per la Ricerca Agronomica (INRA - Francia) come ricercatore ospite. Attualmente è impegnata nelle attività di ricerca e consulenza promosse dal Laboratorio di Ingegneria Sanitaria Ambientale (SEED) dell'Università di Salerno nel campo dell'ingegneria ambientale.

1. Introduction

The spread of the direct biomass utilization in the industrial sectors is turning the organic solid waste into one of the most promising feedstocks for the implementation of the biorefinery concept, referred to the conversion of biomass in a wide spectrum of competitive bio-based products with obvious market significance (Marques et al., 2018; Yang et al., 2015). The potential of plant-based raw materials in replacing a large fraction of fossil resources for the production of energy carriers, chemicals as well as materials is well-established. In recent years research has mostly focused on agricultural crops, lignocellulosic biomasses and algae. However, organic solid waste is raising as a further biomass which can be used in the biorefinery facilities to produce multiple value-added target products (Yang et al., 2015). Such approach may also stand as a novel waste management strategy, perfectly fitting the circular economy principles via the organic waste to bio-chemicals supply chain (Dahiya et al., 2018a; Kaur et al., 2018).

The organic fraction of municipal solid waste (OFMSW) globally produced in large amounts as well as continuously and its availability at low cost makes it attractive to reduce the negative environmental impact of the use of fossil resources (Wainaina et al., 2018). The organic waste is indeed a mixture of carbohydrates, proteins and fats (Dessie et al., 2018; Panda et al., 2016), whose basic components, namely sugars, proteins, amino-acids and fatty acids, can be conveniently turned into a number of value added products, including ethanol, lactic acid, polylactic acid (PLA), polyhydroxyalkanoates (PHAs), succinic acid, 1,4-butanediol (BDO), farnesene, isobutene, acrylic acid, adipic acid, ethylene and polyethylene (Marques et al., 2018; Moscoviz et al., 2018). Nevertheless, such approach poses an important challenge from the technological, economic and environmental perspectives (Gonzalez-Garcia et al., 2018), related to the need to identify a highly efficient and sustainable conversion process of the organic waste.

This kind of waste has been traditionally handled via biological processes and the anaerobic digestion (AD) is currently one of the preferred treatments for the intensive biodegradation of the organic fraction of municipal solid waste (OFMSW). It is considered an up to date waste to energy technology, ending in the production of a biogas mainly composed of methane which is obtained from a stand-alone methanization process or by means of a two-stage process, with a dark fermentation

(DF) stage for the additional production of hydrogen (Mata-Alvarez et al., 2000). The latter case allow to overcome economic and energetic limitation of the process (Song et al., 2010). Currently, the AD of OFMSW for VFAs production has attracted increasing interests than its use for the generation of energy carriers (Tuck et al., 2012; Zhou et al., 2018) due to the high added value of the products. This approach of organic waste management could be even more important if targeting emerging chemicals characterized by growing market potential (Chen et al., 2017b).

The hydrogen is a promising carbon-free clean fuel (Ghimire et al., 2015a), that can be used either directly in combustion engines (Meherkotay and Das, 2008) or to produce electricity via fuel cell systems (Alves et al., 2013).

During DF the production of hydrogen comes along with that of accumulation intermediate products, namely different soluble metabolites (volatile fatty acids, carboxylic acids and alchools) with growing market potential (Chen et al., 2017b; Tuck et al., 2012; Zhou et al., 2018).

The enhanced generation of VFAs is of particular interest (Calt, 2015; Garcia-Aguirre et al., 2017a; Zhou et al., 2018), thanks to the wide range of applications that these metabolic products hold in the cosmetic, pharmaceutical and food industry (Zacharof and Lovitt, 2013), in the production of solvent (Agler et al., 2011), in the generation of energy from microbial fuel cells (Cavdar et al., 2011), in the production of biodegradable polymer (PHA) (Lee et al., 2014; Shen et al., 2017) as well as in the nutrient removal from wastewater.

In this context, the fractionation of the feedstock for anaerobic processes is a necessary step to improve the efficiency of the bioconversion, so that recent advances in research are being directed towards their integration with suitable pretreatments. Chemical, physical and biological methods, as well as their adequate combination, can be used to provide the treatment of the organic waste destined to anaerobic digestion (Ariunbaatar et al., 2014; Carrère et al., 2010; Cesaro and Belgiorno, 2014; Karthikeyan et al., 2017).

Among chemical pretreatments, acids have been applied to release the nutrients locked up in organic waste and other lignocellulose materials for subsequent fermentation (Dessie et al., 2018; Leung et al., 2012). Extensive research has been conducted on the use of acids to break down the lignin and hemicelluloses network and to distrupt the crystalline cellulose structure, in order to promote the bioconversion of lignocellulosic materials for both biofuel and biochemical production (Zhang et al., 2016).

Currently, both the dilute acid and the alkaline pretreatments are believed as the most mature technologies for commercialized application (Alvira et al., 2010; Lee et al., 2014), having shown high effectiveness on several agricultural residues (Dagnino et al., 2013). The use of organic solvents, in particular, has been studied for the

production of high-quality intermediates and it was found to be highly efficient for the biomass fractionation and easy to recover by means of distillation processes (Zhang et al., 2016). Among the available organic solvents, the formic acid has shown a great potential as active agent for the dissociation of hydrogen ions (Wang et al., 2018), the acceleration of biomass hydrolysis (Ding et al., 2018; Özyürek and van Heiningen, 2018) and the improvement of the carboxyl content (Dai et al., 2014). It is the simplest carboxylic acid and compared to other organic molecules, the temperature for its decomposition is lower, resulting in less CO toxicant species, so that it is regarded as an effective pretreatment option, able to increase the surface area of the substrate and to solubilize it to produce value-added pulps (Sindhu et al., 2010; Zhao et al., 2017).
Most of the research works have been carried out to assess the performances of the formic acid pretreatment of lignocellulosic substrates, whereas its potential for the processing of the OFMSW destined to anaerobic processes has not been explored yet.

1.1 Objectives

Aim of the research project was in the study of the applicability of formic acid pretreatment in order to propose alternative and suitable uses of pretreated organic waste. The effects of the operating conditions of the pretreatment on the quality of useful components of the substrate were studied in order to assess the technical and economic feasibility of the pretreatment for the anaerobic process; the production of the value added biochemicals and their potential reuse was evaluated as well.
Therefore, formic acid processing was chosen as OFMSW treatment option and investigated in order to compare its effectiveness on anaerobic digestion and dark fermentation yields as well as to evaluate the technical and economic feasibility of the combined pretreatment and anaerobic process providing the best valorization strategies of the pretreated samples in a biorefinery context.
The main objective of this research was to study the process parameters influencing the organic solvent pretreatment of the organic solid waste and the valorization of the by-products (bio-energy and bio-chemicals) in a biorefinery framework. Research activity was structured in two main steps:

- the study of suitable chemical pretreatment and the exploration of the effects of different operating conditions via variance analysis; the estimation of bio-methane or bio-hydrogen production from pretreated substrates by means anaerobic processes;

- the evaluation of the production of value added bio-chemical intermediates produced from substrates treatment.

To achieve these major aims, the experimental activity was structured in two main steps:

- the study of various combinations of the different operating conditions of the pretreatment according to a factorial design. The evaluation of the statistical significance of the operating condition by means of the analysis of variance was conducted; the effects of the pretreatment combinations on the organic matter composition of the substrates were evaluated; the Biochemical Methane Potential (BMP) tests was also implemented on the pretreated substrates in order to estimate the bio-methane production;
- the evaluation of the effects of the pretreatment on Biochemical Hydrogen Production (BHP) yields in terms of hydrogen (H_2) and soluble metabolites production.

The objectives of the first step, performed at the Sanitary Environmental Engineering Division (SEED) of Salerno University, were:

- the identification of the chemical pretreatment most suitable for the treatment of organic solid waste;
- the definition of the operating conditions of the pretreatment which result statistically significant on the physicochemical characteristics of the pretreated substrates;
- the assessment of pretreatment effectiveness, under the previously identified operating conditions, according to the characterization of the pretreated substrates;
- the evaluation of the pretreatment application in improving anaerobic biodegradability of the treated samples.

The second part of the research focused on the evaluation of the production of value added bio-chemical intermediates produced from the treatment of the waste substrates and it was performed at the Laboratory of the Environmental Biotechnology (LBE) of the National Institute for Agricultural Research (INRA – France).

Main aim of the second phase of the research was the assessment of the feasibility of formic acid pretreatment for OFMSW dark fermentation. To this end, the experimental activity pursued the following objectives:

- the implementation of dark fermentation tests on the pretreated substrate, to estimate the H_2 production;

- the evaluation of the efficiency of the treatment by means of Gompertz parametrization;
- the evaluation of the theoretical and experimental H_2 production;
- the detailed estimation and evaluation of the metabolites pathways for each pretreatment combination.

1.2 Outlines

The thesis is divided in eight chapters. The integration of a biorefinery approach in the management system of the organic solid waste was the argument of the Chapter 2. The possible utilization of organic waste in biotechnological processes for the production of value added chemicals and fuels as an application of the circular economy concept, linked to the bio-based economy was described.
Anaerobic processes, such as anaerobic digestion and dark fermentation, the chemical relationships, the main factors affecting its yields and the application of this process in a biorefinery context is reported in Chapter 3.
Main physical, biological and chemical pretreatments were overviewed in the fourth chapter, in order to define main advantages and drawbacks as well as to highlight the current status of their application on solid substrates, with particular reference to OFMSW. Organic solvent pretreatments were analyzed in detail.
In the Chapter 5, the research plan was illustrated with reference to the experimental activity which can be divided in two main steps:

- the study of the pretreatment and the implementation of Biochemical Methane Potential (BMP) tests on the pretreated substrates in order to estimate the biomethane production;
- the evaluation of the effects of the pretreatment on DF yields in terms of hydrogen and soluble metabolites production.

Chapter 6 shows and discussed the results of each phase of the experimental activity. Concluding remarks and future perspectives are presented in the Chapter 7.

2. Organic waste-based biorefinery

The biorefinery systems efficiently convert biomass into a spectrum of biobased products (feed, chemicals and minerals) and bioenergy (biofuels, power and/or heat) by means of efficient and zero-waste processes (IEA Bioenergy, 2014).
Policy and industrial sector together promote biorefineries, which could more efficiently convert renewable biomass, non-food material and biowaste. The conversion of this biomass would yield diverse products plus energy, thus substituting fossil fuels.
The integration of a biorefinery approach in the management system of the waste allows to promote a production process of the added-value products from renewable waste more sustainable an economic and environmental perspective due to the reducing of the resources consumption and waste production.

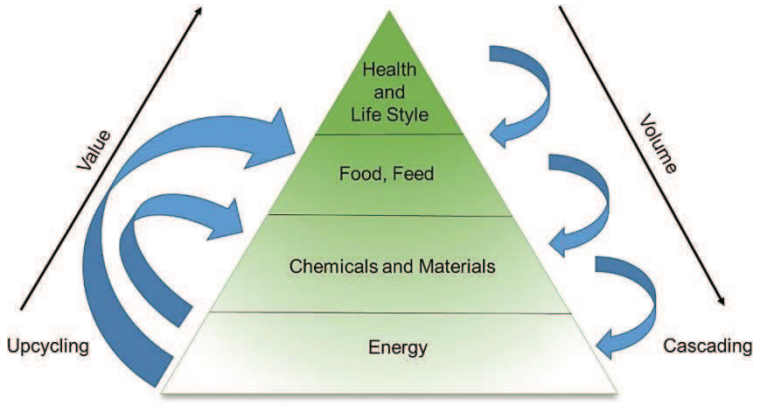


Figure 2-1: Cascading and upcycling in the biorefinery product pyramid (Hagman et al., 2018)

The utilization of the organic waste in this contest by means of biotechnological processes for the production of value added chemicals and fuels represents an application of the recent circular economy concept (Nizami et al., 2017; Pleissner et al., 2015). The circular economy promote the "reduce-reuse-recycle" approach, closing the loop of product life cycles (Nizami et al., 2017) and it is linked to the biobased economy (Peinemann and Pleissner, 2018).

A non-conventional upcycling in the biorefinery system is carried out because lower valued biomass moves to higher values. Instead, the traditional cascading pyramid of the biorefinery showed, for each step of the production system, a value decreasing with a volume increasing. The traditional model was represented by downward directionality, while incorporating upcycling into the scheme the model become bidirectional. Thus, it provides a scheme in which processes like biogas solutions contribute value and support the biorefinery systems (Figure 2-1).

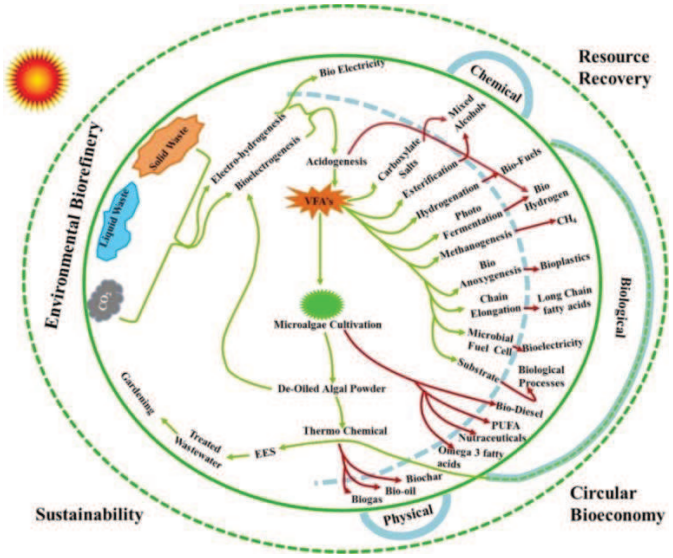


Figure 2-2: Holistic biorefinery approach with hybrid-integrated strategies for multi-purpose applications (Venkata Mohan et al., 2016)

The development of the biorefinery of biomass-derived waste is still evolving. The support of the policy instrument to the stimulation of the biorefineries also for organic waste plays a significant role (Jin et al., 2018).
Currently, the policy instruments stimulate the development of the biorefineries by means of the carbon pricing and mandatory quota. These strategies protect a space for renewable chemicals and biofuels which cannot compete with the fossil-derived products. Some European country have carbon tax exemptions for biofuels produced from renewable resources, such as food waste (Hellsmark and Söderholm, 2017; Jin et al., 2018).
The Environment Protection Agency sets annual quotas indicating the percentage of renewable fuels blended into fossil fuels (EPA, 2018). However, the current policies are not able to stimulate the commercialization of large-scale advanced biorefineries.

A biobased economy development seem to be one of the current priorities in Europe. The interest in the use of biomasses for the production of food and feed, but also for fuel and biochemicals to substitute fossil resources is growing (Santamaría-Fernández et al., 2018). The green biorefinery aims, especially, at utilizing green biomass to produce valuable products such as fibres, proteins, amino acids, lactic acid or energy such as bioethanol, biomethane and biohydrogen.
In recent decades the research has focused on agricultural crops, lignocellulosic biomasses and algae, which are defined as the first, the second and the third generation biomass for the conversion, respectively.

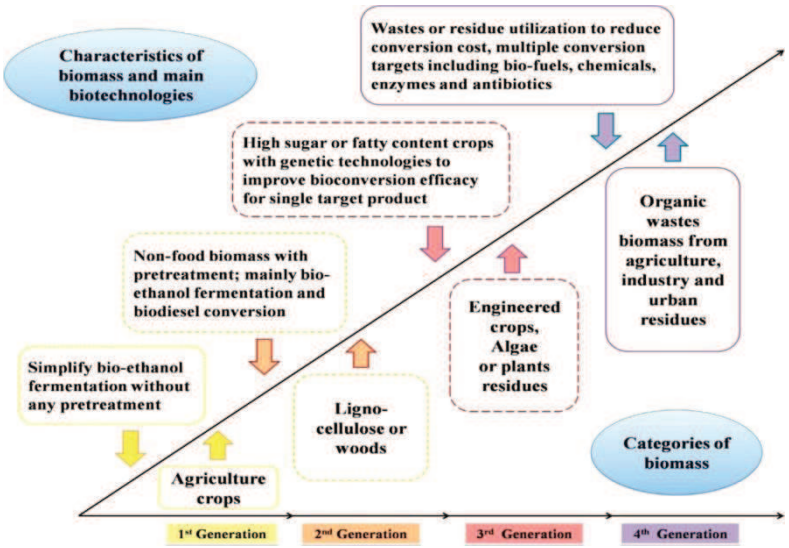


Figure 2-3: Different generation of biomasses (Yang et al., 2015)

Crops resources such as corn sugar and vegetable oil are the main components of the first generation of biomass (Cherubini, 2010; Moncada et al., 2014; Sánchez and Cardona, 2008). The second generation uses non-food materials, such as agricultural residues, wood and energy crops, typically high in lignocellulose (Guo et al., 2015; Qureshi et al., 2010). Biorefinery systems that uses algae and plants residues as feedstock have been referred to as third generation biorefineries (Jonker and Faaij, 2013; Parajuli et al., 2015).
These feedstock generations have been used as potential precursors of platforms to obtain fuels and chemicals (Cherubini, 2010; Moncada et al., 2014).
These type of biorefineries are still under improvement due to technical and economic developments (Gerssen-Gondelach et al., 2014).

The high content of sugarcane of the main king of first generation biomass promoted bioethanol and biodiesel production (Yang et al., 2015). These first generation biofuels have negatively impacted the food-versus-fuel debate, which was characterised by the rising the food prices in order to produce the first generation biofuels. Environmental impacts were also concerned because the biodiesel production did not include cost efficient technologies for the emission abatement (Naik et al., 2010).

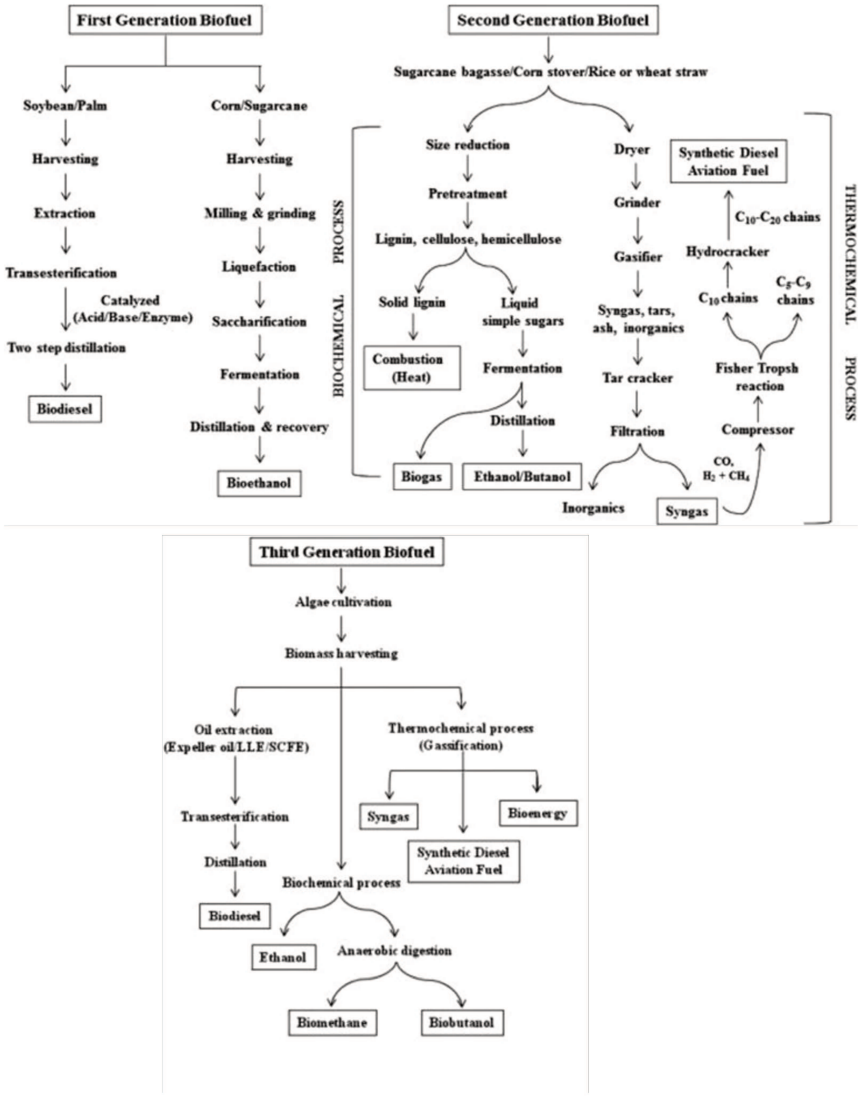


Figure 2-4: Technologies involved for production of first, second and third generation of biofuel (Dutta et al., 2014)

The second generation biomass has more processing flexibilities than the first one. The lignocellulosic feedstocks are showed to cost less and be more readily available. Typical advanced biofuels from lignocellulosic materials of the second generation biomass are bioethanol, which can be obtained through hydrolysis and fermentation, and biodiesel, which is produced through gasification (Carriquiry et al., 2011; Naik et al., 2010).

The algae, which are characterized by a great lipid content, are considered to be a feasible alternative renewable energy resources for the production of the third generation of biofuels. The biochemical conversion of microalgae provides different renewable biofuels, like biodiesel, methane and biohydrogen, overcoming the inconvenient of first and second generation biofuels (Alam et al., 2015; Yang et al., 2015). However, the production of microalgal biomass is generally most technologically challenging as well as more expensive than growing crops (Nigam and Singh, 2011).

The use of biomasses in industrial sectors has turning the organic solid waste into one of the most promising feedstocks for the biocoversion in order to produce competitive bio-based products with an increasing market interest (Marques et al., 2018; Yang et al., 2015). The organic waste to bio-chemicals supply chain as waste management option should be a viable method towards an effective implementation of the circular economy approach (Dahiya et al., 2018a; Kaur et al., 2018).

A fourth generation biomass could be represented by the organic solid waste from agriculture, industry and urban residues, which can be applied in the biorefinery biological and biotechnological plants in order to increase its additional value producing multiple target products (Coma et al., 2017a; Dahiya et al., 2018a; Yang et al., 2015).

The organic solid waste is globally produced in large amounts and its low cost availability makes it attractive to reduce the negative environmental impact of the fossil resources (Wainaina et al., 2018). Biofuels, biomass, biofertilizers, biochemical can be obtained from biotechonological processes, such as anaerobic digestion, fermentation and composting (Hafid et al., 2017; Skaggs et al., 2018). Recovery of high value-added components and their re-utilization is another aspect of the biorefinery concept (Nayak and Bhushan, 2019; Skaggs et al., 2018)

The organic waste is a mixture of different compounds as sugars, proteins, amino-acids and fatty acids which are already produced from non-renewable resources. A wide range of valuable products, such as biofuels, organic acids, biopolymers, biomolecules for pharmaceutical industries, can be obtained by the conversion of organic wastes. Examples of new biochemical platform products from organic solid waste are ethanol, lactic acid, and polylactic acid (PLA), polyhydroxyalkanoates (PHAs), succinic acid, 1,4-butanediol (BDO), farnesene, isobutene, acrylic acid,

adipic acid, ethylene, and polyethylene (Marques et al., 2018; Nayak and Bhushan, 2019).

The carbohydrates (simple sugars and polysaccharides) of the organic waste represent the main carbon contribution to the production of methane, hydrogen, bioethanol, volatile fatty acids, alcohols and organic acids (Alibardi and Cossu, 2016; Carmona-Cabello et al., 2018; Li et al., 2017). The high biodegradability and great consumption in metabolic pathways of monosaccharides and disaccharides make them suitable carbon-rich substrates for the fermentation process (Cherubini, 2010; Sanchez et al., 2016).

The protein-rich organic waste is an extremely valuable substrate of anaerobic digestion because the proteins provide nitrogen for bioprocesses. Proteins are first decomposed to amino acids through hydrolysis, and then degraded in single amino acids in the presence of microbes which use hydrogen (Bong et al., 2018; Yong et al., 2015).

High contents of lipids in the substrates promote a great methane production during anaerobic process (Wu et al., 2018).

However, as to the integrated biorefinery of organic biomass to produce various value-added chemicals and biofuels in a cascade way, although often discussed, have not yet been put into implementation (Kumar et al., 2018).

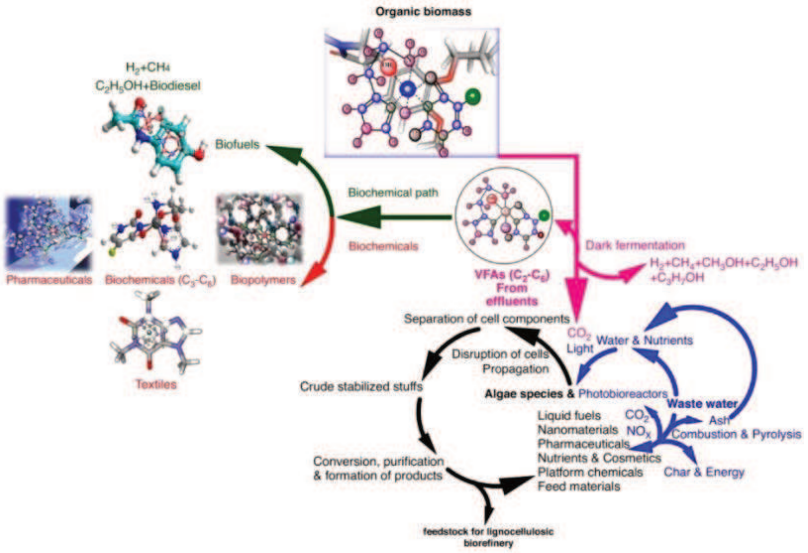


Figure 2-5: Depiction of a possible circular biorefinery design of residual biomasses to value-added chemicals, food and feed ingredients (Kumar et al., 2018)

A circular economy approach in the biorefinery contest for the organic biomass was proposed by Kumar et al. (2018).
In order to define suitable circular economy biorefinery schemes which can be generally applied, it is necessary to define platforms, products, feedstocks, and processes to be included in this context.
Several technologies can be employed in order to upgrade and valorize the organic solid waste according to a biorefinery approach. These biotechnological processes are mechanical/physical (e.g., pressing, pretreatment, milling, separation, distillation), biochemical (e.g., anaerobic digestion, aerobic and anaerobic fermentation, enzymatic conversion), chemical (e.g., hydrolysis, transesterification, hydrogenation, oxidation), and thermochemical (e.g., pyrolysis, gasification, hydrothermal upgrading, combustion) processes.
The organic solid waste can be used as a microbial feedstock for the production of many valuable bioproducts, such as methane, hydrogen, ethanol, biopolymers and bioplastics. The organic solid waste can also be used to produce high-value biochemical and biomaterials such as organic acids, biodegradable plastics and enzymes. However, the market demand for these biochemicals is still smaller than that for biofuel (Deng, 2016).

2.1 Bio-energy carriers

The implemented policies of the governments stimulate the use of bioenergy from biomass resources (Clancy et al., 2018) in order to meet carbon reduction or supply chain optimisation goals. The energy production is undergoing changes in order to reduce the gas emissions (European Commission, 2016). The Europe has set itself a long-term goal of reducing greenhouse gas emissions by 80-95%, when compared to 1990 levels, by 2050. Low-carbon technologies, renewable energy and energy efficiency are some of the significant investments in order to achieve the European objectives (Energy Roadmap 2050 - COM/2011/885).
The production of the biofuels from renewable feedstocks is increasing in order to meet the energy demand, replacing the petrol fuels which are becoming scarce and more expensive.
The main biorefinery concept was the production of biofuels (biodiesel and bioethanol) from biomass resources, and also the process effluents were utilized in order to produce additional biofuels (biohydrogen and methane) to improve the overall bioenergy recovery efficiency (Luo et al., 2011).

2.1.1 Biodiesel

Biodiesel is an alternative fuel to petroleum diesel that reduces net greenhouse effects and its use has become mandatory in many countries (Deng, 2016).
Biodiesel can be produced through the transesterification reaction of triglycerides present in vegetables oils and animal fats with a short chain alcohol (i.e., methanol or ethanol) in the presence of a catalyst with glycerol as coproduct (Yasvanthrajan et al., 2018). Biodiesel is biodegradable and non-toxic, has a carbon monoxide-free and aromatic-free emission, and represents a means of recycling spent oils and fats (Siles et al., 2010).
Different technologies, which differ from one another for catalyst and conditions of transesterification process (Table 2-1), were used to produce biodiesel.

Table 2-1: Different operating conditions of transesterification process (Leung et al., 2010)

Parameter	Transesterification technology				
	Alkaline-catalyzed	Acid-catalyzed	Enzyme-catalyzed	Ultrasonic irradiation	Supercritical
Reaction temperature (°C)	60 - 70	55 - 80	30 – 40	10 – 60	300 – 400
Reaction time (min)	60	240	480 - 4,200	30 - 600	2 – 4
Reaction pressure (MPa)	0.14 - 0.41	0.4	0.1	0.1	8 – 25
Type of catalyst	NaOH	H_2SO_4	Lipase	H_2SO_4–NaOH	None
Methyl esters yield	>95%	>97%	95%	>93 %	98%

The cost of biodiesel is an important issue and its commercial production requires low energy and minimum downstream steps.
According to the concept of biorefinery, through the integration of different processes, apart from the production of the biodiesel, other value-added products such as ethanol, xylitol, synthesis gas, butanol, biogas, and furfural, among others, could also be obtained (Almeida et al., 2012).

2.1.2 Bioethanol

Ethanol is the main biobased molecule used as a biofuels. In the last ten years great market growth occurred in Europe, Brazil and United States, especially. The world

market of the ethanol is of 76700 kt year^{-1} in 2015 using maize and sugarcane as raw materials. The 59% of this market is represented by United States and the 27 % by Brazil. The price of ethanol in the world market range between 0.30 and 1.50 € kg^{-1} depending on the quality of the product and on the raw materials as well. The policy instruments support the development of the ethanol market through subsidies (Moscoviz et al., 2018).

Ethanol has also several industrial applications (e.g., in detergents, toiletries, coatings, and pharmaceuticals) and has been used as transportation fuel for more than a century. Ethanol can be produced synthetically from oil and natural gas, or biologically from sugar, starch, and lignocellulosic materials. The biologically produced ethanol is sometimes called fermentative ethanol or bioethanol (Karimi and Taherzadeh, 2016).

Bioethanol is considered one of the best biofuels from alternative source, like lignocellulosic biomass, which holds remarkable potential to meet the energy demand. It is renewable and have a low environmental impact (Deng, 2016). It is economically feasible to produce biobased plastics through bioethylene, which is a raw material useful for the production of polyethylene and other plastic products, characterized by a market demand of more than 140 million tonnes per year. Therefore, the production of bioethanol from cheap feedstocks is having much research attention (Lundgren and Hjertberg, 2010).

Different kinds of agricultural raw materials, such as sugars, starch, and cellulose, are considered suitable for ethanol production. Traditionally, bioethanol has been produced from starch-rich crops such as potato, rice, and sugar cane (Saini et al., 2015).

The process of bioethanol production generally involves direct fermentation of sugars, or other carbohydrates that can be converted to sugar, such as starch and cellulose. Some sugars can be converted directly to ethanol, whereas starch and cellulose must first be hydrolyzed to sugar before conversion to ethanol.

Hydrolysis breaks down the hydrogen bonds in the hemicelluloses and cellulose fractions into their sugar components: pentoses and hexoses. These sugars can then be fermented into ethanol.

To produce ethanol from cellulosic biomass, a pretreatment process is used to reduce the sample size, break down the hemicellulose to sugars and to cellulose component. Pretreatment methods refer to the solubilisation and separation of one or more of the four major components of biomass to make the remaining solid biomass more accessible to further chemical or biological treatment.

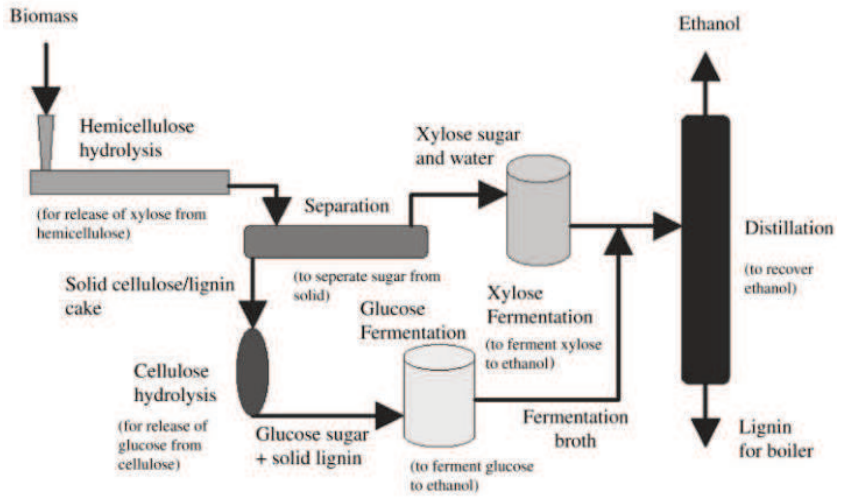


Figure 2-6: Ethanol from cellulosic biomass materials (Demirbaş, 2004)

2.1.3 Biomethane

Biogas and biomethane represent renewable fuels of significant merit. Biogas and biomethane systems predominantly focus on second generation biofuel substrates. However, the main renewable residues used for biogas production (Coma et al., 2017a; Yong et al., 2015) are organic wastes (municipal waste, wastewater sludge, swine manure, cow manure and other related residues), energy crops (sunflower, rape, jatropha, cardoon, etc.), agricultural residues (banana stem, barley straw, rice straw, softwood spruce, etc.), agricultural crops (maize, wheat, barley, sweet sorghum, etc.) and non-conventional feedstocks (glycerol, microalgae, etc.)

Biogas, which consist of 50–70% methane and 30–50% carbon dioxide, is generated from anaerobic digestion; it can also be upgraded to biomethane (> 97% methane) by means of processes which remove H_2S and other biogas impurities such as CO_2, ammonia, moisture and particulates.

Pretreatments are used to facilitate the biomethane production by overcoming the limitation of hydrolysis process, which includes the solubilisation and biodegradation of the substrates (Carrere et al., 2016; Cesaro and Belgiorno, 2014; Zheng et al., 2014).

In many European countries, biogas is directly used for the production of electricity and heat. It is utilized as vehicle fuel providing a substitute for fossil-natural gas (Persson et al.; Sárvári Horváth et al., 2016). Upgraded biomethane could be used as

transportation fuel in the form of compressed natural gas (CNG) or liquid natural gas (LNG) or injected into a natural gas grid.
The main end-use of biomethane is a transport fuel as a consequence of the financial incentives available and limited natural gas grid coverage (IEA Bioenergy, 2014). Biomethane can supply renewable heat to large industry energy users and feed high efficiency combined heat and power (CHP) units. To date, biogas for electricity has been the predominant energy output from AD; by the end of 2015 there were 17376 biogas plants in Europe producing 60.6 TWh (European Biogas Association, 2016). However, as biomethane can be injected to the gas grid and/or used as a transport fuel, it can be viewed as a more flexible energy carrier than biogas-CHP achieving higher final energy output efficiencies (SEAI, 2017). As of 2015, there were 459 biomethane plants in Europe, with significant growth in the UK in particular where 43 new plants built were built (European Biogas Association, 2016).
The biomethane gas mixture has a high calorific value of 17- 25 MJ/m^3, which can be burnt to release heat energy or converted to electricity using internal combustion engines (Zhang et al., 2018).
The largest market for the use of biomethane as a transport fuel is the European Union, with a combined 160 million m^3 of biomethane in 2015 (Scarlat et al., 2018). Biomethane production greatly increased since 2011 (Figure 2-7). In 2016, biomethane production in Europe increased of over 40% and the current growth is therefore demonstrably rapid.

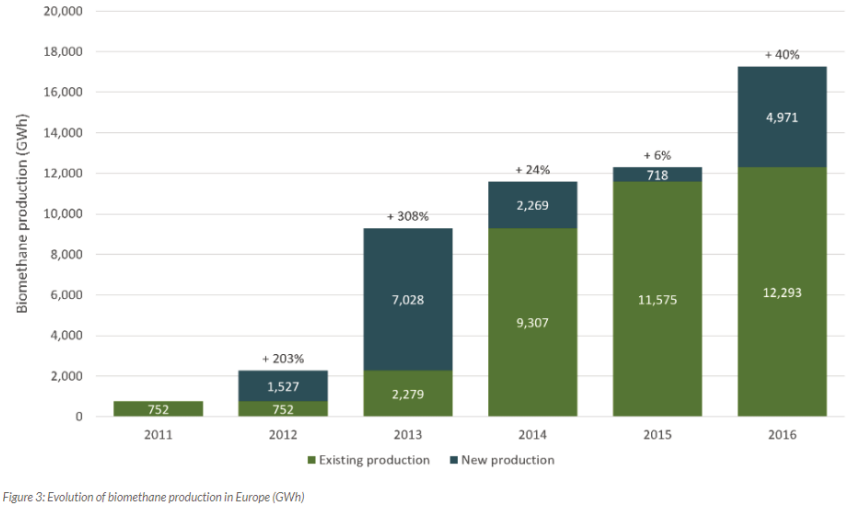


Figure 2-7: Evolution of biomethane production in Europe (EBA Statistical Report 2017)

A reported by the Transparency Market Research forecasts the global biomethane market to reach a valuation of US$ 2624.5 million by 2025 is increasing from US$ 1485.4 million in 2016, at a compound annual growth rate (CAGR) of 6.7% between 2017 and 2025.
In Europe, strategies and actions were developed to be undertaken at national level and to achieve the EU target by 2030. In 2018 it was promoted the use of biomethane and many incentives to renewable sources for power production have been disclosed as well.

2.1.4 Biohydrogen

Hydrogen (H_2) production from biomass is employed due to it is clean, sustainable and renewable. Hydrogen will be an important energy carrier in the near future.
According to diverse sources of hydrogen, different technologies have been developed to produce hydrogen gas: water electrolysis, thermo-chemical hydrogen production, and biological hydrogen production.
The biological processes used for hydrogen production are biophotolysis of water using algae and cyanobacteria, photo-fermentation of organic materials by photosynthetic bacteria, and dark fermentation of organic materials using fermentative bacteria.
In the environmental engineering context, dark fermentation is of great interest in order to stabilize organic waste as well as to produce a clean and sustainable energy carrier (den Boer et al., 2016; Venkata Mohan et al., 2016a).
The dark fermentation is the biological process in which the organic compounds are converted into by-products, including H_2 as well as volatile fatty acids (VFA) (Sarkar et al., 2016). Mixed culture acidogenic fermentation involves the synergistic and competitive interactions of a wide diversity of microbial groups.
Hydrogen production through dark fermentation has many advantages compared to other biological hydrogen production methods because of its ability to continuously produce hydrogen from renewable sources, such as carbohydrate-rich wastes, without an input of an external energy (Deng, 2016). An appropriate inoculum selection and a pretreatment of this inoculum significantly influences the metabolic function during the acidogenic process efficiency. The role of the photosynthetic bacteria of the algae was studied (Chandra et al., 2012). The use of the microalgae ant the methane oxidizing bacteria were used in two stage production of value added biochemicals. However, the large scale of the application of the algae has still many limitations.

Microbial electrolysis cell (MEC), through electro-hydro-genesis process, is used for biogas (biomethane or biohydrogen) production from a wide range of wastewaters and other renewable resources (fermented effluents viz., VFAs) by applying external potential. Integrating dark fermentation (source of carbon) and MFC (for application of external potential), with MEC achieving higher biogas recovery along with complete treatment of waste.

The valorization of the waste by means of biological processes is linked to a bioeconomy approach. In the dark fermentation process, 60–70% of the substrate is channeled to different by-products. This products are represented by Ethanol, 1,3 propanediol (PD) and butyric, especially (Venkata Mohan et al., 2016a).

Hydrogen has the highest energy content per unit weight of any known fuel (143 GJ $tonne^{-1}$) and it is the only fuel that is not bound to any carbon.

Hydrogen have many application in both energy and chemical industry. Also, hydrogen can be used in fuel cells, producing electricity and it can be stored as renewable electricity when is less needed. Hydrogen can, then, be converted back to electricity at peak hours. Hydrogen can be stored at high-pressure, as an integral component in certain alloys known as hydrides, on microscopic carbon fibres. Hydrogen can be converted to formic acid in order to be stored (Lipscomb et al., 2014; Sinha et al., 2015). Indeed in addition to its energy applications, hydrogen is widely used in chemical industries as a raw material for hydrogenation in the production of hydrocarbons, fertilizers, dyes, drugs, and plastics (Moscoviz et al., 2018).

The world market of H_2 is about 60000 kt $year^{-1}$. The current cost of its production by natural gas reforming is between 1.0 and 2.0 € kg^{-1}. The cost of electrochemically-produced H_2 is estimated to be between 3.5 and 5.0 € kg^{-1}.

The cost of biobased H_2 production process could be assessed between 1.5 and 3.5 € kgH_2^{-1} due to the lower environmental impacts, which make it becoming economically competitive with the existing market (Moscoviz et al., 2018).

2.2 Bio-chemicals carriers

One of the objectives of the four biorefinery approach is to convert organic feedstocks into carboxylic products (den Boer et al., 2016; Venkata Mohan et al., 2016a). The carboxylate platform has several attractive features, such as feedstock flexibility, minimal feedstock pre-treatment, utilisation of most organic components of the biomass and mixed culture stability.

The carboxylate platform is based on anaerobic fermentation which involves different processes, with undefined mixed cultures of microorganisms (Agler et al., 2011). Under anaerobic conditions the glucose contained in the organic waste is metabolized to pyruvate and pyruvate is subsequently converted to many end products: lactate, acetate, ethanol, succinate, formate, CO_2 and H_2. The conventional anaerobic digestion process, which produce CH_4-rich biogas, can be altered via pH control or the addition of methanogenic inhibitors, upon which short chain VFA (C2–C4) are extended to medium chain VFAs (C5–C8) (Weimer et al., 2015). The VFA included in the medium can be recovered by extraction processes (Singhania et al., 2013).
This metabolic products present a wide range of applications in the productions of cosmetics, in the pharmaceutical and food industry (Zacharof and Lovitt, 2013) as well as in the production of solvents (Agler et al., 2011), in the production of energy in microbial fuel cells (Cavdar et al., 2011), in the production of biodegradable polymer (PHA) (Shen et al., 2017), in the nutrient removal for the wastewater treatment (Lee et al., 2014). Some VFAs, like as propionate, butyrate and acetate are more attractive products in the chemical industry as platform chemicals. Generally, the market prices is 2163 $/ton, 2000 $/ton and 600 $/ton for propionate, butyrate and acetate acid, respectively (Calt, 2015).

2.2.1 Volatile Fatty Acids (VFAs)

Volatile fatty acids (VFA) or short chain carboxylic acids are short chain fatty acids consisting of fewer carbon atoms (Lee et al., 2014). Conventionally, these VFA are produced chemically from petroleum derived compounds. These chemical processes are energy intensive and have negative impacts on the environment. Thus, VFA generated through biological routes using a specially prepared microbial culture from organic matter through acidogenic anaerobic digestion is gaining great interest (Dahiya et al., 2018a). VFA recovery from waste streams is perfectly matched with that type of circular economy aiming to circulate at high quality in the production system (Atasoy et al., 2018). Recently, generation of VFA from various organic feedstocks such as food waste (Zhou et al., 2018), press mud (Kuruti et al., 2015), organic fraction of municipal solid waste (OFMSW), (Bolzonella et al., 2006; Doğan and Demirer, 2009), waste activated sludge (Xiong et al., 2012) has been reported.
These compounds have large interest in chemical industry serving as starting molecules for bioenergy production and for the synthesis of a variety of products, such as biopolymers, reduced chemicals and derivatives (Strazzera et al., 2018).

A variety of VFA is on the market including formic (C1), acetic (C2), propionic (C3), butyric (C4), valeric (C5), caproic acid (C6). The common VFA produced from organic waste streams are acetic, propionic and butyric. The operating temperature and the pH values have an effect on the final product distribution (Lee et al., 2014). The acidic conditions promote fermentation products such as acetic acid and longer chain products, propionic and butyric acids, which are common primary fermentation products (Agler et al., 2011), while other products such as isobutyric, n-valeric and iso-valeric acids appeare to a lesser extent. On the other hand, the alkaline conditions promote the production of acetic acid at greater extent (Dahiya et al., 2018).
The chemical structure, applications, properties and production methods of these products are reported in Table 2-2.
The **Table 2-3** reported the market size and the price of the main VFA.

Table 2-2: General properties of VFAs (adapted from Atasoy et al., 2018)

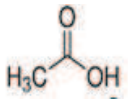
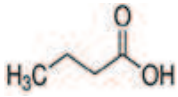
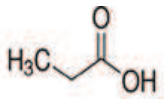
VFA	Chemical formula	Usage/application	Priduction methods
Acetic acid		Vinyl acetate monomer (polymer, adhesives, dyes), food additive, solvent, vinegar,ester production, chemicals	Chemical synthesis (carboxylation of methanol) and micfrobial fermentation (oxifative and anaerobic)
Butyric acid		Animal and human food additive, chemical intermediate, solvent, flavouring agent	Chemical synthesis (oxidation of butyraldehyde), estraction from butter, microbial fermentation
Propionic acid		Esters used food industry as aroma additve, food additove, flavoring, pharmaceuticals, animal feed supplement, fishing bait additive	Chemical synthesis (ethylene hydro formylation, carboxylation of ethylene, direct oxidation of hydrocaebons), by product of acetic acid manufacturing, microbial fermentation

Table 2-3: *Bulk prices and market size of butyric, propionic, lactic, formic, acetic acid and ethanol (adapted from Bastidas-Oyanedel et al., 2015)*

Compound	Price (USD/tonne)	Market size (tonne/year)
Acetic acid	400 -800	3.500
Butyric acid	2000 - 2500	30.000
Propionic acid	1500 - 1700	180.000
Caproic acid	2000 - 2500	25.000
Formic acid	950 - 1200	30.000

Bastidas-Oyanedel et al. (2015) reviewed the dark fermentation products using glucose and organic waste as substrates. The Figure 2-8 shows the average dark fermentation product yields of acetic acid, butyric acid, propionic acid, caproic acid, lactic acid, ethanol, formic acid, H_2, CO_2 and biomass, expressed as mass percentage of substrate consumed. Together with ethanol, organic acids represent the 65% w/w of the substrate consumed.

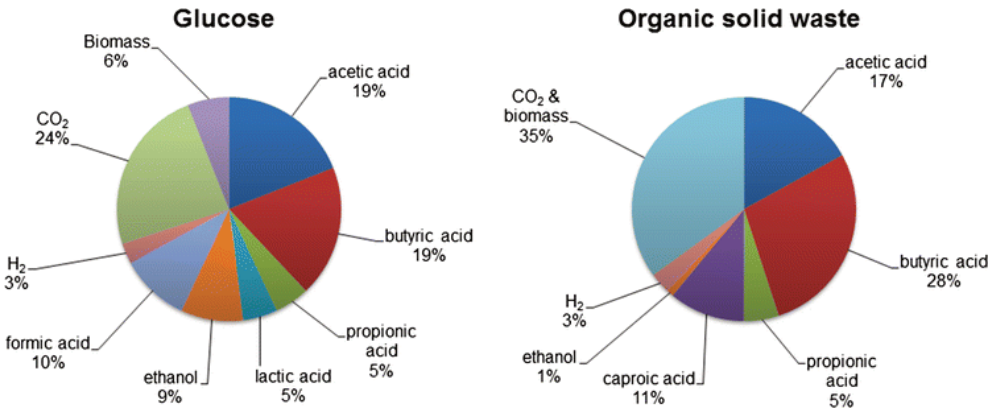


Figure 2-8: Dark fermentation average product yields expressed as mass percentage of substrate consumed, i.e. glucose and organic solid waste (Bastidas-Oyanedel et al., 2015)

Using glucose as substrates, mixed culture dark fermentation is a natural process, which evolved to maximize the cell growth but not the H_2 yield (Figure 2-9). In balanced mixed culture fermentation, intermediates by-products are produced through hydrogenases activity accompanying H_2 production. The metabolic production in mixed anaerobic culture follows many pathways as long as the reactions are thermodynamically favourable (Bastidas-Oyanedel et al., 2015).

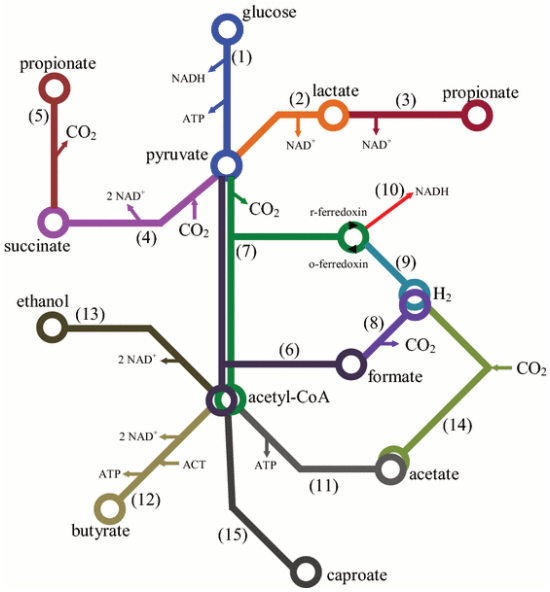


Figure 2-9: Metabolic pathways of dark fermentation (Bastidas-Oyanedel et al., 2015)

Khan et al. (2016) studied that the VFA production is affected by different operating conditions, such as pH, temperature, organic loading rate, retention time and substrate.

pH influences the hydrolysis and the acidogenic phase of the anaerobic processes (Zhao et al., 2018). According to Huang et al. (2018) and Zhao et al. (2018), who investigated the effect of different pH values on VFA production, the optimal VFA production occurred under pH 10. However, the stepwise pH fermentation strategy (from pH 9 to 11) does not only enhance the activity of acid-producing bacteria (pH 9) but also inhibit the activities of methanogens (pH 11), which resulted in higher production of VFA.

The temperature affects the growth of microorganisms and hydrolysis rate (Zhou et al., 2018). The mesophilic temperature is the optimum and most economically favourable condition for VFA production. As pH, temperature also affects the type of main VFA product in fermentation. At 55°C butyrate is the main product, while at 35°C it was observed a greater production of acetate and propionate (Atasoy et al., 2018). As regard the retention time, after 15 days of the anaerobic mixed culture fermentation it was found the highest VFA concentration when 5, 10 and 15 days were investigated (Jankowska et al., 2017).

In addition to the operation conditions, the type of mixed microbial culture and the kind of substrate may affect the VFA concentration and composition (Atasoy et al., 2018; Wang et al., 2014a). The readily fermentable waste stream characteristics

determine the degree of acidification. In the study of Silva et al. (2013) the organic fraction of municipal solid waste streams achieved the highest degree of acidification with total VFA production (2707–3374 mg/L as COD) rather than those obtained for sugarcane, glycerol, winery effluent and sludge.
The main products, which were studied as recent biochemical carriers, are described below.

Acetic acid

Acetic acid is a key building block to a very wide range of applications, including paint, rubber, plastics, synthetic fibres, textile finishes, pesticides, polymer emulsions, paper coating, in the chemical industries and it is one of the major components of flavours, acidity regulators and preservatives used in the food and beverage industries. In 2015 the acetic acid was characterized by a word market volume of 13570 kt $year^{-1}$; this market is relatively stable in Europe and its annual growth rate has been estimated at 5% for the period from 2014 to 2020. The price of acetic acid on the market is ranging between 0.33 and 0.67 € kg^{-1}.

Butyric acid

Butyric acid is a short-chain volatile fatty acid, naturally produced by anaerobic bacteria.
In the chemical industry, butyric acid has numerous potential applications in chemical, textile, plastic, food, dairy and pharmaceutical industries.
It can also be used directly as an additive to plastic materials and textile fibres for heat and sunlight resistance enhancement, as solvent diluents, drugs, perfumes, fibre, additive and raw materials (Jha et al., 2014). In particular, the butyric acid is mainly used as a precursor to produce thermoplastic cellulose acetate butyrate, which has excellent performance in terms of organic solvent solubility due to enhanced hydrophobicity, light and cold resistance, and flexibility. In addition, butyric acid can also be used to enhance the production of butanol as a biofuel.
Current industrial butyric acid production is exclusively via chemical synthesis, with a worldwide market of approximately 80000 metric tons per year at a price of around $ 1.8/kg (Jiang et al., 2018).

Propionic acid

Propionic acid, is a fatty acid present in many processed foods as well as animal feedstocks. It is a natural intermediate and metabolite in many biological processes, and it is a useful intermediate in several chemical reactions, particularly in

polymerizations. However, similarly to butyrate, the use of waste-based propionic acid for food or feed applications would require the approval of regulatory authorities such as EU. Propionic acid had a market volume of 400 kt year^{-1} in 2013, with a price ranging between 1.25 and 1.38 € kg^{-1}.
Similarly to acetate and butyrate, no low-cost processes that could specifically extract propionate from mixtures of short chain carboxylic acids have been developed.

Lactic acid

Lactic acid is an important natural organic acid. It has received increasing attention as one of the most important building blocks for the production of polylactic acid.
The principal isomers which formed lactic acid are L-lactic acid and D-lactic acid. Lactic acid is produced by means of chemical synthesis or microbial fermentation. Chemical synthesis of lactic acid is mainly based on the hydrolysis of lactonitrile by strong acids. Other chemical routes were catalization of sugars, oxidation of propylene glycol, reaction of acetaldehyde, carbon monoxide, and water at high temperatures and pressures, hydrolysis of chloropropionic acid and nitric acid oxidation of propylene. (Gao et al., 2011).
Lactic acid is widely used in the food industry, in the pharmaceutical sector and more recently in the polymer industry for polylactic acid manufacturing (PLA, bioplastic). Lactic acid is currently 100% bio-sourced due to the high isomeric purity. This aspect is particularly important for PLA production, whose biodegradability depends on the L-isomer purity of the lactic acid.
The world market volume is 472 kt year^{-1} and it is expected to grow in the next years, with the market value ranging between 0.84 and 1.51 € kg^{-1}. (Moscoviz et al., 2018).

2.2.2 Polyhydroxyalkanoates (PHAs)

Polyhydroxyalkanoates (PHAs) are a group of biopolymers which can be produced by fermentation. Indeed, in the presence of an excess of carbon source content, some microorganisms are able to accumulate PHAs within their cell as a way to store carbon and energy (Dahiya et al., 2018b). PHAs can be produced from a mixture of carboxylic acids (i.e. acetate, propionate, butyrate, valerate, and caproate). Biogenic wastes like FW (Venkateswar Reddy and Venkata Mohan, 2012), spent wash effluents (Amulya et al., 2015), sugar cane molasses (Albuquerque et al., 2007) and bakery waste hydrolysate (Pleissner et al., 2015) were also used for PHA production and its production can be used as a method to valorise dark fermentation effluents.

The most common PHAs are poly(3-hydroxybutyrate) (PHB) and poly(3 hydroxybutyrate- co-3 hydroxyvalerate) (PHBV), although a wide variety of PHAs can be produced. The production of PHA is 100% biobased; its market volume was only 17 kt $year^{-1}$ in the 2015 and the price in this market was in the range between 2.20 and 5.00 € kg^{-1} (Moscoviz et al., 2018). Nevertheless, the production of PHA is not yet economically competitive when compared to equivalent petro-based plastics (around 1 € kg^{-1}).

3. Anaerobic processes for organic waste treatment

The efficient conversion of renewable bioresources into bioenergy and biochemicals contributes to meeting the ever increasing demand for energy and products. Biochemical process-based biorefineries have a significant potential as a technology to covert low value feedstocks, ranging from municipal and industrial organic wastes, to agricultural and forest residues, and energy crops, into high-value biofuels and biobased products with concurrent waste valorization.
Biochemical conversion involves two main processes: anaerobic digestion and dark fermentation.

3.1 Anaerobic digestion

Anaerobic digestion (AD) is one of the oldest and well-studied conversion technologies for the management of organic solid waste, in order to produce biogas (Ma et al., 2018; Zhang et al., 2018). Among the technologies available for the treatment of organic solid waste, AD is very suitable because of its limited environmental impacts and high potential for energy recovery (Ardolino et al., 2018; Ma et al., 2018; Mao et al., 2015).
AD is a biochemical process which promotes the degradation of the organic materials by a consortium of microorganisms, in the absence of oxygen, resulting in the production of a methane-rich biogas (Lim et al., 2018).

3.1.1 Anaerobic digestion process

AD process generally consists of four biological and chemical stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

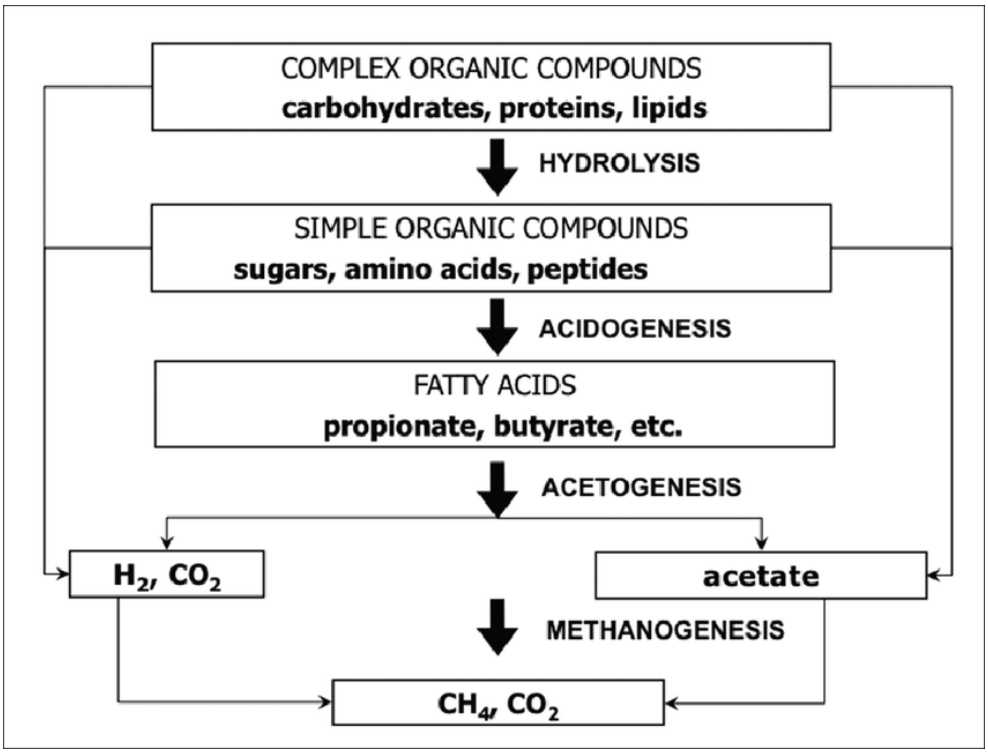


Figura 3-1: Anaerobic pathway of complex organic matter degradation

Firstly, the insoluble, complex and polymeric organic matter macromolecules are broken down into soluble molecules and monomers by means of the hydrolysis process. The soluble molecules and monomers, such as sugars, are readily available to anaerobic microorganisms for further processing. Among the four steps, hydrolysis is the slowest, and it is therefore the rate-determining one.
Extensive research has been conducted on pretreatment methods to accelerate the hydrolysis step (Carrere et al., 2016; Cesaro and Belgiorno, 2014) and to obtain suitable byproducts from this step (Carrère et al., 2010).
The acetate compounds and hydrogen produced in the first steps of AD process can be used directly by methanogens. Other molecules characterized by a chain length that is greater than acetate such as volatile fatty acids (VFA) must first be catabolized into compounds that can be directly utilized by methanogens.
The acidogenic stage consists of the breakdown of the organic monomers by acidogenic (fermentative) bacteria. During this process stage VFA are generated along with ammonia, carbon dioxide and hydrogen sulphide as well as other by-products such as organic acids and alcohols that are converted in the following stage.
In the third stage of the AD process, called acetogenesis, the simplest molecules

generated in acidogenesis are further digested by acetogens in order to produce acetate, carbon dioxide and hydrogen. The final stage of anaerobic digestion is the biological process of methanogenesis. During this step, methanogenic archaea utilize the products of the previous stages and convert them into methane, carbon dioxide and water. These are the main components of the biogas released from the system. The remaining solid residue is the digestate, which includdes non-digestible organic and mineral materials.
The AD process is often separated into two main phases, namely the acid one (hydrolysis, acidogenesis, and acetogenesis) and the gas production phase (methanogenesis) (Adekunle and Okolie, 2015). This operational convention is commonly used in two-stage commercial AD systems in order to facilitate hydrolysis, acidogenesis, and acetogenesis in one reactor and methanogenesis in a separate reactor.

3.1.2 Stoichiometric relationships

The phases of the AD process develop through general stoichiometric relationships, which turn to be helpful for conducting carbon and nutrient mass balances and for explaining the dynamics of alkalinity and other parameters. The stoichiometry of fermentation, in which organic matter ($C_5H_7O_2N$) is converted into acetic acid (CH_3COOH) or acetate (CH_3COO^-) is showed in the following equations (Haandel and Lubbe, 2012):

$$C_5H_7O_2N + 3H_2O \rightarrow 2.5\, CH_3COOH + NH_3$$

$$C_5H_7O_2N + 3H_2O \rightarrow 2.5\, CH_3COO^- + 1.5\, H^+ + NH_4^+$$

During the fermentation phase, ammonia is produced and protons are released.
In the AD process, ammonia is not an energy source for microorganisms and it is consumed in the synthesis of new cells by the microbial populations present in the process. Thus, the ammonia accumulated at a rate that is proportional to the rate of fermentation (hydrolysis/acidogenesis). A well-functioning AD process will produce methane, CO_2, alkalinity, and ammonia. However, the following equation shows the representative stoichiometric reaction of the conversion of acetic acid to methane and carbon dioxide (Haandel and Lubbe, 2012). The methanogenic reactions require the conversion of ethanol to acetic acid and methane and the conversion of carbon dioxide and hydrogen gas to methane and water.

$$2.5\ CH_2OH + 2.5\ H^+ \rightarrow 2.5\ CO_2 + 2.5\ CH_4$$

$$2\ C_2H_5OH + CO_2 \rightarrow CH_4 + 2\ CH_3COOH$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

The stoichiometric production of methane, CO_2, alkalinity, and ammonia can be used to understand the overall health or functionality of an AD system.
A great process efficiency is demonstrated by a great organic matter degradation and a consequent great alkalinity, ammonia, and methane generation. However, changes in alkalinity and ammonia concentrations can be floating due to the complex biochemical nature of the feedstocks of AD process. Thus, the measure of methane production is generally used to quantify the AD efficiency (Khanal, 2008).

3.1.3 Parameters affecting anaerobic digestion

Many factors affect the efficiency of biogas generation during the AD process. Strategies for enhanced biogas production often includes the optimization of these factors (Zhang et al., 2018).
Physical and chemical categories of operational parameters are monitored in AD processes. These parameters are especially influenced by substrate characteristics.
Physical parameters include Total Solid (TS) content (Li et al., 2011a), substrate to inoculum ratio (S/I ratio) (Li et al., 2011a), temperature, retention time, organic loading rate (OLR) (Chene et al., 2014), Volatile Solid (VS) reduction (Li et al., 2011a), and methane generation rate. Chemical parameters include carbon to nitrogen ratio (C/N ratio) (Li et al., 2011a; Yang et al., 2015), pH and alkalinity (Amani et al., 2010), Volatile Fatty Acid (VFA) concentration (Amani et al., 2010), and concentrations of micronutrients and inhibitory compounds (Chen et al., 2008) such as free ammonia (NH_3) and hydrogen sulfide (H_2S). Each parameter is interrelated with others, so that the change of one of them is followed by the changes of other ones. As with all biological processes the optimum environmental conditions are essential for successful operation of anaerobic digestion. The high concentration of ammonia, which related to protein-rich feedstock treated would inhibit methanogenic activity (Chen et al., 2008). While, lipid-rich feedstock presents higher methane production potential, compared to carbohydrate-rich and protein-rich feedstocks (Angelidaki and Ahring, 2000).
Depending on the solid content of the substrate in AD, two different type of AD process occurred. Solid-state anaerobic digestion (SS-AD) is generally characterized

by solid concentrations higher than 15%. In contrast, liquid AD occurs at solid concentrations between 0.5% and 15%. Significant increase in methane production from food waste from 10% to 15% was observed (Fernandes et al., 2009) and the reduction of methane production was found for increasing TS content until 30% in AD of OFMSW (Fernandes et al., 2009). Thus, according to these studies, it would appear that the optimal TS content for AD of OFMSW is somewhere between 15% and 20%. However, the SS-AD is generally used because it allows to include smaller reactor volume, lower energy requirements for heating, minimal material handling, and lower total energy loss. The digestate of SS-AD could be used as fertilizer or pelletized fuel, as it contains lower moisture content than the effluent of the wet AD (Li et al., 2011a).

Substrate to inoculum (S/I) ratio is an important parameter in AD because greatly affects the digester performance. The inoculum contains all microbes necessary for the startup of a digester and it is typically obtained as effluent from another operational anaerobic digester. The S/I ratio is determined by the ratio of VS of substrate to VS of inoculum. According to Kong et al. (2016) this ratio must be minor to 2 in order to avoid the excessive production of the organic acids and, consequently, inhibition of methane production.

Anaerobic digestion can operate in a wide range of temperature. With increasing temperature the reaction rate of anaerobic digestion strongly increases. Thermophilic temperatures (50-60°C) promote faster reaction rates and, consequently, higher productivity of AD than mesophilic temperature. However, in AD process under thermophilic temperature was observed many disadvantages such as low quality effluent, decreased stability, toxicity, poor methanogenesis and higher net energy input, which inhibited biogas production. A suitable condition for AD, therefore, would be thermophilic hydrolysis/acidogenesis and mesophilic methanogenesis in a two phase anaerobic digestion process (Grilc, 2012).

Retention time, defined in practice as the time that the waste material undergoes digestion, is a function of feedstock biodegradability and system operating conditions, and is selected to optimize the economics of the system. Retention times in high solid AD systems generally range from 10-30 days (Amani et al., 2010; Kothari et al., 2012).

ORL refers to the amount of the raw material continuously fed to the anaerobic digester per day per unit working volume. With increasing OLR, the biogas yield increases to an extent, but the equilibrium and productivity of the digestion process can also be greatly disturbed (Zhang et al., 2018). The maximum recommended OLR for mesophilic and thermophilic AD systems are 5 and 8 kg_{VS}/m^3 d (D. and Grilc, 2012).

VS reduction and methane yield are the most direct measures of digester performance and degree of digestion. Methane yield as specific methane yield, in particular, is the volume of methane produced normalized by the mass of VS or Chemical Oxygen Demand (COD) loaded to the digester (L CH_4/kg VS or m^3 CH_4/kg COD) (Khanal, 2008).

Both VS reduction and methane yield decrease logarithmically with time and depend on feedstock biodegradability and system operating parameters. Maximum VS reduction achievable in AD is often cited as 60%, though this value is exceeded in some cases (Kaparaju and Rintala, 2005).

In anaerobic digestion the pH is mostly affecting the digestive process, especially the products in the methanogenic stage. The optimum pH value for the methanogenic microorganisms is between 6.5 and 7.5; pH values minor than 6.5 promote a higher production of acids that leads to imminent process failure.

AD process is very sensitive to C/N ratio because it reflects the nutrient levels of a digestion substrate. A high C/N ratio induces a low protein solubilization rate and leads to low energy and structural material metabolism of microorganisms. Thus ammonia inhibition may be avoided by optimizing the C/N ratio in the AD process. However, an excessively high C/N ratio provides insufficient nitrogen to maintain cell biomass and leads to fast nitrogen degradation by microbials, resulting in lower biogas production. Substrates with an excessively low C/N ratio increase the risk of ammonia inhibition, which is toxic to methanogens and causes insufficient utilization of carbon sources. The optimal C/N ratio for anaerobic digestion has been shown to be between 20 and 30 or between 20 and 35 with a ratio of 25 being the most commonly used (Mao et al., 2015).

VFA are crucial intermediate chemicals produced in the AD process, which can accumulate at high ORL and cause inhibition (Li et al., 2011a). VFAs are naturally produced by acidogens and acetogens and then consumed by methanogens in the AD process (Adekunle and Okolie, 2015). Accumulation of VFAs can therefore be viewed as a measure of the differences in the rates of VFA production by acidogens/acetogens and consumption by methanogens. Because hydrolysis is the preceding metabolic step of the AD process, VFA accumulation is an indicator that methanogenesis is the rate-limiting step rather than hydrolysis. Reductions in VFA concentrations, on the other hand, indicate that methanogenesis is occurring at a more rapid pace than acidogenesis and acetogenesis, an indication that hydrolysis is the rate-limiting step. In the case of batch AD processes, decreases in VFA concentrations can also be an indicator that the bioavailable raw substrate (e.g. proteins, carbohydrates, lipids) has been metabolized and the AD process is coming to an end (Mussoline et al., 2013). Total VFA concentrations are typically expressed in terms of acetic acid equivalents because acetic acid is the most common VFA

produced in natural systems. VFA concentrations greater than 10.000 mg/L as acetic acid are generally considered inhibitory to methanogens (Amani et al., 2010). However, methanogens are acclimated to survive in high VFA environments and certain populations are prone to inhibition at lower concentrations (Khanal, 2008).

3.1.4 Anaerobic digestion in biorefinery context

Recent studies (Bastidas-Oyanedel et al., 2015; Dahiya et al., 2018b; Venkata Mohan et al., 2016b) proved the possibility of using waste as renewable feedstock in the framework of biorefinery. Exploitation of waste organic residues would enhance biorefinery competitiveness and social acceptance. AD biorefinery holds a great potential to serve as a technology platform to convert a variety of low-value feedstocks, agricultural and forest residues and energy crops as well as municipal and industrial organic wastes into high-value biofuels and biobased products with concurrent waste valorization.

Surendra et al. (2015) proposed AD-based biorefinery scheme (Figure 3-1) in which the AD not only generates bioenergy in the form of biogas and biobased products, such as organic acids and biopolymers, but also simultaneously remediates the wastes, reducing the environmental footprint of such industries. The monomeric sugars generated during the process can be used as precursors in the production of other products such as bioenergy to organic acids and biopolymers (e.g., bioplastic). The insoluble solid lignin residue can be used to produce heat and electricity or be converted into biobased products such as lignosulfonates. The digester effluent, which is generally rich in nutrients, can be used as a fertilizer after suitable post treatment. The AD effluent could be useful for macro- and micro-algae production. The effluent of these processes can be recycled back as process water into the AD plant, as well as algal biomass that can be further processed into biofuels and biobased products.

Ren et al. (2018) and Venkata Mohan et al. (2016) proposed a conceptual waste biorefinery model which integrates food waste conversion processes and technologies into fuels, electricity and chemicals production. This waste refineries could promote food waste conversion into more valuable products through the combination between the current researches about anaerobic digestion of food waste, technological route for methane fermentation and biorefinery technologies. The proposed model include 4 stages: i) pretreatment; ii) resourceful product; iii) biomethane fermentor: digestate from previous process can undergo anaerobic digestion to product methane; iv) and microalgae CO_2 capture: the carbon dioxide produced in the anaerobic system and the organic components in the digestate are

used as dual carbon sources to increase the growth rate of microalgae and oil or starch production. The pretreatment can largely alleviate the problem of system collapse caused by poor hydrolysis. During the resourceful product, food waste can be used by components to produce hydrogen, lactic acid, acetic acid, ethanol, butanol. In the biomethane fermentator the digestate from previous process can be used within anaerobic digestion to product methane. The microalgae CO_2 capture promotes the use of carbon dioxide produced in the anaerobic system and the organic components in the digestate as dual carbon sources to increase the growth rate of microalgae and oil or starch production. In this model, food waste was used to produce many useful products toward zero emissions.

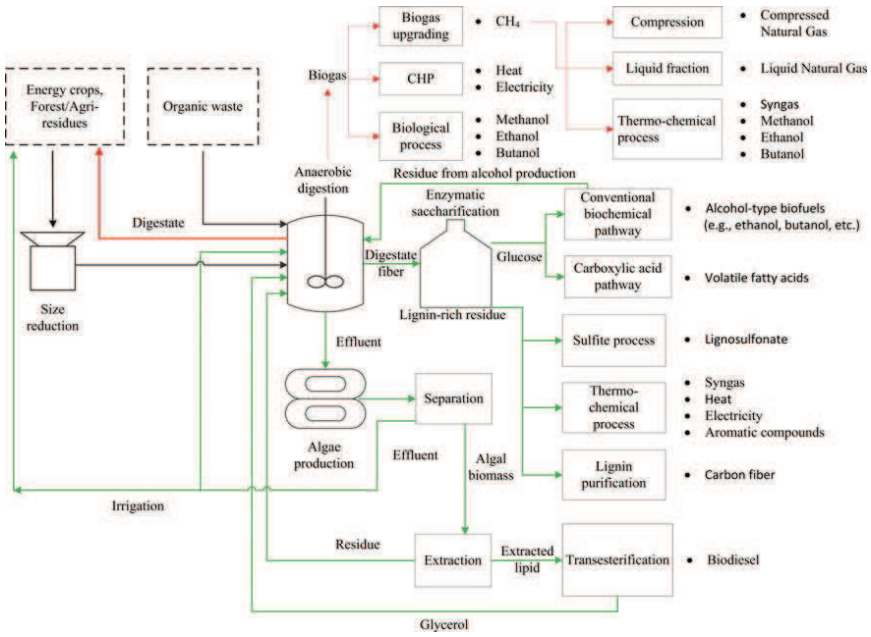


Figure 3-1: Schematic of an AD-based biorefinery for producing biofuels and biobased products (Surendra et al., 2015)

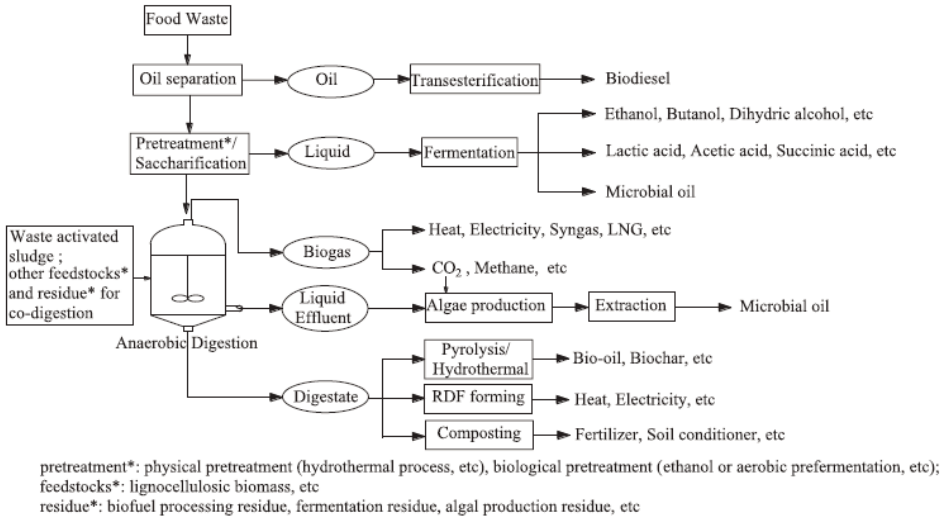


Figure 3-2: A technological route for food conversion based on biorefinery (Ren et al., 2018)

However, the implementation of the AD-based biorefinery concept is still rare. A more comprehensive study supported by research and development is crucial for developing an AD-based biorefinery analogous with the petroleum refinery.

3.2 Dark fermentation

Dark fermentation is a catabolic process in which bacteria convert sugars and proteins to carboxylic acids, hydrogen gas, carbon dioxide and organic solvents. This biological chemical reaction is inhibited by the presence of oxygen and thus it is only carried out under anaerobic conditions.

3.2.1 Biochemical reactions of dark fermentation

The biodegradation steps of the dark fermentation process is mediated by microbiological pathways, which involve the fermentative breakdown of substrate (Figure 3-3). Anaerobes (Clostridia, methylotrophs, rumen bacteria, methanogenic bacteria, archea), facultative anaerobes (Escherichia coli, Enterobacter, Citrobacter), and aerobes (Alcaligenes, Bacillus) bacteria are involved in the fermentative process (Li and Fang, 2007).

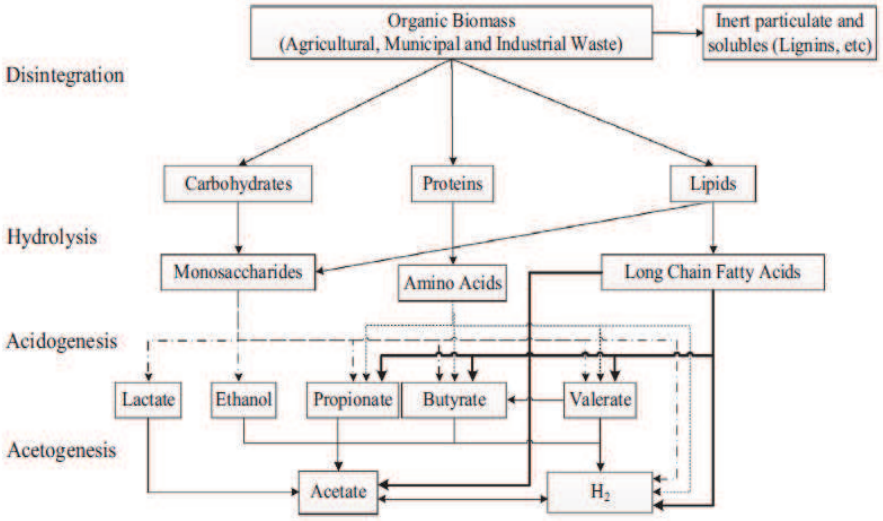


Figure 3-3: Biodegradation steps and microbiological pathways involved in the fermentative breakdown of waste biomass (Ghimire et al., 2015a)

Acetate and butyrate are the most common products of DF. The hydrogen production from the glucose of the substrate is determined by the butyrate/acetate ratio (Ghimire et al., 2015a; Hawkes et al., 2007). When acetic acid is the end-product, a theoretical maximum of 4 moles hydrogen per mole glucose is obtained:

$$C_6H_{12}O_6 + 2\,H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$$

The yield of H_2 is 2 mol for a mole of glucose when the final product is butyric acid:

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$$

The molar ratio of butyric to acetic acid (B/A ratio) is a quantitative indicator of the biohydrogen yield associated with microbial metabolic pathways. However, that B/A ratio was found directly proportional to H_2 yields (Cappai et al., 2018; De Gioannis et al., 2013; Kim et al., 2006). It was reported that a B/A ratio higher than 2.6 indicated an efficient H_2 production by anaerobic fermentation.
The biochemical pathways may lead to the production of other metabolites rather than H_2. However, some process conditions promote ethanol and acetate (Nath and

Das, 2004) production together with a low H_2 yield (2 mol of H_2 for a mole of glucose):

$$C_6H_{12}O_6 + 2\,H_2O \rightarrow CH_3CH_2COOH + 2H_2\,O$$

The hydrogen consuming pathways with the production of the propionate may also occur during fermentation process:

$$C_6H_{12}O_6 + 2\,H_2 \rightarrow 2\,CH_3CH_2COOH + 2H_2\,O$$

Similarly, some operating conditions might lead only ethanol and lactic acid production without hydrogen production:

$$C_6H_{12}O_6 \rightarrow 2\,CH_3CH_2OH + 2CO_2$$

$$C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + 2CO_2$$

Thus, the highest theoretical yields of hydrogen are associated with acetate and butyrate as end-product of the fermentation process. High hydrogen yields are associated with a suitable mixture of acetate and butyrate fermentation products, and low H_2 yields are associated with propionate and reduced end-products such as lactic acid (Ghimire et al., 2015a; Łukajtis et al., 2018).

H_2 production, H_2 yield, metabolites pathways from DF of organic waste is influenced by the presence of an effective hydrolysing and H_2 producing microbial community. Heat, alkaline and acid treatment have been recently applied to food waste in order to increase the H_2 yield. However, this pretreatments was not used to increase the hydrolysis step but to select microbes which facilitate the H_2 production (Im et al., 2012; Kim et al., 2009; Yun et al., 2018).

3.2.2 Parameters affecting dark fermentation

Several factors can heavily impact the performance of biological hydrogen production during dark fermentation process. The main factors include substrate, inoculum, pH, hydraulic retention time (HRT), and the presence of inhibiting product.

In order to obtain great H_2 yield, the substrate is a key factor also related to the overall economy of the process. The H_2 production is mainly affected by carbohydrate content, bioavailability and biodegradation rate of the substrate (Guo et al., 2010; Ren et al., 2011). The substrates used in DF studies were carbohydrate-rich substrates. According to Guo et al. (2010) and Monlau et al. (2012) the soluble and readily accessible sugars represent the main fraction of biomass that can be converted into hydrogen.

Although substrates rich in simple carbohydrates, sucrose or starch hydrolysates are commonly used in the fermentation process, their utilization on a large scale is unprofitable. Continuous hydrogen production requires renewable resources rich in lignocellulose or starch waste.

As the utilization in dark fermentation of organic solid waste seems to be attractive, the main substrates used for DF were the organic fraction of municipal solid waste (OFMSW) (Chen et al., 2012; Łukajtis et al., 2018; Tawfik and El-Qelish, 2012), agricultural residues like lignocellulosic biomasses (e.g. rice straw, wheat straw and corn stalks), agro-industrial wastes like those from food processing industries (e.g. olive mill wastewater and cheese whey), effluents from livestock farms and aquatic plants (Ghimire et al., 2015a; Łukajtis et al., 2018; Show et al., 2012).

As regard the evolution of the fermentative pathways, the most important factors is represented by the inoculum (De Gioannis et al., 2013). The study of the inoculum effects on dark fermentation focused on the monitoring of anaerobic fermentation under acidic conditions maintaining high hydrolysis and acidification rates without methane production.

Soil, wastewater sludge and compost can be used as inoculum for fermentative H_2 production because they all contain H_2 synthesizing bacteria (Li and Fang, 2007). In most cases the mixed culture inocula need to be enriched and adapted from inocula obtained from extreme conditions before applying to dark hydrogen fermentation. A suitable pretreatment was applied to the inocula in food waste and OFMSW fermentation (Cappai et al., 2018; De Gioannis et al., 2013; Wang et al., 2014a). This is necessary in order to enhance the biohydrogen production as well as to inhibit hydrogen consumers such as methanogens and homoacetogens, often present in these mixed inocula (Wang et al., 2014a, 2009). Heat treatment of mixed cultures is the main pretreatment of the inoculum used before DF process because it is a simple, inexpensive and effective method (Ghimire et al., 2015a; Li and Fang, 2007; Wang et al., 2009). However, the effect of heat treatment might be different depending on the inoculum source such as activated sludge or anaerobic sludge. Compared to acid, base and aeration, the heat-shock pretreatment promoted the maximal hydrogen production potential, maximum hydrogen production rate, hydrogen yield, substrate

degradation efficiency for both activated sludge and anaerobic sludge (Wang and Wan, 2008; Wang et al., 2014a).

Dark hydrogen fermentation reactions can be operated at different temperatures: mesophilic (25 -40°C), thermophilic (40-65°C), extreme thermophilic (65-80°C) (Shin, 2004; Valdezvazquez et al., 2005).

Although a wide range of temperatures (30–60 °C) has been found optimum for dark fermentation, most of the studies suggested the optimum operating temperature for dark fermentation between 37–40 °C and 55–60 °C to achieve better bio-H_2 production without any inhibition.

However, ethanol decreased with further increasing temperature from 35 °C to 55 °C. In comparison, Valdezvazquez et al. (2005) reported that butyrate was the major by-product at 37 °C and acetate at 55 °C. The acetate was the predominant by-product at 20 °C, but the production of acetate decreased at 55 °C with increasing production of butyrate, propionate, and ethanol (Elbeshbishy et al., 2017). Up to now, most of dark fermentation experiments are conducted at 35-55°C (Alexandropoulou et al., 2018).

Hydrogen fermentation pathways are sensitive to pH value. The control of pH was crucial to hydrogen production minimizing the activity of the hydrogen consumers. Acid condition (pH below to 6), generally, inhibits the methanogenic activity under both mesophilic and thermophilic conditions, but the inhibition of hydrogen consuming homoacetogenic activity can only be achieved under thermophilic conditions at the initial pH of 5.5. Liu et al. (2006) found that on mesophilic conditions the optimal pH ranged between 5.0 and 5.5. The pH value around 5.5 has proved to be satisfactory for hydrogen production using OFMSW as substrate (Alzate-Gaviria et al., 2007), while the optimal initial pH for converting starch to hydrogen was found at 6.0 under thermal conditions.

The pH values also influence the metabolic by-products as well as biohydrogen yields. The pH at 7 promote acetate and butyrate as the major end products which enhance the hydrogen synthesis (Ghimire et al., 2015a; Yokoyama et al., 2007). Neutral pH favored the acetate pathways, while acidic pH conditions favored the butyrate pathways. (De Gioannis et al., 2013) reported that a pH value equal to 6 promotes higher acetate concentration in all the tests; whereas at pH 6.5 butyrate and propionate concentration exceeded those of acetate.

Hydraulic retention time is a measure of the average length of time that a substrate remains in a fermentation chamber. The HRT can affect substrate hydrolysis and thus the production of intermediates and products, affecting fermentative H_2 production. The hydrogen production rate increases over a certain range of HRT values but after exceeding the optimal HRT value the production rate decreases with an increase in HRT (Łukajtis et al., 2018).

The low HRT value promote the H_2 production due to the dilution effect of the methanogens. With decreasing HRT the H_2 production increases (Kim et al., 2006). However, the optimum HRT for biohydrogen production in DF depends on the type of substrate and on the other operating conditions because the HRT alone is not sufficient to fully suppress the methanogenic activity (Liu et al., 2008). 4 h, 2 h and 12 h were suitable HTR value for simple carbohydrates (Chen et al., 2008; Tapia-Venegas et al., 2013). For the OFMSW, the highest H_2 production was related to a HRT of 12 h (Zahedi et al., 2013) and in this case acetic and butyric acid were the main liquid products. For longer hydraulic retention times, the presence of propionic and caproic acids was also observed (Łukajtis et al., 2018).

Liu et al. (2008) investigated the effects of different HRT (1, 2, 3, 4 and 6 days) at a constant pH of 7 and the effect of different pH (5, 5.5, 6, 6.5 and 7) at a constant HRT of 3 days. The combination of pH 5.5 and HRT of 3 days resulted the optimum biohydrogen production conditions.

During dark fermentation, acetate and butyrate productions are always accompanied with hydrogen production. However, excessive concentration of these products could result in the inhibition of the microbes' activities. Therefore, product inhibition is always the critical factor leading to a worse performance scenario in biological hydrogen reactions.

H_2 production in dark fermentation is generally accompanied by the production of acetate and butyrate as the main soluble metabolites and electron sinks. However, the significant accumulation of these soluble metabolites can inhibit the metabolic activity of H_2 producer, possibly due to the increase in ionic strength or inhibition by un-dissociated acids (Elbeshbishy et al., 2017; Srikanth and Venkata Mohan, 2014).

Lactic acid bacteria are well known for their anti-microbial activities, and have been found to partially or completely inhibit dark fermentation. The possible inhibitor activity can to be acidification via production of lactic acid, production of anti-microbial products such as production of reactive oxygen species and secretion of polypeptide antibiotics such as bacteriocins (Gomes et al., 2015; Lee et al., 2013).

Other feasible inhibitors were represented by various metal ions, which at low concentrations can stimulate the cell growth, enzymatic activity, and other metabolic processes involved in dark fermentation. High concentrations of these metal ions may considerably inhibit the fermentation process (Elbeshbishy et al., 2017; Lemire et al., 2013).

3.2.1 Dark fermentation biorefinery

The production of hydrogen based on the concept of a biorefinery could improve the profitability, energy efficiency and reduce the emissions of the processes compared to that based on the stand-alone way. The selection of ethanol and organic acids as valuable co-products of the biorefinery in the hydrogen production process makes a flexible and suitable process to produce energy and chemical carriers.
Ghimire et al. (2015) showed the integration of DF in a biorefinery context. According to this scheme (Figure 3-4) the waste generated from biofuel production such as crude glycerol, de-oiled algal cake or cotton seed cake can be utilized as a substrate. The metabolites produced during the fermentative process can be utilized in the production of micro-algal biomass and biodiesel, which in turn can serve as feedstock for DF processes.

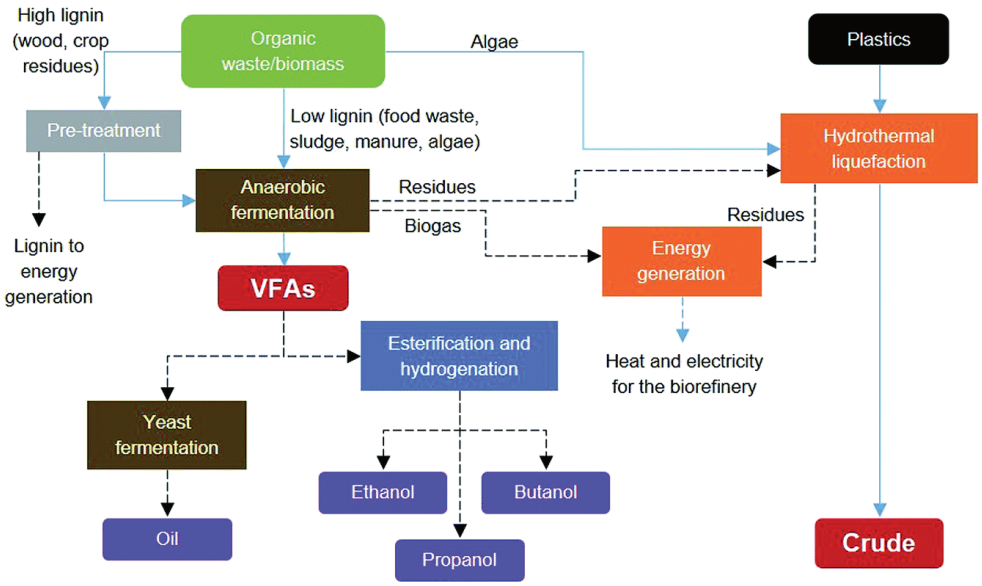


Figure 3-4: System integration for a waste biorefinery concept combining biochemical and thermochemical processes to produce platforms for biofuels or chemical production (Coma et al., 2017b)

Coma et al. (2017) also showed the integration of the VFA conversion into the biorefinery scheme. Different processes were involved in the representation: biochemical processes, which can produce a palm oil substitute, to be used for cosmetics or to the food industry or for biodiesel production; chemical synthesis esterification and hydrogenation, in order to produce mixed alcohols including ethanol, propanol and butanol. This chemical process requires hydrogen. Thus, a

wide integrated route would be needed, possibly via anaerobic fermentation, which could be tuned to produce VFAs and hydrogen, or via steam reforming of biogas or gasification of solid residues.
Extensive analysis and optimization of all processes involved were required in order to define the best integration alternatives for a sustainable waste biorefinery (Coma et al., 2017).
The DF process could also act as pretreatment of the complex organic matter, producing effluents more easily degradable than the initial substrate. The DF effluents could, then, be used for biogas production, injected into microbial electrolysis cell or photo-fermentation reactor in order to produce more H_2. From DF effluents, biomolecules (acetate, butyrate and ethanol) could be extract; these molecules are stably co-produced with H_2 during mixed-culture DF or produced purely by bioelectrochemical processes. Additional fermentation processes could be implemented to extract other molecules such as caproate or Polyhydroxyalkanoates (PHAs) from DF effluents. In the biorefinery content it could redirect the DF process toward the production of metabolites without H_2 production. Varying the process conditions propionate and lactate production, which are anticorrelated with H_2 production, could be promote.

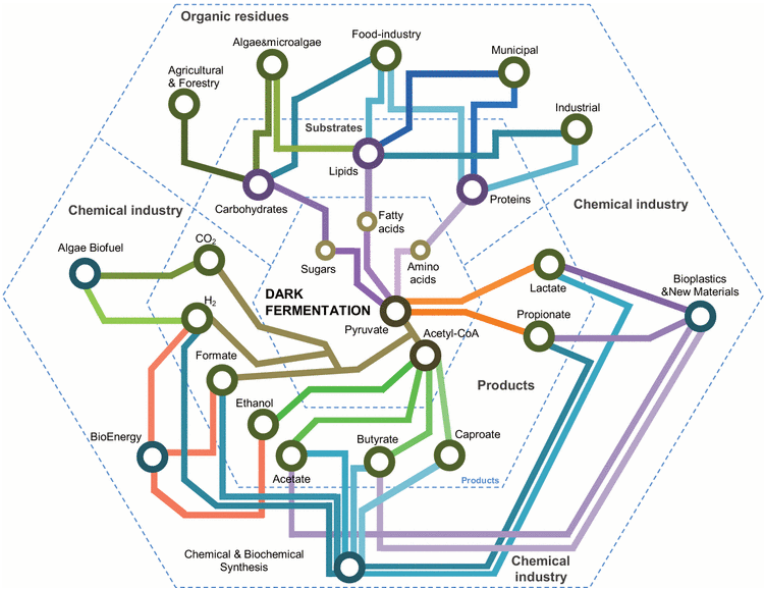


Figure 3-5: Dark fermentation as a core bioprocess in the bio-society (Bastidas-Oyanedel et al., 2015)

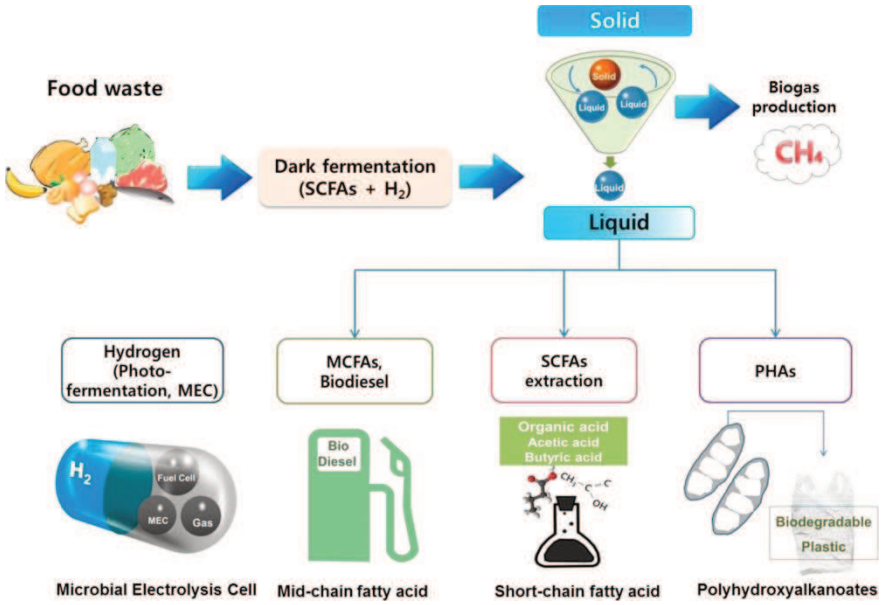


Figure 3-6: Integrated system of dark fermentation of food waste with effluent conversion process (SCFAs = Short-chain fatty acids, MEC = Microbial electrolysis cells, MCFAs = Medium-chain fatty acids, PHAs = Polyhydroxyalkanoates) (Yun et al., 2018)

An integrated system, which include the DF process was showed by (Yun et al., 2018). The lipid and carbohydrate contents of the food waste was used for H_2 production. After successful dark fermentation of FW, short-chain fatty acids (SCFAs) including acetate and butyrate remain, representing 40–60% of the energy content of FW. These SCFAs can be further processed to H_2 by MEC and photo-fermentation. The produced SCFAs can be further converted to liquid biofuels including medium-chain fatty acids (MCFAs) and biodiesel by microalgae growth. Acetate, lactate, and propionate, which are the possible soluble metabolites of dark fermentation, have been biologically elongated to MCFAs. The other possible candidate of chemical that can be derived from DFE is polyhydroxyalkanoates (PHAs), which can be used in the bioplastic production.

4. Pretreatment methods to enhance anaerobic processes yields

Many studies reported that the rate-limiting step for complex organic substrates in the anaerobic digestion (AD) is the hydrolysis (Ariunbaatar et al., 2014; Cesaro and Belgiorno, 2014; Izumi et al., 2010), due to the formation of toxic by-products or volatile fatty acids (VFA) which increase during the hydrolysis step. In some case the methanogenic step was also the rate-limiting one for easily biodegradable substrates.

The limiting activity for the hydrogen (H_2) production in the dark fermentation (DF) process was generally related to the selection of hydrogen-producing bacteria capable of using biomass as substrate. The hydrolysis of the proteins and lipids must be improved during the DF process (Lee et al., 2009; Parthiba Karthikeyan et al., 2018).

The organic fraction of municipal solid waste (OFMSW), mainly composed of lignocellulosic polymers, is extremely complex. It is necessary to apply pretreatments to remove the lignin content and decrease the cellulose crystallinity in order to use the OFMSW for gas biofuels production in the context of waste biorefineries. To this end, it is necessary to understand the effects of the conventional pretreatments applied to OFMSW, with the goal of improving the H_2 production, as well as other biofuels.

Extensive research has been conducted on treatment methods prior to AD and DF, thus obtaining a higher energy recovery and eliminating the extra cost of the processes.

The main objectives of using pretreatment (Ariunbaatar et al., 2014; Cesaro and Belgiorno, 2014; Fisgativa et al., 2016; Karthikeyan et al., 2017; Monlau et al., 2012; Parthiba Karthikeyan et al., 2018) are:

- accelerate the hydrolysis step;
- improve the quality of useful components like nitrogen and phosphorus to be recycled;
- to improve the surface properties for better microbial interactions;
- to reduce/remove the toxic compounds that may affect the process;
- to improve the hydrolysis rate kinetics for proteins and lipids;

- to increase the accessibility of hardly accessible compounds;
- to reduce the organic carbon losses during storage and transport.

A generally effective pretreatment is difficult to implement to different biomass feedstocks. Many pretreatment technologies have been studied during the last decades according to different type of substrate, including sewage sludge, animal by-products, lignocellulosic biomass, food wastes and algae.
In the following paragraphs physical, biological and chemical pretreatments were critically synthetized as main pretreatments applied to the organic solid waste, which increase the cellulose accessibility and could significantly improve the harvested biohydrogen as well as methane in biological processes typically included in biorefineries, such as dark fermentation of wastes and anaerobic digestion. They were discussed, pointing out main strengths and weaknesses, as raised in scientific literature (Alvira et al., 2010; Carrere et al., 2016; Carrère et al., 2010; Cesaro and Belgiorno, 2014; Karthikeyan et al., 2017; Lee et al., 2009; Panagiotopoulos et al., 2009).
Particular attention was given to the feasibility and sustainability of the pretreatment, which are essential in a biorefinery contest. The effects of various pretreatment methods are highly different depending on the characteristics of the substrates. Hence, it is difficult to compare and systematically assess the applicability and sustainability of such methods at a full scale.

4.1 Physical pretreatments

4.1.1 Mechanical pretreatment

Mechanical pretreatment is used to disintegrate and grind solid particles of the substrates.
After mechanical disintegration and releasing cell compounds, the specific surface area increases. This effect provides better contact between substrate and anaerobic bacteria, thus enhancing the anaerobic process (Ariunbaatar et al., 2014; Carrère et al., 2010; Cesaro and Belgiorno, 2014).
Milling/chopping/grinding, screw press, lysis-centrifugation, liquid shear/collision and high pressure homogenization methods can be alternatively used to improve the physical or mechanical properties of organic waste.
Low particle size of the substrates results in higher chemical oxygen demand (COD) degradation and higher methane production rate (Esposito et al., 2011).Thus, particle

reduction can accelerate the hydrolysis and acidogenesis steps as well as the production of volatile fatty acids (VFAs) and this may also result in excessively high organic loading in the anaerobic digestion reactor (Cesaro and Belgiorno, 2014; Mshandete et al., 2006).
The mechanical pretreatment does not affect the original substrate composition, but energy requirements are high. However, it facilitates the digestion/ fermentation in shorter retention time, aids in reducing the particles of the organic residues, without generating any products that may have inhibitory effect; thus, this method is generally associated with increase in biogas production.
Mechanical pretreatments, largely applied in full-scale AD facilities, were extensively studied as an effective technology for OFMSW separation and treatment prior to AD, which could enhance the biogas production by 18 – 36% (Zhu et al., 2009).

4.1.2 Thermal pretreatment

The thermal process hydrolyse the organic constituents of organic wastes, thus enhancing anaerobic process yield. The main effect of this pretreatment is the disintegration of cell membranes, thus resulting in the solubilization of organic compounds.
These methods are classified as wet type and dry type. Lignocellulosic substrates were usually pretreated with dry type, while the wet type was generally used to food waste prior to AD or DF (Yeshanew et al., 2016). Since it is operated under elevated temperatures, it helps to solubilize more sugars through better hydrolysis and provides a more homogenous pulp for feeding AD or DF reactors.
According to Vavouraki et al. (2014), thermal pretreatment methods applied to different types of organic wastes have changed the biomass structure by breaking the intermolecular bonds, and thus aid in the release of soluble organic monomers, that are more accessible and readily biodegradable by anaerobic bacteria. This pretreatment resulted in high solubility of organic waste constituents which promote the conversion of the hydrolysed substances under anaerobic condition to biogas through volatile organic acid production (Yeshanew et al., 2016).
The overall performance of thermal pretreatment on anaerobic process was stable but any significant methane increase was observed.
Solubilization rate was accelerated from the middle to late experimental periods under mesophilic (35 °C and 45 °C) conditions. In contrast, overall solubilization rate was significantly lower under thermophilic (55 °C and 65 °C) conditions than under mesophilic conditions, although solubilization occurred rapidly in the early

experimental period. The production of biogas was high under mesophilic conditions of 35 °C and 45 °C (Komemoto et al., 2009)
According to Komemoto et al. (2009) and Kuo and Cheng, (2007), thermophilic pretreatments cannot improve methane recovery but they can accelerate the hydrolysis of OFMSW, thus shortening the hydraulic retention times for solubilisation and reducing the total volume of the reactors.
After thermal pretratment, it was showed that the COD solubilisation and temperature have a direct correlation. The solubilisation effect increases at lower temperatures, but longer treatment times are needed.
In addition, the thermal pretreatments are easier and quicker than other pretreatment techniques. Another important advantage is that the FW pulp is also sterilized and native unwanted microbes (e.g. lactic acid producing bacteria) that could affect CH_4 or H_2 yields are deactivated. However, the thermal pretreatments promote the formation of recalcitrant compounds over 150°C (with short retention time) or minor to 100°C (longer retention time) that may affect the product recovery.

4.1.3 Microwave pretreatment

Microwave pretreatment uses the electromagnetic field to increase heat, decreasing energy consumption (Cesaro and Belgiorno, 2014). This method uses short waves of electromagnetic energy in a frequency range of 300 MHz – 300 GHz, thus increasing the kinetic energy of water dipoles and bringing it to its boiling point very quickly. Microwave irradiation bonded structures which can be weakened or broken if exposed even if the quantum energy irradiation may not be strong enough to break chemical bonds (Marin et al., 2010).
Microwave was used to pretreat both lignocellulosic substrates and kitchen waste (Marin et al., 2010; Tomás-Pejó et al., 2011).
The heat produced via microwave irradiation promotes the disruption of the recalcitrant structures of lignocellulose, accelerates the destruction of crystalline structures and changes the super molecular structure of lignocellulosic material.
Marin et al. (2010) studied the effect of microwave irradiation on the anaerobic biodegradability kitchen waste. At 175°C solubilisation of sugars and proteins occurred, but this improved solubilisation did not always result in enhanced anaerobic biodegradability.

4.1.4 Ultrasound pretreatment

Ultrasonic pretreatment has been considered as an efficient process for enhancing the biodegradability of organic matter in anaerobic digestion (Cesaro and Belgiorno, 2013). The main effects of the ultrasonic pretreatment are related to monolithic cavitation, which physical and chemical impacts in the slurry. The cavitation bubbles during the sonication modifies the chemical structure by the creation of free radicals. the process involves releasing bioavailable nutrients through hydrolysis as a result of disruption of biosolid flocs and bacterial cells which enhance nutrient solubilization as well as the overall process of anaerobic degradation (Elbeshbishy et al., 2011, 2017). The physical disintegration which occurred during sonication leads to the enhancement of microbial activity, thus improving biogas yield.
Low frequency ultrasonication as a pretreatment step affects the overall performance of anaerobic digestion process. Oz and Yarimtepe (2014) used a ultrasonic frequency of 20 kHz at different time to treat landfill leachate and the higher organic matter solubilisation was found at 600W L^{-1} under 45 minutes of sonication. This pretreatment led to 40% higher biogas production than the control sample.

4.2 Biological pretreatments

Different biological pretreatments of organic waste were studied, such as anaerobic and aerobic methods, enzymatic methods which include peptidase, carbohydrolase and lipase to the AD system. Compared to conventional pretreatment methods, the biological pretreatments are not very popular for OFMSW, but they have been applied widely on other types of organic waste such as wastewater sludge and pulp and paper industries (Ariunbaatar et al., 2014).
Biological pretreatment is usually a slow process that requires longer retention time and the microbes utilize the free and readily available sugars as main carbon source during the pretreatment step. Optimization and maintenance of pure biological agents for pretreatment of FW are usually difficult, since they are competing with the indigenous microorganisms during the pretreatment process. There are only few case studies using pure microbial enzymes for pretreatment of FW because enzymatic pretreatment is expensive and requires high concentrations of enzymes to achieve efficient pretreatment. Instead, crude enzymes produced from biomass lysate are also directly used for pretreatment to reduce the costs (Kiran et al., 2014; Yin et al., 2016). But only limited information is available from the literature and it

is too early to conclude whether the biological pretreatments of FW are viable technologies.

4.3 Chemical pretreatments

This method is effective in breakdown of organic constituents by means the action of acids, alkali and oxidants. These pretreatment methods are used to enhance the biogas production and improve the hydrolysis rate.
However, chemical pretreatment is not very suitable for easily biodegradable substrates containing high amounts of carbohydrates, due to their accelerated degradation and subsequent accumulation of VFA, which leads to failure of the methanogenesis step. However, the acid pretreatment is considered as one of the most important techniques and it aims for high yields of sugars from lignocellulosic substrate. Usually, acids or diluted acids (0.5 - 15% w/w) at temperature ranging between 100°C and 200°C was carried out (Fernandes et al., 2009).
Among the chemical based methods, oxidation (ozonation and peroxidation) has been found useful in pretreatment resulting in sludge solubilisation. A dose dependent relationship exists between sludge solubilization and oxidant concentration up to a certain limit.

4.3.1 Ozonation

Another chemical pretreatment method is ozonation, in which either the ozone or the radicals of the ozone react with the organic matter. The reaction, which does not cause an increase of the salt concentration, solubilizes and reduces the size of organic compounds enhancing their biodegradability (Cesaro and Belgiorno, 2013).
Ozonation promotes the sludge solubilisation and reduces the time of the digestion. Its yield increases with ozone dose. High ozone dose could reduce the solubilisation effects due to the oxidation of the solubilised components.
According to different studies (Ariunbaatar et al., 2014; Cesaro and Belgiorno, 2014), solubilisation efficiency ranged between 25% and 40% at ozone doses of 0.06-0.16 g_{O3}/g_{TSS}. Great performances were found at an ozone dose of 0.16 gO3/gTS, which determined a 37% increase in biogas volume, compared to the one from untreated substrate (Cesaro and Belgiorno, 2013).

4.3.2 Alkaline pretreatment

The alkali pretreatment is efficient in solubilization of proteins and lignin. However, the first reactions promoted by the alkaline pretratment are solvation and saphonication, inducing the swelling of solids. This process involves the increment of the specific surface area. Thus, the substrates are easily accessible to anaerobic microbes. On the other hand, during the alkaline pretreatment, the biomass itself consumes some of the alkali and higher alkali reagents might be required in order to obtain the desired AD yields. Long reaction time was required and salt formation was an important drawback (Ariunbaatar et al., 2014; Carrère et al., 2010; Cesaro and Belgiorno, 2014).

4.3.3 Acid pretreatment

Acid pretreatment is known to be more efficient to solubilize carbohydrates.
This pretreatment was found to be more effective for the anaerobic digestion of lignocellulosic substrates because it promoted the breakdown of the lignin and the acid conditions enhanced the acclimation of the hydrolytic microbes. The addition of the acids promotes great reaction during the process such as the hydrolysis of hemicellulose into perspective monosaccharides as well as the precipitation of the lignin. High concentration of acids used during the pretreatment resulted in the production of inhibitory by-products, such as furfural and hydroxymethylfurfural (HMF). For this reason, high concentrations of acids are not used and the acid pretreatment is better coupled with thermal processes.
Chemical pretreatments could exhibit disadvantages and generally need high cost of chemicals and subsequent chemical disposal.
However, the chemical treatment is the main step in the biorefinery approach of the lignocellulosic materials. This pretratment is used to reduce cellulose crystallinity, increase biomass porosity, and improve enzyme accessibility (Zhang et al., 2016). An effective pretreatment must enhance enzyme efficiency, minimize carbohydrate losses, and inhibit by-product formation.
According to raw material characteristics and desired end products, organic solvent pretreatment is recognized as an interesting chemical treatment because it promotes the fractionation of lignocellulosic biomass into cellulose, lignin, and hemicellulose components with high purity, and it is also characterized by easy solvent recovery and solvent reuse (Zhang et al., 2016).

4.3.4 Organic solvent pretreatment

Organic solvent pretreatment involves the use of an organic or aqueous-organic solvent with or without the addition of a catalyst (acid, base or salt).
Compared with dilute acid, steam, or alkaline processes, organic solvent pretreatment can achieve high-extent removal of hemicelluloses and lignin simultaneously in one-pot process, with great reduction of particle size, degree of polymerization of cellulose, and increase in porosity and cellulose accessible surface area (Zhao et al., 2017).

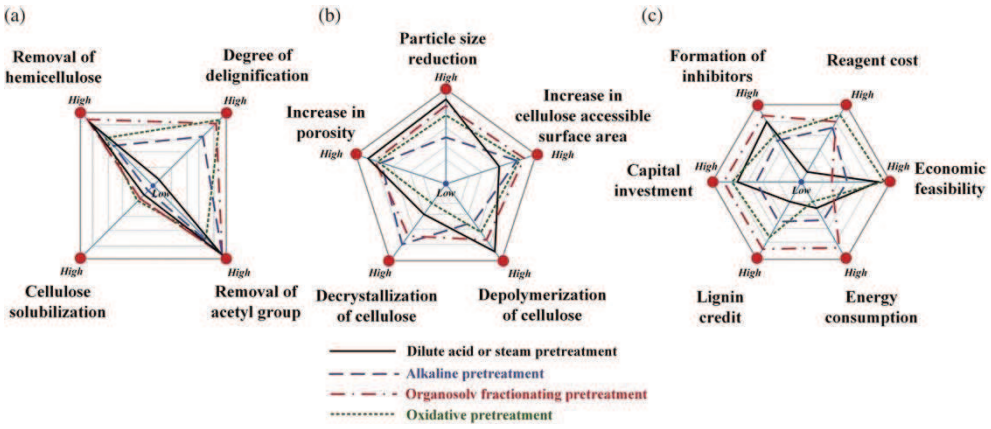


Figure 4-1: Qualitative comparison of organosolv fractionating pre-treatment with other leading pre-treatment technologies by radar charts: (a) removal of chemical components, (b) structural features, and (c) economy relevant parameters (Zhao et al., 2017)

Organic solvent processes are alternative methods for the delignification of lignocellulosic materials, in order to promote biofuels and biochemical production (Zhang et al., 2016). It has been used as pretreatment for the production of lignin and other potentially valuable co-products (e.g. acetone, butanol, ethanol and biogas), as shown in Figure 4-2. The mixture of organic liquid, water and catalysts partially hydrolyses lignin bonds and lignine carbohydrate bonds, resulting in a solid residue composed mainly of cellulose and some hemicellulose.

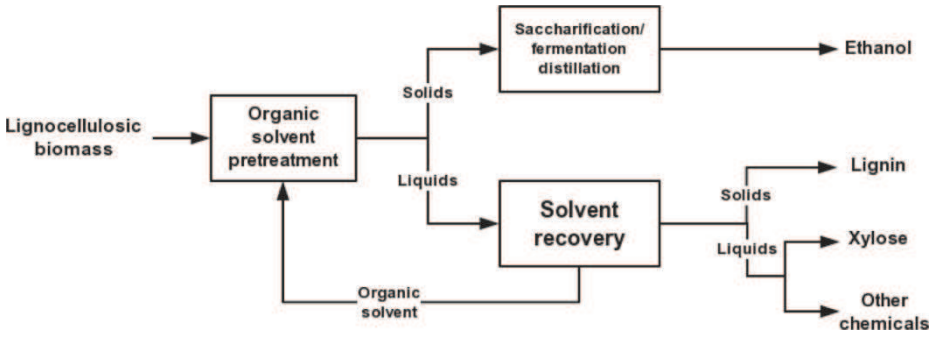


Figure 4-2: Organic solvent pretreatment of lignocellulosic biomass (Zhang et al., 2016)

Due to the process, cellulose is delignified, with the organic solvent functioning as lignin extraction solvent, and the hemicellulose is depolymerized through acid hydrolysis by the added acid or the acid that is formed from the acetyl side groups of the hemicellulose at elevated temperature (Huijgen et al., 2010).
In this way the organic solvent pretreatment could be used in a suitable biorefinery process, which is considered the sustainable processing of biomass into a spectrum of marketable products (food and feed, materials, and chemicals) and energy (fuels, power, and heat). In biorefinery appropriate fractionation of the complex lignocellulose material, into its constituents, is of great importance.

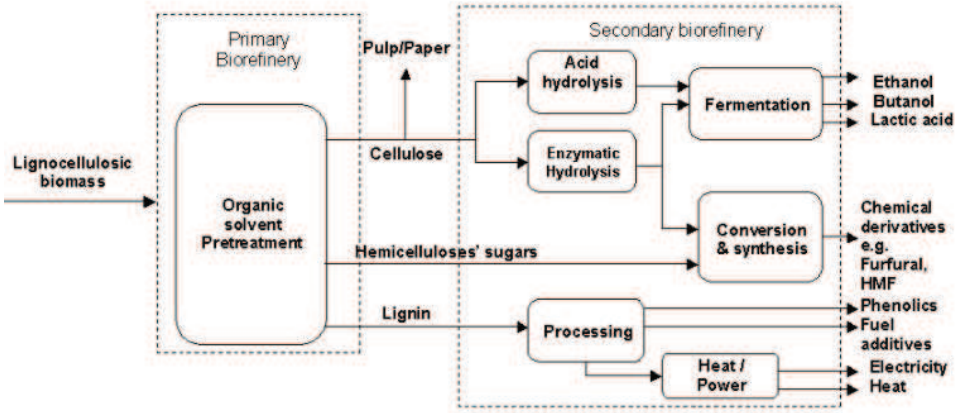


Figure 4-3: Organosolv-based lignocellulosic biorefinery (Salapa et al., 2017)

As lignocellulosic materials, the organic fraction of municipal solid waste are attractive for the production of biofuels and biochemicals, due to their abundance

and carbohydrate content. The conversion of this organic waste prevents hydrolysis of polysaccharides, hence demanding a pretreatment step.
In the scientific literature there are no reports on the application of organic solvent pretreatment for improving biohydrogen or methane yields from OFMSW.
The organic solvent pretreatment is an emerging approach because lower amounts of inhibitory compounds are generated when compared with dilute acid pretreatment (McDowall et al., 2014; Sinha et al., 2015).
Different organic solvents, such as alcohols, phenol, acetone, propionic acid, dioxane, amines, esters, formaldehyde, and chloroethanol with and without catalyst, have been investigated within the organic solvent pulping (Zhao et al., 2009) process. Ethanol (Sidiras, 2004), methanol (Gilarranz et al., 1999), acetone (Huijgen et al., 2010), formic acid (Dapia et al., 2002; Snelders et al., 2014), acetic acid (Snelders et al., 2014) and glycerol (Sun et al., 2015) appear to be the most popular organic solvents considered over the years.
Ethanol is a suitable organic solvent for the pretreatment of biomass at low boiling point. Use of ethanol in organic solvent pretreatment is beneficial due to low solvent price, lack of toxicity, full miscibility with water, and ease of recovery (Sidiras, 2004). Methanol pretreatment can also be carried out with or without catalyst temperatures ranging between 170 and 200°C. Without catalyst, methanol pretreatment requires an increased operating temperature in order to achieve acidification process. The main use of methanol pretreatment is in the field of pulping in order to produce high quality cellulose fibre (Zhang et al., 2016).
Organic acid pretreatment was used to treat lignocellulosic biomass under mild temperatures and time conditions. In most extreme cases, the organic solvent process was conducted at temperatures ranging from 150 to 200 °C for 2–5 h with acid concentrations from 75% to 95%.

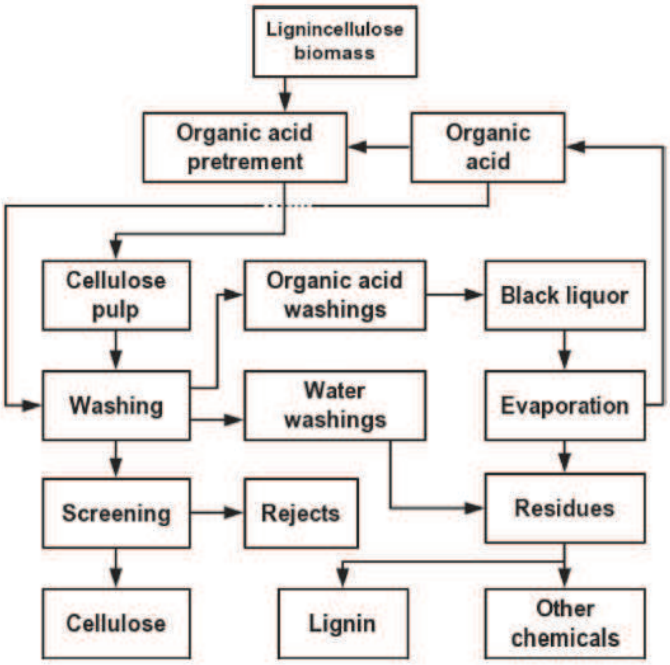


Figure 4-4: Flowchart of organic acid pretreatment of biomass (Zhang et al., 2016)

Many advantages were related to use of organic acid. After filtration, the solid fraction of the sample can be washed with fresh organic solvent and water to prevent lignin precipitation and rejects for high-purity cellulose. The liquor rich in lignin can be separated by means evaporation and it can be recycled (Figure 4-4).

The application of the organic acid pretreatment to organic solid waste could promote its different valorization compared to the conventional management.

Organic acid is an emerging solvent because of its inherent advantage of solubility value close to the value of lignin (Zhao et al., 2009). Lignocellulosic biorefinery for sustainable processing using an acetic and formic acid based organosolv fractionation process was also implemented in European Union project, such as ‘‘BioCOmmodity REfinery’’ (or in short ‘‘BIOCORE’’, 2010-2014).

Formic acid and acetic acid have much higher solubility to lignin than other organic solvent, and the pretreatment can be conducted under much mild condition, such as the temperature minor to 100°C under atmospheric pressure. Formic acid and acetic acid pretreatment also causes hydrolytic breakdown of biomass polymers into smaller and more soluble molecules. Organic acid pretreatment is usually performed at 80–110°C, under 60–98% acid concentration, with 0–1% mineral acid as the catalyst for 0.5–2 h. For formic acid pretreatment, lower temperature should be used when no mineral acid is added to obtain a high disintegration degree (Zhao et al.,

2017). Among different organic acids and organic solvents tested, formic acid was found to be effective on the acid hydrolytic reaction in order to promote sugarcane bagasse conversion (Sindhu et al., 2010), enzymatic hydrolysis and biogas production from rice straw (Amnuaycheewa et al., 2016). Formic acid concentration demonstrated a very significant effect also on polysaccharide solubilisation and formylation of cellulose (Zhao and Liu, 2012).

Compared with the other organic solvent, the formic acid was also involved in the H_2 production and storage (McDowall et al., 2014; Sinha et al., 2015). The formic acid has shown a great potential as active agent for the dissociation of hydrogen ions (Wang et al., 2018), the acceleration of biomass hydrolysis (Ding et al., 2018; Özyürek and van Heiningen, 2018) and the improvement of the carboxyl content (Dai et al., 2014). It is the simplest carboxylic acid and compared to other organic molecules, the temperature for its decomposition is lower, resulting in less CO toxicant species, so that it is regarded as an effective pretreatment option, able to increase the surface area of the substrate and to solubilize it to produce value-added pulps (Sindhu et al., 2010; Zhao et al., 2017).

So, the use of the formic acid as organic solvent in the pretreatment could be promote the conversion of the OFMSW into a value-added products in a biorefinery content similar to that obtained for lignocellulosic materials (Figure 4-3).

5. Research experimental activity

The efficient utilization of the organic fraction of municipal solid waste (OFMSW) and the reduction of the treatment costs are mandatory to attain a cost-effective valorisation of this residual stream.
Growing interest in producing biochemicals using mixed organic waste is argued as well as the production of heat, electricity and fuel (Chen et al., 2018, 2017c).
Anaerobic digestion (AD) and dark fermentation (DF) processes combined to various technologies can be used to tackle the rising interest of by-products from organic waste.
AD is very effective in stabilizing organic waste producing biogas, which mainly consists of methane and can be used as an energy source. AD is a suitable treatment for a wide range of solid and liquid wastes, as long as they contain a sufficient carbon source and nutrients (Zhang et al., 2018).
DF represents one of the most promising and cost-effective technologies for bio-hydrogen production due its faster conversion efficiencies. DF process can utilize a wide range of renewable complex waste biomass as feedstock and produce other valuable platform bio-chemicals of economic interest (Ghimire et al., 2015b).
The by-products of DF process, which mostly includes volatile fatty acids (VFAs), lactic acid, alcohols and un-hydrolyzed residues, can be utilized in other biological systems for their valorization via energy recovery or can be used as a feedstock in production of platform chemicals of economic interests (Agler et al., 2011; Bastidas-Oyanedel et al., 2015; Ghimire et al., 2018).
The selection of suitable pretreatment that allows an effective fractionation of biomass and the use of the pretreated material in different processes to recovery bio-products are considered promising strategies for that purpose.
The fractionation of feedstocks substrate by means pretreatment is a necessary process in order to improve the efficiency of bioconversion. Proper pretreatments prior to the anaerobic treatment can to overcame the limiting steps of the process and to make the substrate suitable for the degradation (Saha et al., 2016). The organic solvent pretreatment of biomasses have been studied in order to produce high-quality intermediates, demonstrating higher efficiency for lignocellulosic biomass fractionation and readily recovery solvent by means distillation process (Zhang et al., 2016). As available organic solvent, formic acid showed potential as active agent

for dissociation of hydrogen ions (Wang et al., 2018), acceleration of biomass hydrolysis (Ding et al., 2018; Özyürek and van Heiningen, 2018), increase the carboxyl content (Dai et al., 2014).
The treatment with organic solvents is an effective alternative method to promote the conversion of the fourth generation of biomass, like OFMSW. The organic solvent pretreatment may to upgrade the acceleration of biomass utilization, to increase the surface area and to induce the solubilisation in order to produce other value-added pulps enhancing the additional value of OFMSW (Stephen and Periyasamy, 2018; Yang et al., 2015).
In this study, the organic solvent process was considered as organic solid waste pretreatment : its effectiveness, under different operating conditions, was discussed in terms of mineralization and/or solubilization, on anaerobic digestion and dark fermentation yields, in order to evaluate the technical and economic feasibility of the combined treatment providing the best performances for the valorization of OFMSW.

5.1 Experimental plan

The main objectives of this research were to study the process parameters influencing the organic solvent pretreatment of the organic solid waste and the valorization of the by-products (bio-energy and bio-chemicals) in a biorefinery framework. The specific objectives were:

- to identify an appropriate chemical pretreatment;
- to explore the effects of different operating conditions via variance analysis;
- to estimate bio-methane or bio-hydrogen production from pretreated substrates by means of anaerobic processes;
- to investigate the production of value added bio-chemical intermediates produced from substrates treatment.

To achieve these major aims, the research activity was structured in two main steps:

- the study of various combinations of the different operating conditions of the pretreatment according to a factorial design and the analysis of the effects of these pretreatment combinations on the organic matter composition of the substrates by means an analysis of variance; the implementation of Biochemical Methane Potential (BMP) tests on the pretreated substrates and the estimation of bio-methane production;
- the evaluation of the effects of the pretreatment on DF yields in terms of H_2 and soluble metabolites production.

In the last step of the experimental activity, the combination of the organic solvent pretreatments and the dark fermentation tests of the organic waste was performed in order to promote the simultaneous recovery of valuable streams of biochemicals.
The first two phases of the experimental activity were conducted in the Laboratory of Sanitary Environmental Engineering (SEED) of the University of Salerno (Italy). The last part of the research activity was performed at the Laboratory of the Environmental Biotechnology (LBE) of the National Institute for Agricultural Research (INRA – France).

6. Materials and methods

In the following paragraphs the experimental setup and the analytical procedures implemented for the research activity are described.
The first section reports the materials and methods used for the identification of the most suitable experimental conditions for OFMSW pretreatment, according to analysis of variance as well as the evaluation of the influence of the operating conditions on the physical characteristics as well as on the and biochemical potential of the pretreated substrates.
The second part of the chapter focuses on the materials and analytical methods followed for the study of the effects of the pretreatment on the biohydrogen and biochemical production.

6.1 Analysis of the formic acid pretreatment operating conditions

6.1.1 Substrates

The experimental tests were performed using the organic fraction (OF) and the lignocellulosic fraction (LF) of municipal solid waste as substrates. The synthetic OF was freshly prepared with a well-defined composition (Table 6-1). Four different major organic waste species were combined (Alibardi and Cossu, 2015; Favaro et al., 2013; Fisgativa et al., 2016) in order to obtain the OF substrate for the experiments. The mixed meals, drinks and snacks fraction, which have been identified as minor components of the OFMSW, were not considered in the composition in order to simplify the substrate preparation for experimental purposes. The LF substrate was characterized by tree leaves (50%) and wheat straw (50%), which represent the typical agricultural products used as alternative to energy crops in anaerobic processes. The LF was used as a control single substrate as well as to prepare a mixture with OF. Thus, the experimental tests were carried out on:

- LF samples;
- mixed samples containing 70% w/w of OF and 30% w/w of LF;

- OF samples.

The ratio (% w/w) between lignocellulosic residues and organic waste was driven by the usual values adopted to mix these substrate in full-scale biological treatment facilities.

Table 6-1: Composition in wet weight of the OFMSW substrate

Fraction	Percentage[a] [%]
Cooked meat-cooked fish-cheese	16,2
Fruit peeling	32,2
Vegetable peeling	32,1
Bread and cooked pasta	19,5
Total	100

[a] Determined by weight, wet basis

After being collected, the substrate was ground to reduce the particles size up to about 3 mm, and stored at 4 °C until use. In order to determine the characteristics of the water soluble matter, a water extraction was performed at room temperature on 1:20 substrate/water ratio for 1 h under agitation. The mixture was then centrifuged (15000 rpm for 15 min) to obtain the sample soluble fraction (D'imporzano and Adani, 2006). The main characteristics of the substrate used for the experiments were reported in the Table 6-2.

pH was measured by pHmeter model HI 99121 (Hanna Instruments). The total solid (TS) and volatile solid (VS) contents as well as the soluble COD were evaluated according to Standard Methods (AWWA-APHA-WEF, 1998). The elemental characterization of the substrates was performed by the Elemental Analyzer OEA Flash 2000 (Thermo Finnigan). Total Organic Carbon (TOC) was determined using a Total Organic Carbon Analyzer coupled to a Shimadzu (model 5000A). Total Kjeldahl nitrogen (TKN) and ammonia nitrogen contents were measured with digestion, distillation and titration processes. The carbohydrate concentration by the Dubois method (Dubois et al., 1956) and the protein concentration was determined by the modified Lowry method ((Fr/olund et al., 1995).

Table 6-2: Characterization of the substrates

Parameter	$LF_{untreated}$	$(OF + LF)_{untreated}$	$OF_{untreated}$
pH	6,2 ± 1,8	6,0 ± 1,2	6,8 ± 1,1
TS [%]	23,7 ± 5,8	75,5 ± 1,4	26,7 ± 2,1
VS [%TS]	58,4 ± 1,6	23,8 ± 1,6	50,3 ± 3,3
sCOD [mg/l]	13550 ± 8820	30150 ± 7700	28350 ± 9750
TOC [mg/l]	450 ± 48	1500 ± 95	750 ± 35
TKN [mg/kg]	9,5 ± 1,4	3,6 ± 1,9	6,5 ± 0,8
C [%]	48,5 ± 10,3	33,7 ±11,5	28,3 ± 9,8
H [%]	4,6 ± 1,2	6,0 ± 0,8	5,1 ± 1,3
N [%]	9,1 ± 4,2	3,6 ± 0,6	2,2 ± 1,3
Carboydrates [%]	29,2 ± 5,8	16,8 ± 0,6	22,1 ± 5,1
Proteins [%]	14,7 ± 1,6	19,5 ± 5,7	16,5 ± 3,8

6.1.2 Pretreatment experimental setup

A sequential pretreatment of the substrates was carried out (Figure 6.1). To this end, 300 ml flasks with specific solid-liquid ratio were used. 30 grams of substrate were immersed in a 15 % v/v of formic acid/water solution and pretreated in autoclave at 120°C for 70 minutes (Amnuaycheewa et al., 2016; Sannigrahi et al., 2010; Sindhu et al., 2010).

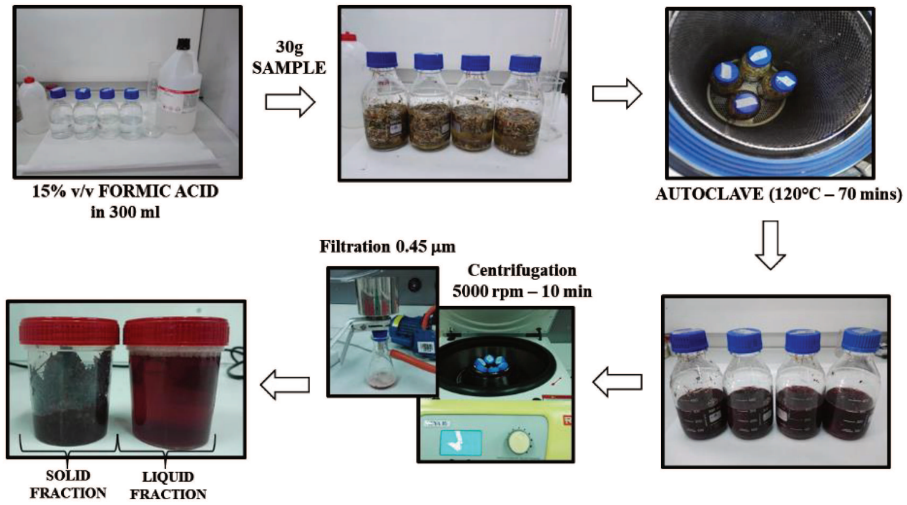


Figure 6-1: Steps of the formic acid pretreatment with solid-liquid separation

The samples processed in autoclave were centrifuged at 5000 rpm for 10 min, the supernatant were then filtrated through a filter with 0,45 μm porosity (Zhang et al., 2016).
The solid and liquid fractions were collected and stored at 4°C until use. The solid fraction of the substrates was used for the BMP tests, while the liquid fraction was analyzed in order to evaluate the metabolic pathways (Figure 6-2).

6.1.2.1 Design of experiment and statistical analysis

Preliminary tests were performed using waste samples pretreated with 15% v/v acid formic/water solution for 70 minutes at 120°C, as described above.
Then, in order to verify the significance of the main operating parameters, as identified in scientific literature, the experiments were conducted according to a 2^n type factorial design in which "n" is the number of the factors and "2" is the number of levels of the factors. The factors tested (Table 6-3) were the formic acid concentration (X), the autoclave temperature (Y) and the operating time (Z).

Table 6-3: Values of the pretreatment operation parameters

Factors	High value	Low value
Formic acid concentration [%]	15	5
Operating temperature [°C]	120	80
Operating time [min]	70	35

This factorial design established 8 different experimental test runs, as reported in the Table 6-4.

Table 6-4: Operating conditions for the investigated test run

Test run	Formic acid concentration [%]	Operating temperature [°C]	Operating time [min]
a	15	120	70
b	15	80	70
c	15	80	35
d	5	80	70
e	5	120	70

f	5	120	35
g	5	80	35
h	15	120	35

A statistical study of the data was developed by means of the R-Studio statistical software. An analysis of variance (One-way ANOVA test) was carried out in order to estimate the significance of the operating conditions on analysed parameters. The value of probability (Pr), so called P-value, lower than 0.05 indicate that model terms are significant at the 95% confidence level. The principal components were orthogonal to each other, therefore carry no redundant information. However, the assumption of independence (null hypothesis), normality and homogeneity of variances (homoscedasticity) were assumed in order to implement the ANOVA test. For the independence assumption, each sample is randomly selected and independent. The null hypothesis for ANOVA is that the average value of the dependent variable is the same for all groups. The ANOVA test procedure produces an F-statistic, which is used to calculate the P-value. If P-value results minor to 0.05, the null hypothesis can be reject and it possible to conclude that the average of the dependent variable is not the same for all groups. The dependent variables should be approximately normally distributed for each category of the independent variables. As regard the one-way ANOVA, it requires approximately normal data because it is quite "robust" to violations of normality, meaning that assumption can be a little violated and still provide valid results. The Shapiro-Wilk test was used to verify the normality hypothesis. The assumption of homogeneity of variance is important because the selected independent variables within the multiple regression model which was considered could not be statistically significant. The test of Barlett is used to tests if the samples was from variables with equal variance.

6.1.3 Biomethane Potential (BMP) tests setup

The BMP tests were carried out under controlled conditions. 1000 ml glass bottles equipped with silicon discs and plastic screw caps punched in the middle (Schott Duran, Germany) were used. The experiments were conducted in triplicate with a substrate (S) to inoculum (X) ratio (S/X) of 0,5 g VS substrate/g VS inoculum.
Digested sludge was used as inoculum and it was chosen because it has a biologically active methanogenesis population (Momayez et al., 2019; Xu et al., 2016).
The digested sludge was sampled at the anaerobic digester of the conventional wastewater treatment plant in Salerno (Italy) and its main chemical-physical characteristics are reported in Table 6.3.

Table 6-5: Characterization of digested sludge used as inoculum

Parameter	Digested sludge
pH	6,9 ± 0,8
TS [%]	3,1 ± 1,8
VS [%TS]	47,5 ± 2,7
sCOD [mg/l]	67 ± 3,4
C [%]	18,2 ± 1,9
H [%]	2,9 ± 0,5
N [%]	2,7 ± 0,6

The bottles were incubated at constant temperature (37 ± 1 °C) with a thermostat in a water bath. Headspaces of the bottles were flushed with nitrogen gas for about 5 min and then the bottles were sealed with rubber septa and gas collection system connected.
The daily biomethane production was measured as reported by (Esposito, 2012).

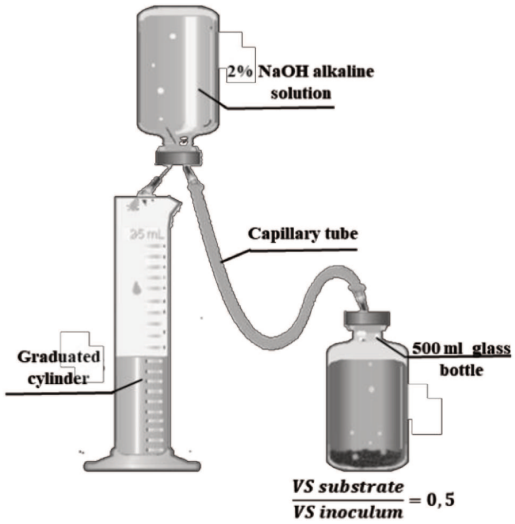


Figure 6-2: Scheme of the BMP test

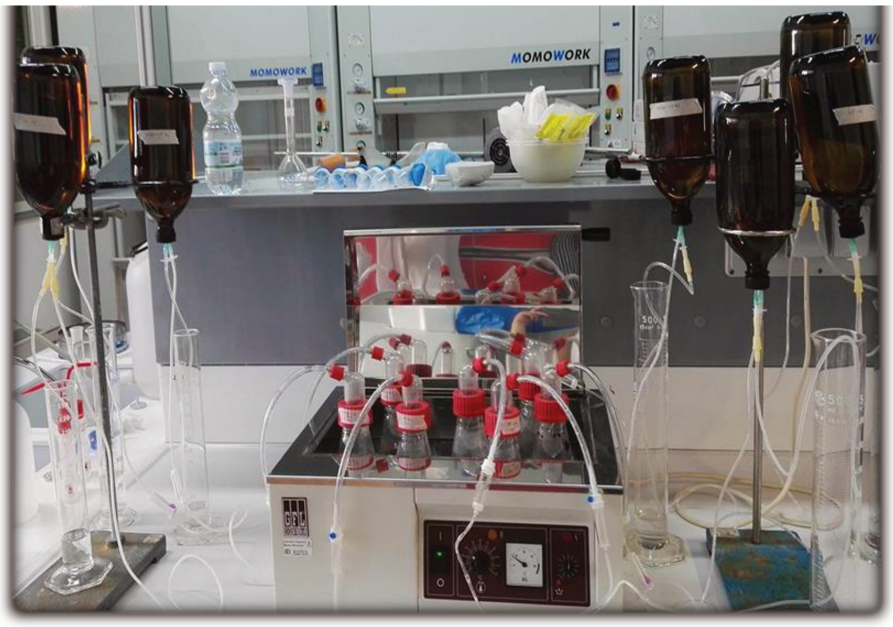


Figure 6-3: Experimental setup of the BMP test

According to (Holliger et al., 2016), biomethane production was measured by a volumetric technique in which the produced CO_2 is trapped in alkaline solution and only the volume of CH_4 is determined. Each bottle was connected by a capillary tube, which equipped on both ends with a needle to an inverted glass bottle containing 2% NaOH/water solution. This promote the gas transfer between the bottles. Another capillary tube connected the inverted glass with a graduated cylinder. Manual mixing once a day was carried out to avoid scum layer formation.
At the end of AD tests, pH was measured by a pH-meter and TS and VS content was estimated.

6.1.1 Analytical procedures

The characterization of both the raw materials and the pretreated samples was carried out on the solid and liquid fractions obtained from the centrifugation of the substrates.
The solid fractions were characterized in terms of Total Solids (TS) and Volatile Solids (VS), pH, soluble Chemical Oxygen Demand (sCOD), the elemental characteristics (C, H, N), the Total Kjeldahl nitrogen (TKN), the Total Organic

Carbon (TOC). In the case of the raw material characterization, the carbohydrate and protein contents were also determined.
The characterization of the solid fraction of the pretreated samples was related to the evaluation of the solubilisation or mineralization effect due to each operating condition of the pretreatment.
For the liquid fraction of the samples, the pH and the sCOD were estimated as well as the total Volatile Fatty Acid (VFA) concentration and the Acid Soluble Lignin (ASL) content. The VFA concentration was analysed in order to evaluate the fractionation effect of the pretreatment on the biomass; ASL content was also estimated because lignocellulosic biomass was used in the experimental tests.
Total Solids (TS) and Volatile Solids (VS) were determined according to Standard Methods of the American Public Health Association (APHA, 1998). The pH determination was carried out on each sample after 1:20 dilution, agitation for 1h and centrifugation (15000 rpm, 15 minutes); the pH was, then, measured by pHmeter model HI 99121 (Hanna Instruments).
The chemical oxygen demand (COD) was initially determined according to Standard Methods (AWWA-APHA-WEF, 1998). The sCOD test was carried out on each sample after centrifugation (5000 rpm, 10 minutes) and filtration (< 0.45 μm); the 1:20 dilution was necessary for the analysis of the solid fractions.
The disintegration degree expressed in terms of the sCOD (DDCOD) was also evaluated in order to better discuss the solubilisation effect of the pretreatment on the substrates. The following equation showed the calculation expression of the DDCOD, which was estimated as the ratio of COD increase by pretreatment to the COD increase by the chemical disintegration:

$$DD_{COD} = \frac{COD_{pretreatment} - COD_0}{COD_{NaOH} - COD_0} \; x \; 100\%$$

where CODpretreatment is the COD of the pretreated solid fraction (g/l), COD_0 is the COD of the untreated substrates (g/l), COD_{NaOH} is the COD of the reference substrate (g/l).
The elemental characterization of the substrates was performed by the Elemental Analyzer OEA Flash 2000 (Thermo Finnigan).
The CH_4 volume produced during the BMP test was estimated as the NaOH solution accumulated in the graduated cylinder. Temperature and pressure at the measurement were also recorded in order to convert the measured gas volume to dry gas at standard conditions (273.15 K, 101.33 kPa).
The CH_4 volume was daily calculated according to the following expression:

$$V_{CH_4} = V_{CH_4}^{(NaOH)} \cdot \frac{P}{P - P_w}$$

where:

- $V_{CH_4}^{(NaOH)}$ is the NaOH solution volume daily accumalated into graduated cylinder;
- P is the atmospheric pressure;
- P_w is the vapor pressure at 35°C.

The cumulative CH_4 flow rate was estimated as follows:

$$F_{CH_4} = \frac{V_{CH_4}^{cumulative}}{M_s \cdot TS \cdot VS}$$

where:

- $V_{CH_4}^{cumulative}$ is the cumulative NaOH solution volume daily accumalated into graduated cylinder;
- M_s is substrates mass used to batch test;
- TS is the total solid content of the substrate into the bottle;
- VS is the volatile solid content of the substrate into the bottle.

This specific methane production that corresponds to the BMP of the substrate was evaluated. It is expressed as volume of dry methane gas produced per mass of VS of the substrate added ($mlCH_4\ gVS^{-1}$).
The liquid fraction of the substrates was analysed in terms of total VFA concentration. Standard Distillation Method 5560C was used to determine the VFA concentration, which was expressed as acetic acid equivalents. The VFA analysis was carried out on each liquid sample after centrifugation (5000 rpm, 10 minutes) and filtration (< 0.45 μm). The VFA bioconversion yield (Y_{VFA}) was also evaluated and it was defined as the ratio of VFA produced to the amount of consumed substrate (Bengtsson et al., 2008) according to the following equation:

$$Y_{VFA} = \frac{VFA_{OUT} - VFA_{IN}}{(COD_{IN} - VFA_{IN}) - (COD_{OUT} - VFA_{OUT})}\ x\ 100\%$$

where VFA_{OUT} and COD_{OUT} are the VFA and the sCOD concentration of the pretreated substrates (g/l), VFA_{IN} and COD_{IN} are the VFA and the sCOD of the untreated substrates (g/l).

Since in the experimental tests LF substrates were used, the ASL content was determined by means the analysis with ultraviolet–visible (UV–Vis) spectrometry was employed. The ASL determination was carried out on each liquid sample after centrifugation (5000 rpm, 10 minutes), filtration (<0.45 μm) and suitable dilution (from 1:20 to 1:50) in order to avoid the spectrometry interference. The The following equation shown the calculation in order to estimate the ASL content:

$$ASL\ content\ (wt\%) = \frac{D\ x\ V_{filtrate} x\ (A_{sample} - A_{blank})}{a\ x\ m}\ x\ 100\%$$

where "D" is the dilution ratio (volume of the filtrate + volume of dilution)/ volume of the filtrate), "$V_{filtrate}$" is the filtrate volume (L), "A" is the absorbance and "a" is the absorptivity which are 280 nm and 23,6 L g^{-1} cm^{-1} respectively for organic waste samples , and "m" is the mass in grams of the dry feed (Hidajat et al., 2017).

6.2 Studies focused on the pretreatment effects

The second part of the research activity, which was conducted at LBE (INRA - France), was addressed towards the pretreatment effectiveness on the production of both hydrogen and metabolites by means Bio-Hydrogen Potential (BHP) tests.

6.2.1 Substrates

The experimental activity was carried out using OF+LF and OF substrates. These substrates were prepared according the methods adopted in the previous paragraphs. The composition of the OF sample was reported in Table 6-1. The mixed sample contained 70% of OF and 30% of LF, in which the LF was composed of 50% of tree leaves and 50% of wheat straw.
The samples was reduced in size (3 - 4 mm) and was stored at 4 °C until use. The characteristics of the substrates were determined after water extraction according to (D'Imporzano and Adani, 2006). The main characteristics of the substrates used for the experiments were reported in the Table 6-6.

Table 6-6: Characterization of the substrates

Parameter	$LF_{untreated}$	$(OF + LF)_{untreated}$	$OF_{untreated}$
pH	6,5 ± 1,2	6,4 ± 0,3	6,7 ± 0,2
TS [%]	22,5 ± 5,5	78,5 ± 2,3	29,8 ± 2,8
VS [%TS]	52,9 ± 3,8	22,8 ± 0,7	49,2 ± 3,7
sCOD [mg/l]	17350 ± 8820	33100 ± 7700	22325 ± 10745
TOC [mg/l]	398 ± 88	1648 ± 41	677 ± 76
TKN [mg/kg]	6,9 ± 0,8	3,0 ± 1,3	5,8 ± 1,9
Carboydrates [%]	36,2 ± 5,1	18,8 ± 1,9	27,1 ± 4,7
Proteins [%]	16,3 ± 2,9	11,9 ± 3,1	15,3 ± 2,3

The total solid (TS) and volatile solid (VS) contents were evaluated according to Standard Methods (AWWA-APHA-WEF, 1998). The chemical oxygen demand (COD) was analyzed using an Aqualytic 420721 COD Vario Tube Test MR (0 -1500 mg/l). Two mL of sample were pipetted into each tube and then they were placed inside a ECO 25 Thermoreactors for COD (Velp Scientifica) at 150 °C for 2 h. Soluble COD concentrations were determined using an Aqualytic Multidirect spectrophotometer. pH was measured using a pHmeter Eutech Instrument pH510. Total Kjeldahl nitrogen (TKN) and ammonia nitrogen contents were measured with Digest Automat K-438 and an AutoKjehdahl Unit K-370, BUCHI. Total Organic Carbon (TOC) and Inorganic Carbon (IC) were determined using a Shimadzu TOC-VCSN Total Organic Carbon Analyzer coupled to a Shimadzu ASI-V tube rack. The Total Carbon (TC) content corresponded to the sum of TOC and IC.

6.2.2 Pretreatment experimental setup

The sequential pretreatment studied in the first step of the research activity was implemented again as shown in Figure 6-1

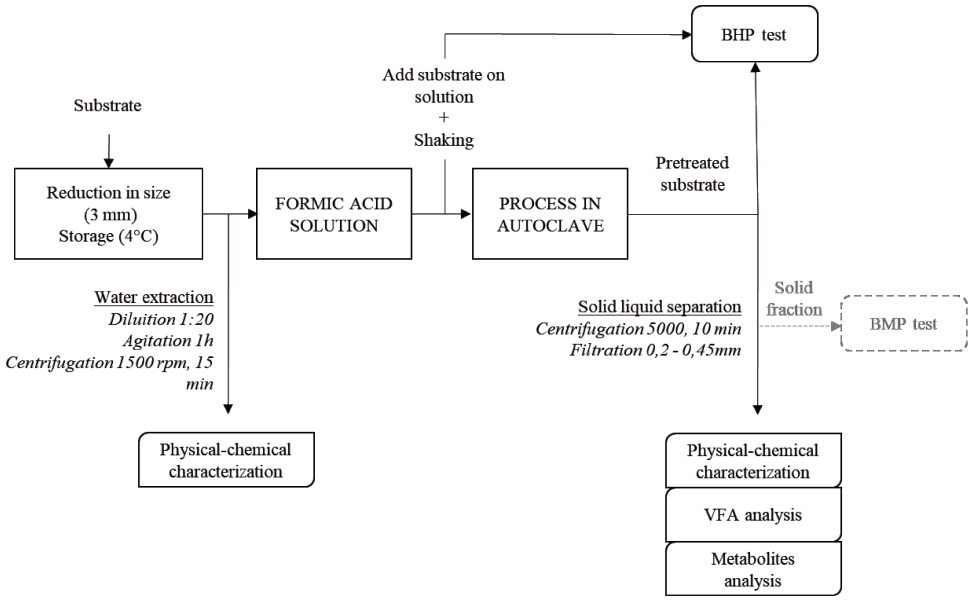


Figura 6-1: Sequential approach of the pretreatment

At the end of the autoclave process no solid-liquid separation of the pretreated substrates was carried out and the samples were completely tested for BHP.
All pretreatment combinations reported in the Table 6-4 were implemented; some substrates were pretreated adding the formic acid without process in autoclave in order to better understand the effect of the formic acid on the substrate degradation during the DF process.

6.2.3 Biohydrogen Potential (BHP) test setup

BHP tests were performed in batch reactors under mesophilic conditions. Pretreated samples and control raw material samples were used as substrates.
In these experiments batches were specific bottles, hermetically closed with a plug equipped with a central silicon septum which allowed gas sampling by a needle. The tests were carried out in quadruplicates in 400 ml flasks. The working volume consisted of 220 ml of substrate, 5 - 130 ml of 2-(N-morpholino) ethanesulfonic acid

(MES) buffer and 1 – 10 ml of 3.2% NaOH. Heat treated inoculum was then added to reach a substrate/inoculum (S/X) ratio of 10 gVS substrate/gVS inoculum. The initial pH was adjusted at 5.5 using MES buffer and it was set-pointed at 6 ± 0.2 using NaOH.

Figure 6-4: Multiplexing the channels (a), conductivity detector of the micro-gas chromatograph (SRA Instrument) (b), water bath equipped with thermostat (c), inoculum (d), purging with nitrogen gas (e)

In this case, waste activated sludge was used as inoculum (Ghimire et al., 2015a) and sampled from a French municipal wastewater treatment plant. (Baldi et al., 2017; Ghimire et al., 2018) highlighted a better response of activated sludge on thefinal hydrogen generation in comparison with anaerobic sludge. However, the pre-treatment of the sludge prior to DF is an indispensable step to inhibit methanogenesis activities and H_2 consuming populations (Rafieenia et al., 2018).
Thus, the sludge was pretreated by heat/shock treatment (90°C, 30 min) and it was used in order to obtain a substrate (S) to inoculum (X) ratio (S/X) of 10 g VS substrate/g VS inoculum. The characteristics of the activated sludge is reported in Table 6-7.

Table 6-7: Characterization of the activated sludge used as inoculum

Parameter	Waste activated sludge
pH	6,7 ± 0,2
TS [%]	10,1 ± 2,0
VS [%TS]	34,3 ± 0,9
sCOD [mg/l]	27200 ± 6100
TOC [mg/l]	183 ± 22
TKN [mg/kg]	1,9 ± 1,1

After inoculation and pH adjustment, the bottles were sealed with a rubber stopper and locked with an aluminium screw. The removal of the oxygen traces and the anaerobic conditions were obtained through a 15 minutes flushing of N_2 gas in the head space of bottles. Batch tests were incubated at mesophilic temperature (37 ± 1 °C) with a thermostat in a water bath. All batch reactors were connected to a micro-gas chromatograph to monitor gas production on line (Figure 6-4). The fermentation was stopped when the hydrogen production stabilized to avoid further consumption.

6.2.4 Analitycal procedures

Both raw materials and pretreated samples were characterized in terms of Total Solids (TS) and Volatile Solids (VS), pH, soluble Chemical Oxygen Demand (sCOD), Total Kjeldahl nitrogen (TKN), Total Organic Carbon (TOC) and Inorganic Carbon (IC). The carbohydrate and protein contents were also determined for the raw materials.
In order to better evaluate the BHP tests performance, the concentration of each VFA of the samples was estimated and the metabolites pathway was determined.
Total Solids (TS) and Volatile Solids (VS) were determined according to Standard Methods of the American Public Health Association (APHA, 1998). The pH measure was carried out with a pHmeter Eutech Instrument pH510 after 1:20 dilution, agitation for 1h and centrifugation (15000 rpm, 15 minutes).
The chemical oxygen demand (COD) was estimated using an Aqualytic 420721 COD Vario Tube Test MR (0-1500 mg/l). Each samples was diluted 1:20, centrifugated (5000 rpm, 10 minutes) and filtrated (<0.45 μm); two mL of sample were then pipetted into each tube and then they were placed inside a ECO 25 Thermoreactors for COD (Velp Scientifica) at 150 °C for 2 h. Soluble COD (sCOD) concentrations were determined using an Aqualytic Multidirect spectrophotometer.
In order to determine the C/N ratio, in this experimental step, TC e TKN concentrations were estimated. Total Kjeldahl nitrogen (TKN) and ammonia

nitrogen contents were measured with Digest Automat K-438 and an AutoKjehdahl Unit K-370, BUCHI. Total Organic Carbon (TOC) and Inorganic Carbon (IC) were determined using a Shimadzu TOC-VCSN Total Organic Carbon Analyzer coupled to a Shimadzu ASI-V tube rack. The Total Carbon (TC) content corresponded to the sum of TOC and IC.

After acid hydrolysis of the raw materials with sulfuric acid (solution 10% v/v H_2SO_4 98% with 1 g TS/l of substrate and agitation for 24 h), the carbohydrate concentration was determined by the Dubois method (Dubois et al., 1956) and the protein concentration was determined by the modified Lowry method (Fr/olund et al., 1995).

BHP tests using both raw materials and pretreated substrates were implemented with micro-gas chromatograph. All batch reactors were connected to a multiplexed R3000 micro-gas chromatograph (μGC) with two analytical capillary columns (SRA instrument, Marcy l'Etoile, France) to monitor gas production on line. The first column was dedicated to carbon dioxide analysis and corresponded to a 5Å molecular sieve (10 m length and 0.32 mm diameter) with argon as carrier gas at a pressure of 30 PSI. The second column dedicated to oxygen, hydrogen, nitrogen, and methane analysis was a PLOT Q (8 m length and 0.32 mm diameter) with helium as carrier gas (20 PSI). The injector and column temperatures were 90°C and 80°C, respectively. The detector was a microthermal conductivity detector (μTCD). Multiplexing the channels allowed the simultaneous connection of 16 batch tests with a measure of the total gas production every 2 hours, by pressure measurement. To maintain a constant pressure in headspace, the gas composition was evaluated by sampling only when pressure was higher than 1.0 bars.

Each batch test run was carried out in quadruplicates and average values were considered for discussion.

Hydrogen volumes produced in the time interval between each measurement (t - t-1) were calculated using a model that takes into consideration the gas concentration at time t and time t-1, the total volume of biogas produced at time t, the concentration of the specific gas at times t and t-1, and the volume of the head space of the reactors (Table 6-8).

Table 6-8: Hydrogen volume estimation

Parameter	Formula
Hydrogen moles	$n_{mol,\ H_2} = \frac{V_{HS} \cdot (P_t - P_{t-1})}{R \cdot T_{DF}}$
Biogas volume	$V_{biogas} = \frac{n_{mol} \cdot R \cdot T_{st}}{P_{atm}}$
Hydrogen volume	$V_{H_2} = \frac{V_{biogas} \cdot C_{H_2}}{C_{biogas}}$

where:

- V_{HS}, is the head-space volume at time t;
- P_t is the pressure measured at time t before the analysis;
- P_{t-1} is the pressure measured at time t-1 after the analysis;
- R is the ideal gas constant;
- T_{DF} is the temperature of the water bath during DF tests.
- V_{biogas}, is the volume of the biogas produced at time t;
- T_{st} is the standard conditions temperature (25°C);
- P_{atm} is the atmosferic pressure.
- C_{H2} is the concentration of hydrogen measured at time t;
- C_{biogas} is the concentration of biogas measured at time t.

The hydrogen production kinetic was examined by a modified Gompertz equation, which has been widely accepted and used to describe the cumulative hydrogen production progress as showed in the folling equation:

$$H(t) = H_{max} \cdot exp \cdot \left\{-exp\left[\frac{R_{H_2} \cdot e}{H_{max}} \cdot (\lambda - t) + 1\right]\right\}$$

where:

- t denotes the time [d];
- H (t) is the cumulative hydrogen production at time t [ml H_2];
- H_{max} is the maximum cumulative hydrogen production [ml H_2];
- RH_2 denotes the maximum H_2 production rate [ml/d];
- e = 2,71828;
- λ is the lag-phase time [d] for hydrogen production

The time required for H_2 production to attain 95% of the maximum yield, t_{95}, was also derived from the Gompertz equation as follows:

$$t_{95} = \frac{P}{R_m \cdot e}(1 - \ln(-ln0.95)) + \lambda$$

Cappai et al. (2015) defined the t_{95} as the time required to achieve 95% of the maximum H_2 yield. The t_{95} provides a measure of how fast the maximum production is achieved and it can be usefully adopted to compare experimental conditions with different associated H_2 generation yields.

Prior to physicochemical characterization and measurement of the metabolites, 20 g of biomass was extracted by deionized water (1:2), mixed during 30 minutes, centrifuged at 5000 rpm during 10 min and filtrated at 0.45 µm (D'Imporzano & Adani, 2007; Ghimire et al., 2018). A portion of the remaining liquid phase was used to quantify organic acid and the other portion was filtrated at 0.2 µm in order to quantify the other metabolic end-products (Figure 6-5).

Volatile fatty acids (VFA) composition, ie. acetic (C2), propionic (C3), butyric and iso-butyric (C4 and iC4), valeric and isovaleric (C5 and iC5) and caproic (C6) acids, was determined with a gas chromatograph (GC-3900 Varian) equipped with a flame ionization detector. The formic acid (C1) concentration was also determined with GC using a specific control sample.

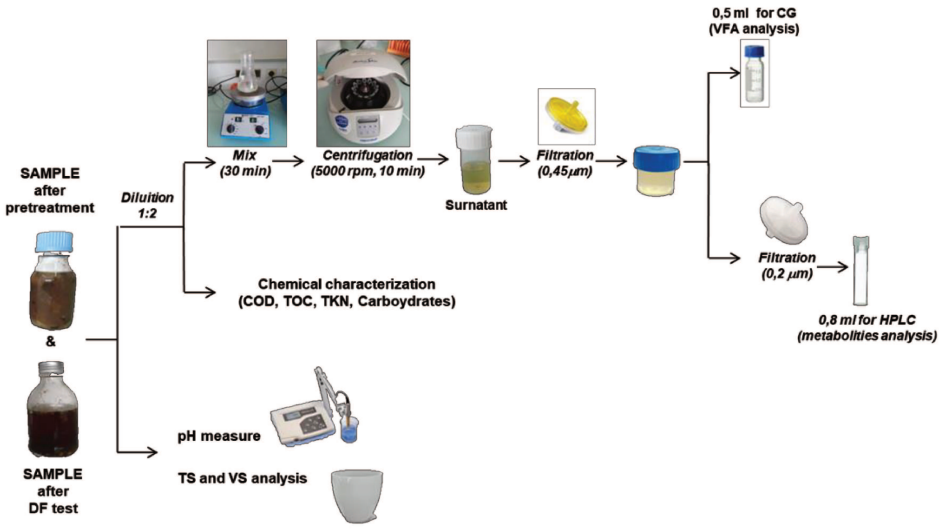


Figure 6-5: Sequential analysis for VFA and metabolites quantification

High performance liquid chromatograph (HPLC) was used to measure the concentrations of non-VFA metabolic products, carboxylic acids and alcohols (glycerol, ethanol, 1,3-propanediol, formic acid, fumaric acid, succinic acid, pyruvic acid and lactic acid), sugar monomers (glucose, xylose, arabinose and lactose). The HPLC was coupled to refractometric detection (Waters R410). Chemicals were separated by an Aminex HPX-87H column (300 mm on 7.8 mm, Biorad) equipped with a protective precolumn (Microguard cation H refill catbridges, Biorad). The eluting solution corresponded to 2 mM H2SO4 under a flow rate of 0.4 ml min-1. The column temperature was set at 35 °C and the refractive index detector (Waters 2414) worked at 45 °C.

7. Results and discussion

In the following paragraphs, the results of the experimental activity are divided in two main sections and discussed.
Firstly, the estimation of the statistical significance of different operating parameters, as resulting from the analysis of variance, was reported. These results have been also used to evaluate the influence of the pretreatment combinations on the physico-chemical characteristics as well as on the pretreatment effectiveness in terms of biomethane production potential.
The second step of the discussion focuses on the study of the implementation of BHP tests of the pretreated substrates, in order to evaluate the effectiveness of the pretreatment on the recovery of biochemicals as well as of biohydrogen.

7.1 Assessment of pretreatment performances

7.1.1 Analysis of the pretreatment operating conditions

A two freedom degree statistical distribution was considered and the outputs of the R-Studio Software, like F value and probability (Pr), were evaluated in order to define the statistical significance of the implemented pretreatment conditions. A F-critical value equal to 19 and a value of the probability (Pr) lower than 5% were considered for the purposes of the Anova test. According to this requisite, the operating conditions were considered statistically significant if the F-value did not exceed the F-critical value, thus promoting a Pr value minor to 0.05.
The performances of the pretreatment which were considered to evaluate the statistical significance of the operating conditions were: the variation of VS and sCOD as well as the C/N ratio in the solid fractions (Table 7-1); the increment of VFA concentration and ASL content in the liquid fractions (Table 7-2) of the pretreated substrates.
These experimental results were used as input in the R-Studio software in order to implement the Anova test. In the the Table 7-1 and in the Table 7-2 the main outputs of the Anova test (the F-value and the Pr-value) are reported, for each considered

operating parameter and for their combinations.

Table 7-1: Results of ANOVA test for VS reduction, sCOD increment and C/N ratio of the solid fraction of the substrates

Operating parameter**	Substrate	VS reduction		sCOD increment		C/N ratio	
		F value	Pr (>F)*	F value	Pr (>F)*	F value	Pr (>F)*
X	**LF**	8,01	**0,022**	4,12	**0,013**	11,25	0,214
	OF+LF	9,215	**0,017**	13,85	**0,0048**	6,642	**0,03**
	OF	14,07	**0,002**	6,838	**0,042**	16,144	**0,013**
Y	**LF**	10,5	**0,008**	1,37	**0,0011**	9,852	**0,019**
	OF+LF	4,14	**0,0165**	0,008	**0,0452**	12,788	**0,043**
	OF	0,057	0,823	5,539	0,078	18,604	**0,008**
Z	**LF**	14,52	0,81	13,204	0,928	0,825	**0,007**
	OF+LF	7,198	0,573	2,43	1,4584	0,68	0,334
	OF	2,47	**0,031**	10,684	**0,031**	0,361	0,581
X:Y	**LF**	6,587	**0,036**	11,526	**0,038**	2,156	0,325
	OF+LF	8,277	**0,025**	2,871	0,591	2,007	1,185
	OF	1,841	0,246	0,507	0,516	0,751	0,435
X:Z	**LF**	10,36	0,142	0,681	0,074	2,789	**0,0038**
	OF+LF	0,256	**0,047**	2,433	**0,045**	5,163	**0,026**
	OF	2,47	0,191	0,594	0,484	0,158	0,711
Y:Z	**LF**	1,569	0,12	2,64	0,156	0,058	**0,031**
	OF+LF	2,826	0,096	0,001	2,332	1,436	**0,047**
	OF	0,001	0,972	3,846	0,121	0,002	0,991
X:Y:Z	**LF**	5,794	**0,016**	3,564	0,247	5,974	0,348
	OF+LF	7,284	0,277	10,116	**0,011**	1,055	0,13
	OF	6,485	**0,042**	7,535	**0,038**	7,336	**0,041**

**The Pr values minor of 0.05 were highlighted in bold*

*** The operating parameters are acid concentration (X), temperature (Y) and time (Z).*

Table 7-2: Results of ANOVA test for VFA increment and ASL concentration of the liquid fraction of the substrates

Operating parameter**	Substrate	VFA increment		ASL concentration	
		F value	Pr (>F)*	F value	Pr (>F)
X	**LF**	3,521	**0,043**	7,52	0,264
	OF+LF	13,895	**0,002**	11,743	**0,021**
	OF	8,714	**0,008**	13,66	**0,027**
Y	**LF**	2,63	**0,02**	0,0254	**0,047**
	OF+LF	0,057	**0,013**	15,277	0,081
	OF	13,365	**0,004**	4,25	**0,005**
Z	**LF**	5,952	**0,041**	11,05	**0,01**
	OF+LF	2,44	**0,039**	0,101	0,756
	OF	11,35	**0,014**	9,721	**0,002**
X:Y	**LF**	5,741	**0,015**	1,632	**0,006**
	OF+LF	1,819	**0,041**	0,548	**0,034**
	OF	0,687	**0,012**	5,252	0,114
X:Z	**LF**	10,256	0,091	4,786	**0,0044**
	OF+LF	12,44	0,189	3,327	**0,048**
	OF	3,652	**0,018**	11,643	**0,035**
Y:Z	**LF**	1,587	**0,031**	5,492	**0,043**
	OF+LF	0,001	0,96	3,602	0,128
	OF	4,523	0,324	1,235	0,825
X:Y:Z	**LF**	9,574	**0,018**	1,356	**0,007**
	OF+LF	6,405	**0,047**	9,282	**0,037**
	OF	10,35	**0,011**	11,525	**0,001**

**The Pr values minor of 0.05 were highlighted in bold*

*** The operating parameters are acid concentration (X), temperature (Y) and time (Z).*

The Anova test is based on the assumption that: i) the results mean are equal, ii) the results followed a normal distribution, and iii) are characterized by the homogeneity of the variances. The first step was, thus, directed to the verification of these hypothesis.

To determine whether any of the difference between the means are statistically significant, each P-value was compared with the significance level (0.05) considered to assess the null hypothesis (results mean all equal). Many P-values obtained from Anova test resulted minor of 0.05, proving that some of the means were statistically

significant. This, in turn, made it possible to reject the null hypothesis and to conclude that not all of the means are equal.
After the null hypothesis was verified, the normal distribution was evaluated. Thus, the Shapiro test was carried out for all data considered. The P-value obtained from Shapiro test resulted greater than 0.05 and this prove that the resulting data were characterized by a Gaussian distribution.
In order to evaluate the homogeneity of the variances of the results, the Bartlett test was carried out. The homoscedasticity hypothesis was also respected because the P-value obtained from the Bartlett test were found to be greater than 0.05.
Since all assumption were verified, the statistical significance of the considered operating parameters, namely acid concentration (X), the temperature (Y) and the pretreatment time (Z), was assessed by means of the P-values reported in Table 7.1 and 7.2.
The results obtained for the studied substrates (OF+LF; OF) were reported in the same Tables together with those found for the LF substrate, considered as control.
The sCOD concentration and the VS reduction of the OF substrate are mainly influenced by the formic acid concentration (X) and the operating time (Z), which were found to be statistically significant in determining both the solubilisation and mineralization effects. The acid concentration was observed to be significant also when considering the same effects on the LF substrates. This outcome pointed out that the addition of the acid to substrate for a fixed duration promoted the fractionation of the organic biomass into simpler components as previously shown for lignocellulosic biomasses (Zhang et al., 2016). The fractionation of the biomass likely promoted the solubilisation increment in the pretreated substrates.
The biochemical properties of the OF substrate, which has been assessed in terms of the C/N ratio, are affected by the formic acid concentration (X) and the operating temperature (Y). The formic acid concentration was also found to be significant for the OF+LF substrate, but it did not show a significant influence on the LF substrate. Instead, the C/N ratio of the control LF substrate was affected by the operating time (Z). This confirms that the decomposition of the lignocellulosic material requires a long pretreatment (Amin et al., 2017). However, the duration of the treatment is not statistically significant with reference to the C/N ratio of the OF substrates.
All operating parameters of the pretreatment were found to be statically significant to VFA increment and ASL content in the liquid fraction of the OF substrates. This condition was also obtained for both the OF+LF and the LF substrates. The fractionation of the biomass has happened unevenly in the lignocellulosic and the organic sample.
However, the combination between the acid concentration and temperature (X:Y) was not significant for ASL content in the OF substrate. This result was not

particularly relevant due to the absence of the lignocellulosic biomass in this substrate. Conversely, the combination between the acid concentration and operating time was not significance for the OF+LF substrate. Thus, it was justified by a similar behaviour in the LF substrate.
Eventually, the sCOD increment and the VS reduction of the solid fraction of the OF substrate were affected by the formic acid concentration and the operating time. These pretreatment parameters were also found relevant for the fractionation of the organic biomass which was resulted in terms of total VFA increment of the liquid fraction of the OF substrate. However, the formic acid concentration and the operating time resulted more statistical significant when the VFA concentration was considered rather than the sCOD increment. The operating temperature, which was found statistically significant for the VFA, but not for the physico-chemical characteristics of the solid fraction, namely sCOD, VS content and C/N.
The statistical significance of the operating parameters resulting from the analysis of variance was used to emphasise the evaluation of the influence of the pretreatment combinations on the physicochemical characteristics and the pretreatment effectiveness on biomethane production for the OF substrate.

7.1.2 The influence of the operating conditions of the pretreatment

The objective of a formic acid pretreatment is to determine a fractionation process of the substrate, which can enhance the production of its components for further processing into chemicals and biofuels. The composition of the pretreated substrates and the effects of the different operating condition of the pretratment are discussed in the following paragraphs.

7.1.2.1 Solubilisation of the substrates

The variation of the VS content and the sCOD concentration were considered to evaluate the solubilization effects determined by the applied pretreatment on the substrates, under different operating conditions. The analysis of the DD_{COD} trend makes better understand the solubilisation rate obtained during the pretreatment.

Initially, the sCOD value of the raw materials was 32.0, 33.1 and 36.2 g/l for LF, OF+LF and OF substrates, respectively. The concentrations of sCOD discussed have been purified by the initial formic acid concentrations used for the pretreatment, which are 64.55 g_{COD}/l for the 15% of formic acid and 21.22 g_{COD}/l for 5% concentration.

Various chemical-physical effects were obtained varying the single operating condition of the pretreatment and each substrate differently responded to the application of the pretreamtent.
Thus, the formic acid pretreatment promoted either the solubilisation or the mineralization of all kind of substrates. An improvement in the DD highlights the increase in the solubilisation of organic matter and the pretreatment has been considered successful when the increment of sCOD and the reduction of VS was higher than 50%, which is the threshold value of efficiency generally assumed in previous works dealing with other kinds of OFMSW pretreatment (Cesaro and Belgiorno, 2013; Razavi et al., 2019).
DD_{COD} higher than 40-50% can be achieved by formic acid pretreatment. These values of DD_{COD} were similar to those resulted in previous works after alkaline (Wonglertarak and Wichitsathian, 2014), thermal (Ciaciuch et al., 2017) and ultrasonic pretreatment (Guo et al., 2014), which promoted a DD increase up to 58%. Experimental results pointed out that the implemented pretreatment was effective to increase the organic matter solubilisation in all substrates.
In the OF+LF substrates, the operating temperature play a key role in the increment of the DD_{COD}; the lower temperature (80°C) promoted a slighter DD_{COD} than the pretreatment combination at 120°C. Similar results was observed in the control LF substrate. Thus, the effect of the pretreatment on the OF+LF substrate was, probably, related to the lignocellulosic component included in the substrate. In fact, different results were observed for the OF substrate. The DD_{COD} in the OF substrate was affected by the high value of the acid concentration and temperature; the combination “a” (15% - 120°C – 70 min) and the combination “h” (15% - 120°C – 35 min) enhanced the DD_{COD} in the OF substrate, whereas no particular effects were observed in the other substrates.

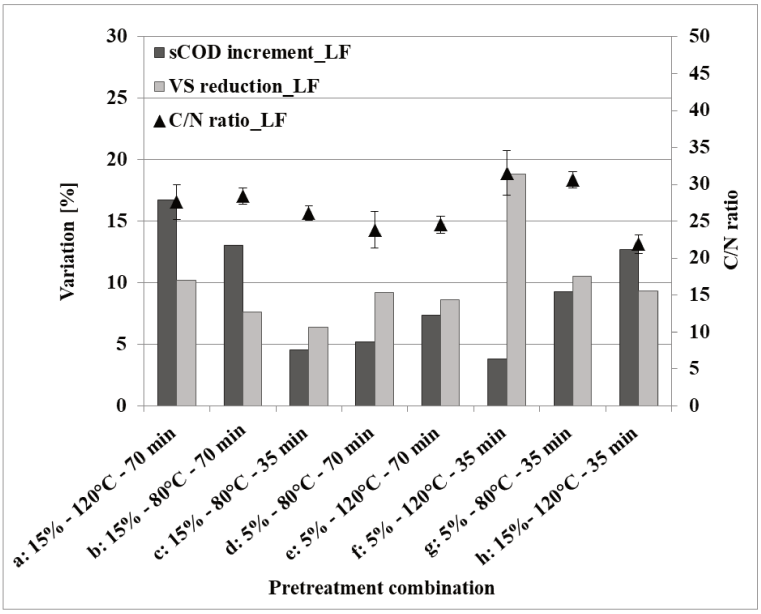


Figure 7-1: sCOD increment, VS reduction and C/N ratio of the pretreated LF substrate

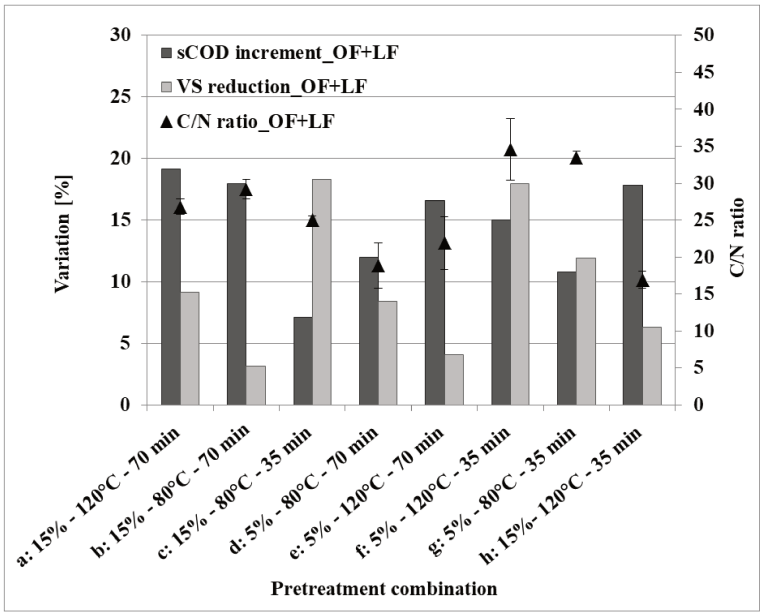


Figure 7-2: sCOD increment, VS reduction and C/N ratio of the pretreated OF+LF substrate

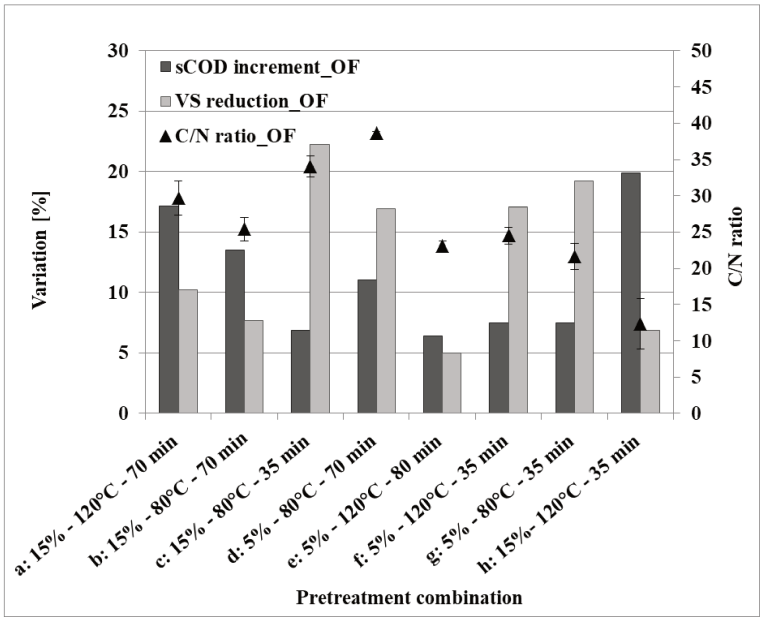


Figure 7-3: sCOD increment, VS reduction and C/N ratio of the pretreated OF substrate

The increment of the sCOD concentration and the reduction of the VS content were depicted in detail in order to better understand the solubilisation effect on the substrates under each pretreatment combination.

The different operating conditions of formic acid pretreatment promoted diverse solubilisation pathways of substrate organic matter.

By increasing the acid concentration from 5% to 15% v/v, the solubilisation effect increased (combination a: 15% - 120 °C - 70 min; b: 15% - 80 °C - 70 min; h: 15% - 120 °C - 35 min) and a sCOD enhancement up to 60% in the case of the OF samples occurred when the high concentration was applied under the higher temperature value (combination h: 15% - 120°C - 35 min). These results indicate that the organic substance of the samples were effectively solubilized by high concentrations of formic acid. The solubilised fraction in the treated substrate was thus expected to result in additional amounts of easily utilizable substrate for microbial growth, reportedly leading to an enhanced organic waste digestibility (Cesaro and Belgiorno, 2014; Salihu and Alam, 2016).

Conversely, the moderate duration of the treatment caused the mineralization of the substrates. This effect was particularly evident for the OF+LF substrate (Figure 7-2) and OF substrate (Figure 7-3) pretreated under the combination c (15% - 80°C - 35 min) which promoted VS reduction rather than sCOD increment. The operating time was not found to be significant for the VS reduction in the control LF substrate,

which was observed to be affected by the high autoclave temperature (Figure 7-1) as in the case of combination f (5% - 120°C – 35 min).
If the biomass is not quickly fragmented by the high acid quantity added, the degradation occurs more slowly and it is reactivated by increasing the operating time, as observed when decreasing the only acid concentration as in the case of the combination d (5% - 80°C - 70 min) applied to OF substrate. When this occurs, the solubilisation of more complex substances still predominates over the mineralization process, but it is driven by the physical fragmentation of the waste that exposes new surfaces for microbial attack (Menon et al., 2016).
For the OF+LF and OF substrates, at the same pretreatment acid concentration and duration, the higher temperature caused the increase of organic matter solubilisation as reported by Alvarez-Gallego et al. (2015). It was found that the best solubilisation effects of the organic matter was obtained at temperatures up to 160°C that do not induce losses by thermal decomposition, polymerization or caramelization of organic matter.
These evaluation of the solubilisation effect of the pretreatment has been used to realize that the solid fraction of the substrates would be suitable substrate in the anaerobic digestion processes (Li et al., 2018; Ma et al., 2018).
Thus, the solid fraction of the pretreated samples was used as substrates in AD tests in order to estimate the biomethane production corresponding to each pretreatment combination, as described in the following paragraphs.

7.1.2.2 Biochemical properties

The influence of the pretreatment parameters was also investigated with reference to the biochemical properties of the substrates. Due to the importance of the C/N ratio in anaerobic processes (Chen et al., 2017a; Fisgativa et al., 2016), it is regarded as an essential indicator for controlling biological and chemical changes into the biomass. Usually, a C/N between 15 and 30 is advised for a good substrate degradation during anaerobic digestion. However, microrganisms must have a 20 - 30 ratio of C/N in order to operate at maximum conditions (Li et al., 2011b; Zeshan et al., 2012). A C/N ratio between 35 and 45 is advised for a good improvement in hydrogen and VFAs during fermentation processes (Saha et al., 2016; Saidi et al., 2018).
The formic acid pretreatment allowed to enhance the C/N ratio, which generally ranged from 10 to 40 for the different pretreatment combination. In particular, the values of the C/N ratio of the OF substrates differ greatly in their patterns according

to various operating parameters (Figure 7-3), while in the LF substrate the C/N ratio only ranged from 20 to 35 (Figure 7-1).
The solid fraction of all substrates pretreated under the combination a (15% - 120°C - 70 min) and b (15% - 80°C -70 min), showed C/N ratios (20-30) which make them suitable for methane production according to reported by (Wang et al., 2014b). However, (Zhang et al., 2014) have observed that the digestion of organic waste proceeds well also at C/N ranged from 15 to 20, which was observed for the solid substrate pretreated under the combination d (5% - 80°C – 70 min).
The suitable combination of the operating parameters resulted significant for LF and OF+LF substrates, when considering the C/N ratios. Thus, for the OF+LF substrates, the pretreatment combination f (5% - 120 °C - 35 min) and g (5% - 80°C - 35 min), characterized by the lower acid concentration and the lower temperature, resulted in a C/N ratio of approximately 40 (Figure 7-2), whereas the pretreated OF substrates (Figure 7-3) were not found to be characterized by an optimal C/N ratio for anaerobic digestion (15 ÷ 20), so that a relevant enhancement of the H_2 yields was expected only in the former cases. Nevertheless, Chen et al. (2017) reported that the C/N ratio between 7 and 25 at acid pH value promotes great hydrogen production during dark fermentation processes, while higher C/N ratios could involve the enhancing of the specific type of VFAs rather than the others during anaerobic processes. According to (Chen et al., 2017a) C/N ratio higher than 25 resulted in the improvement of propionic acid greater than the acetic and butyric acid among the VFAs produced during organic waste anaerobic fermentation; under the same conditions, no hydrogen is generated. In the latter case the carbon-rich sample could be considered as a promising feedstocks for the production and accumulation of metabolites (Sivagurunathan et al., 2018).

7.1.2.3 Fractionation of the biomass

The substrates were also analysed in terms of VFA concentration. It is well know that the VFAs are produced in the initial hydrolysis on anaerobic digestion (Gerardi, 2003) and the excess of their concentration can be inhibitory for the biomass in the anaerobic digester (Chen et al., 2008). However, the digestion process could also be optimized for producing volatile fatty acids (VFAs) and biohydrogen as well as biomethane involving specific bioreactor arrangements and optimum set point of process parameters (Khan et al., 2016).
The total VFA increment was related to the specific operating parameter values and their combination The VFA concentration after the pretreatment combinations d (5% - 80°C - 70 min) and g (5% - 80°C - 35 min) did not exceed 7.7 g/l. This means that

these pretreatment combination may have promoted in the substrates a production of VFA, which indicates a preliminary hydrolysis phase, generally considered the limiting step of AD (Cesaro and Belgiorno, 2014). This VFA concentration could not resulted inhibitory for the methanogenesis phase (Franke-Whittle et al., 2014), although the methane production also depends on the different type of the VFA and their ratio (Wang et al., 2009).

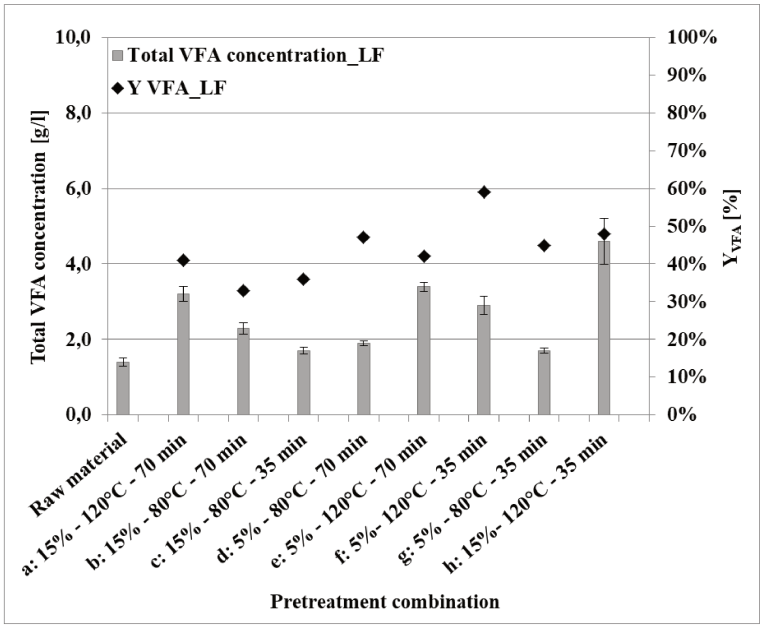


Figure 7-4: Total VFA concentration and bioconversion yield (Y_{VFA}) of the pretreted LF substrate

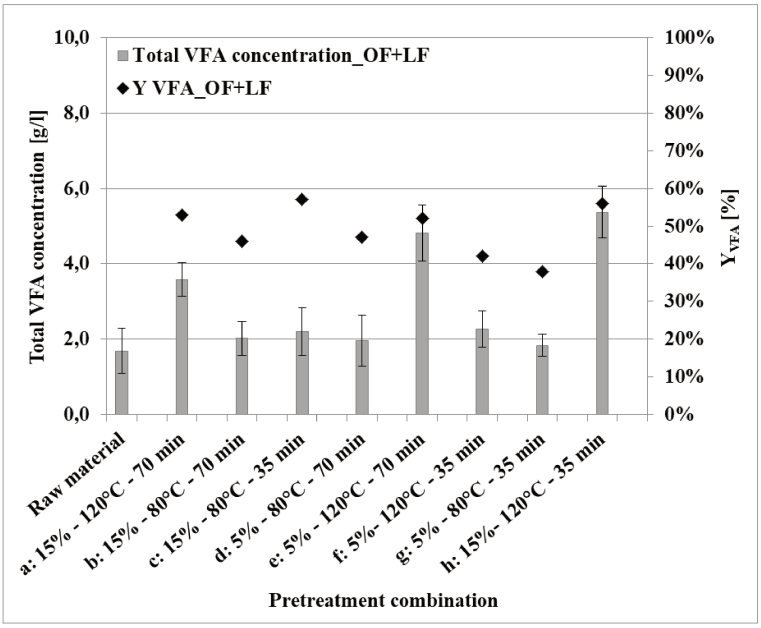


Figure 7-5: Total VFA concentration and bioconversion yield (Y_{VFA}) of the pretreted OF+LF substrate

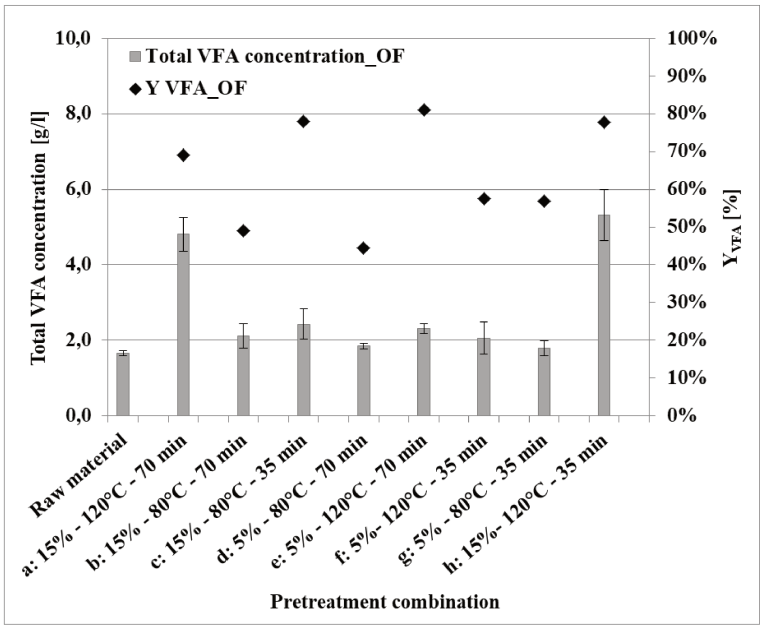


Figure 7-6: Total VFA concentration and bioconversion yield (Y_{VFA}) of the pretreted OF+LF substrate

The suitable combination of the acid concentration and temperature affected the VFA concentration. The application of the high acid concentration and high temperature promoted the production of great amount of VFA. The pretreatment combination a (15% - 120°C - 70 min) promoted a VFA concentration until 3.2, 3.6 and 4.8 g/l in the LF, OF+LF and OF substrates, respectively. After the pretreatment combination h (15% - 120°C- 35 min) the LF substrates was characterized by 4.6 g/l of VFA, in the OF+LF substrates was found 5.6 g/l of VFA and in the OF substrates was measured a VFA concentration equal to 5.3 g/l.
The other pretreatment combinations promoted VFA concentrations ranging from 2.0 to 4.0 g/l, which revealed the occurred bioconversion in all substrates.
The pretreated substrates showed a great VFA bioconversion yield (Y_{VFA}), which means that most of the initial COD was converted into VFA during the pretreatment. The Y_{VFA} of all substrate was affected by the high value of the operating temperature (120°C). However, the combination h (15% - 120°C -35 min) promoted a Y_{VFA} of 0.48% for the LF substrate and 0.56 for the OF+LF substrate, while in the OF substrate it was reached the 70% and the 78% of Y_{VFA} due to the combination a (15% - 120°C -70 min) and the combination h (15% - 120°C - 35 min), respectively.

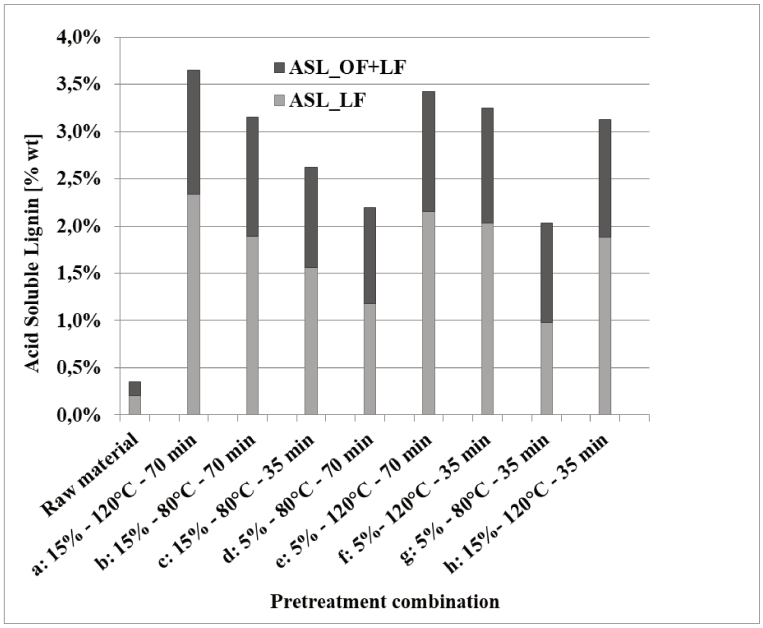


Figure 7-7: Acid Soluble Lignin (ASL) content of the LF substrate and the OF+LF substrate

In the substrates containing the lignocellulosic biomass, the possible existence of a soluble form of lignin in the acid hydrolysates, which could persist for a very long

period in the anaerobic environment (Li et al., 2018) inhibiting the methane production, was expected.
Indeed, the formic acid pretreatment is likely to have a significant impact on the delignification of the lignocellulosic biomass (Kumar et al., 2009). In particular, it should convert the recalcitrant lignocelluloses structure to reactive cellulose intermediates in order to allow their hydrolysis and their conversion into fermentable sugars (Kumar et al., 2009; Singh et al., 2014). The acid soluble lignin (ASL) content was not found to be significant in the OF substrate, while it reached the 2.5 % and the 1.5 % in the LF and OF+LF substrates, respectively (Figure 7-7). These value was not considered significant to evaluate the pretreatment performances. However, the percentage of the ASL obtained was related to the acid concentration, especially in the LF substrate. (Snelders et al., 2014) reported that under acidic conditions, like as during organic acid pretreatment, proteins could be linked to lignin as well as the condensation of lignin with degradation products of carboydrates probably could occurr.

7.1.3 Anaerobic digestion test

The effect of the substrate pretreatment can be evaluated from changes in the experimental methane potential in the BMP test.
During BMP tests methane production was measured by a volumetric technique in which the produced CO_2 is trapped in alkaline solution (NaOH solution) and only the volume of CH_4 is determined.
Figure 7 11, Figure 7 12 and Figure 7 13 plot cumulative methane production from untreated samples and pretreated samples for LF, OF+LF and OF substrate, respectively.

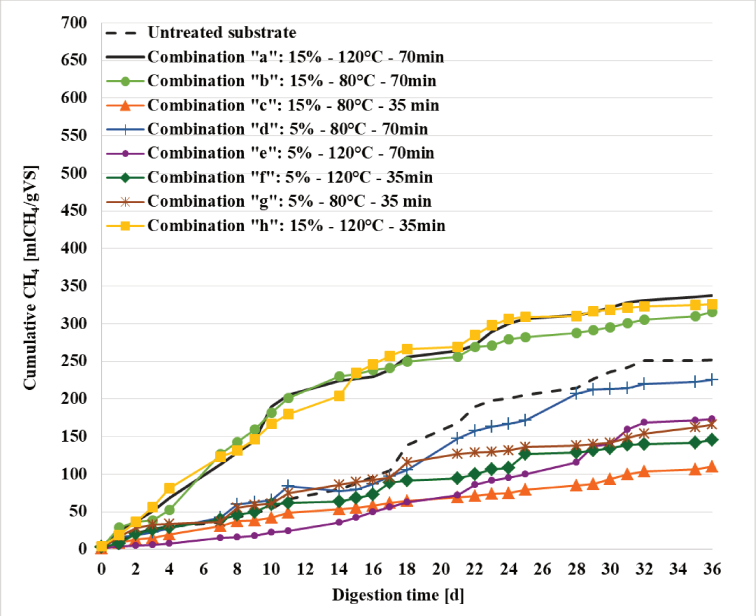


Figure 7-8: Cumulative methane production of the LF substrates after each pretreatment combination

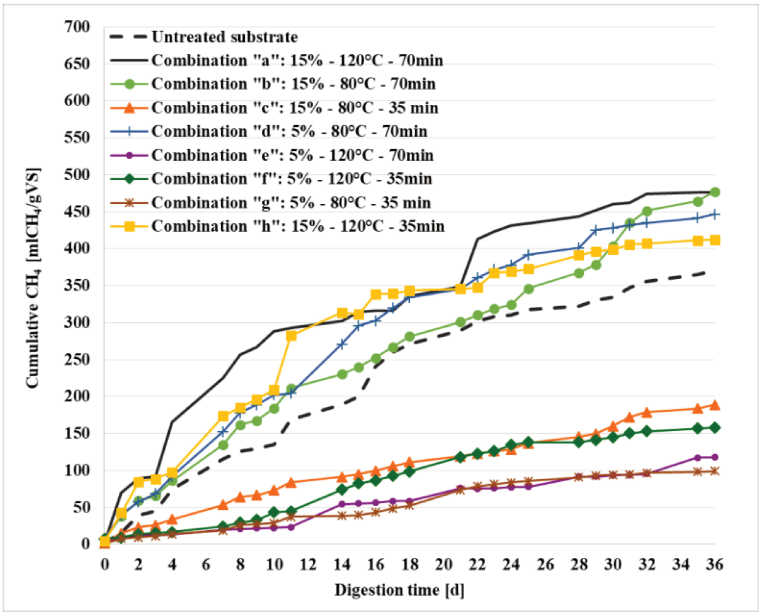


Figure 7-9: Cumulative methane production of the OF+LF substrates after each pretreatment combination

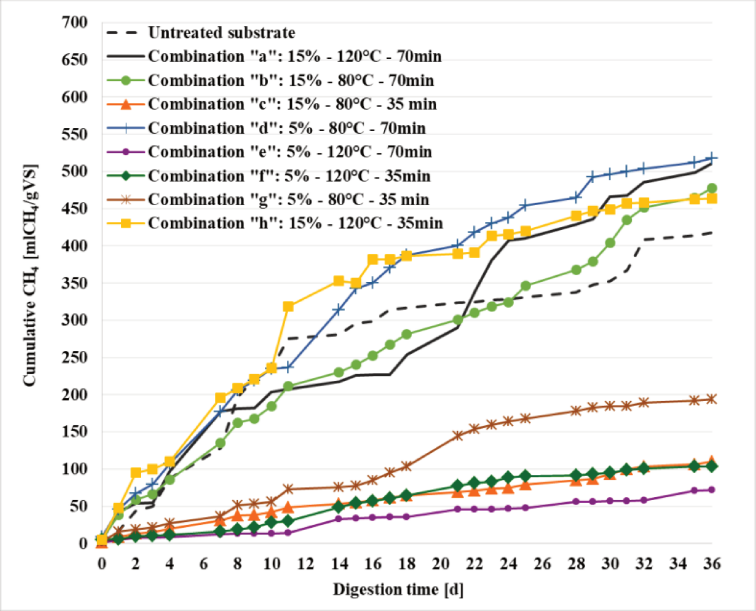


Figure 7-10: Cumulative methane production of the OF substrates after each pretreatment combination

The maximum CH_4 production of the untreated substrates after 36 days of the process resulted in 252 $mlCH_4/gVS$ for the LF substrate, 371 $mlCH_4/gVS$ for the OF+LF substrate and 418 $mlCH_4/gVS$ for the OF substrate, in accordance with previous studies dealing with the methane production of similar substrates (Islam et al., 2012; Li et al., 2018, 2017; Zhang et al., 2018).

Specific trends were obtained according to the different substrates and the various pretreatment combination previously applied.

Two principal trends were observed for all pretreated substrates: the pretreated substrates (pretreatment combination a, b, d and h) characterized by cumulative methane productions greater than those of the corresponding untreated substrate and the pretreated substrates which showed no significant CH_4 production. After the pretreatment combination a (15%- 120°C - 70 min), b (15% - 80°C - 70 min) and h (15% - 120°C - 35 min) higher CH_4 production volume during BMP test was produced. For the control LF substrate the maximum CH_4 production ranged from 315 $mlCH_4/gVS$ to 340 $mlCH_4/gVS$, for the OF+LF a range 410 $mlCH_4/gVS$ to 480 $mlCH_4/gVS$ was obtained and the OF substrate showed maximum volumes ranging from 460 $mlCH_4/gVS$ to 510 $mlCH_4/gVS$.

The COD conversion efficiency, which promoted solubilisation effects of the solid fraction of the substrates, has generally resulted in great volume of methane, likely related to the enhanced release of the biodegradable organic matters.
The substrates pretreated under the combination a (15% - 120°C - 70 min), b (15% - 80°C -70 min) and h (15% - 120°C – 35 min), characterized by high value of the acid concentration, had previously showed the greater sCOD increment.
As shown in the Figure 7-8 and the Figure 7-10, the OF+LF and OF substrates pretreated under combination d (5% - 80°C - 70 min) also resulted in one of the best cumulative CH_4 production, even though no significant solubilisation effect was observed. Similar results were obtained by (Barua et al., 2018), which found that great methane production was observed in the less effective pretreatment in terms of solubilisation and, conversely, the significant solubilisation effect related to the acid pretreatment resulted in less CH_4 production than the untreated substrate.
For the OF substrate, the methane production from the samples pretreated under the combination a, b, d and h were similar to the one of the untreated sample for the first 10 days. Then, the pretreated substrates were observed to have a higher CH_4 production than untreated sample. Instead, the slope of the curve representing the cumulative CH_4 production for the pretreated OF+LF substrates resulted right away unlike from those of the untreated sample.
This discordant result for the OF+LF substrate was related to the absence of significant mineralization phenomena, that were, instead, observed for the OF substrate.
The substrates pretreated under combination c (15% - 80°C - 35 min), e (5% - 120°C - 70 min), f (5% - 120°C - 35 min) and g (5% - 80°C - 35 min) showed very low CH_4 production. For the LF substrate, the curve slope observed for the combination f (5% - 120°C - 35 min) and g (5% - 80°C - 35 min) was similar to the one of the untreated samples during the first days. From the beginning of the BMP test of the OF+LF and the OF substrates, the trend of the cumulative CH_4 production of the combination c, e, f and g was completely different from those of the untreated substrates, which were characterized by a CH_4 volume higher than pretreated samples.
These results were related to the occurrence of a significant VFA production which may have inhibited the methanogenic process according to (Franke-Whittle et al., 2014). However, the samples pretreated under the combination e (5% - 120°C - 70 min) and f (5% - 120°C - min) for the OF+LF substrate, showed a significant VFA production, as reported to the preliminary analysis of the liquid fraction on the substrates after pretreatment.
Based on the results evaluation of the BMP tests and of the fractionation effect of the pretreatment, BHP tests were implemented in order to address the suitable valorization of the pretreated OF+LF and OF substrates.

7.2 Biohydrogen and metabolites recovery

Dark fermentation tests were carried out for the OF+LF and OF substrates in order to evaluate possible valorization strategies of the pretreated samples in a biorefinery context.

BHP tests were implemented on the pretreatred substrates, the H_2 production was estimated; the specific cumulative H_2 production yields by means Gompertz parametrisation, the theoretical H_2 and experimental H_2 ratios of the different pretreatment combinations were compared. A detailed estimation and evaluation of the metabolites pathways for each pretreatment combination was also reported.

Throughout the batch experiments, only H_2 and CO_2 were detected in the gas phase, while methane (CH_4) was not observed among the gaseous products. The heat-shock treatment of the inoculum has efficiently suppressed the methanogenic activity.

In the control substrates (untreated samples), H_2 production attained yields of 25.88 and 21.67 ml/gSV for OF+LF and OF substrate, respectively (Figure 7-11 and Figure 7-12). These H_2 yields were consistent with the previously reported ones, obtained from pretreated lignocellulosic and organic waste (Angeriz-Campoy et al., 2018; Kumar et al., 2009; Saidi et al., 2018). Differences in the total H_2 production were observed in the experiment runs, depending on the operating conditions, so that different pH variations were obtained (Figure 7-11,Figure 7-12).

The H_2 production showed a decreasing trend for increasing formic acid concentration and pretreatment duration as well as for decreasing temperature. The formic acid concentration remarkably affected the H_2 yield, with the best performances being observed at the lower value of the acid concentration (5%). The maximum hydrogen yield was obtained for the combination g (5% - 80°C - 35 min), which resulted in a H_2 of 36.1 and 33.5 mL H_2/gVS for the OF+LF and OF substrates, respectively. It is thus evident that the process in autoclave at low temperature and for short time enhanced fermentative degradation, which was particularly evident under weakly acidic conditions.

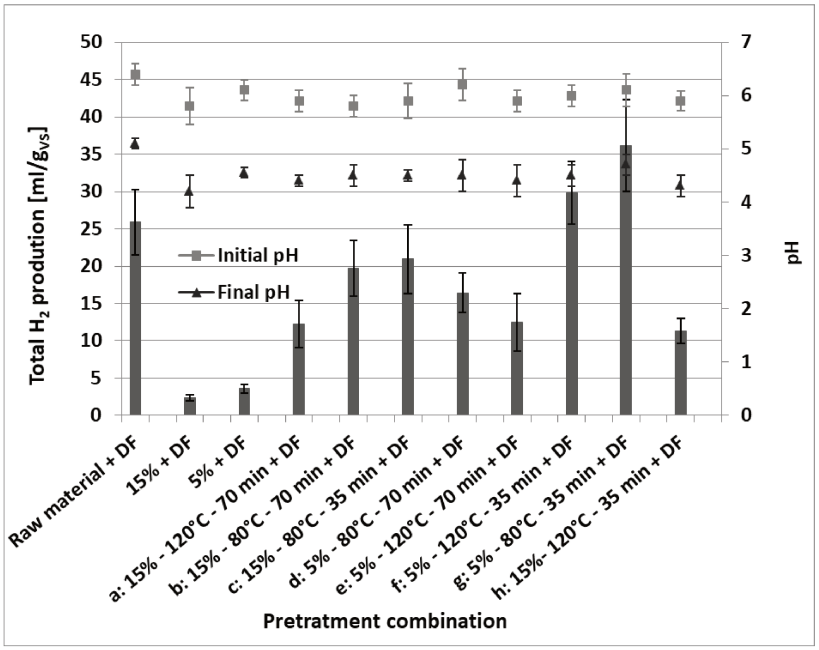


Figure 7-11: Total hydrogen production of the OF+LF substrate

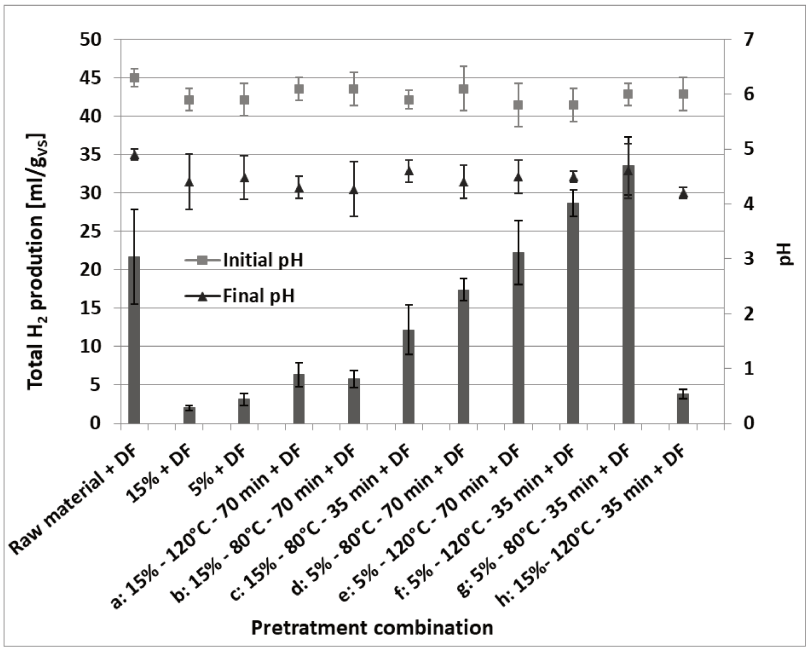


Figure 7-12: Total hydrogen production of the OF substrate

For the substrates pretreated at the more extreme conditions considered for the experiment, no significant H_2 production was showed.
As shown in Figure 7-11 and Figure 7-12, under the same acid concentration and operating temperature, different pretreatment duration led to the same total H_2 yield: this aspect can be observed comparing the results of the test runs a (15% - 120 °C - 70 min) and h (15% - 120 °C - 35 min) as well as those of the test runs b (15% - 80°C - 70 min) and c (15% - 80°C - 35 min). In particular, for the OF+LF substrates, the decrease of the time (from a: 15% - 120°C - 70 min to h: 15% - 120°C - 35 min) resulted in lower H_2 production, whereas the decrease of the temperature (from a: 15% - 120°C - 70 min to b: 15% - 80°C - 70 min) promoted an increase in H_2 production. For the same kinds of substrates, no relevant differences were observed when the formic acid concentration was reduced (from a: 15% - 120°C - 70 min to e: 5% - 120°C - 70 min). These results confirmed that the degradation of the LF in the mixed samples during the DF process is affected by the temperature and the duration of the pretreatment (Karimi and Taherzadeh, 2016; Kumar and Sharma, 2017). According to (Bolado-Rodríguez et al., 2016), the protracted process in the autoclave allowed a low sugar release, which inhibits the H_2 production and promotes the production of metabolites.
On the other hand, the acid concentration plays an important role in H_2 yields of OF samples (Elbeshbishy et al., 2017; Wang et al., 2019). In this case, the decrease of the formic acid concentration promoted the significant increment of the H_2 production, whereas the operating temperature and time did not produce significant effects.
The fact that the hydrogen production rate increased although the solubilization efficiency decreased could be a strong indication that the hydrogen might be also produced from sources other than organic matter. The pretreatment conditions could have promoted the H_2 production from formic acid decomposition. In biological systems the formic acid is converted to H_2 due to the facultative microbes (McDowall et al., 2014), which develop under anaerobic conditions. The conversion reaction is dependent on formate hydrogen lyase system (FHL), which facilitates the hydrogen production via break down of formic acid (Trchounian et al., 2017). The treatment of the substrates under operating temperature of around 80°C (Lipscomb et al., 2014), stimulated the oxidation of the formic acid present into the medium. This most likely happened when a low initial acid concentration was treated on long term at a given temperature (combination d: 5% - 80 °C – 70 min). This proved the efficiency of the use of the formic acid as organic solvent in the pretreatment step for bioenergy production. Nevertheless, whatever the pretreatment combination applied, the presence of the formic acid in the samples which were not processed in autoclave promoted a significant inhibition of H_2 production performances in terms

of lag phase, H_2 yield and maximum H_2 production. The quantity of the acid, without autoclave process, did not allow the occurrence of the fermentative mechanisms (Figure 7-11, Figure 7-12).

At the end of the fermentative process, the H_2 concentration decreases due to both the depletion of the readily available substrate and the H_2 biological consumption (Agneessens et al., 2018). Since no CH_4 production was detected, the H_2 consumption may have been caused by the development of either the propionic fermentation (Dong et al., 2009) or homoacetogenesis process (Cappai et al., 2015b; Siriwongrungson et al., 2007; Cata Saady, 2013). Moreover, as will be explained below, the pretreatment of the OF substrate under high acid concentration and high temperature (h: 15% - 120°C - 35 min) promoted an acidogenic fermentation with consequent accumulation of lactic acid, which was then converted into acetic acid, but not into H_2 during BHP tests.

The Figure 7-13 and the Figure 7-14 plot the cumulative H_2 yields over time for both the untreated substrates and those treated under different pretreatment combination. Gompertz equation-based best fit curves for cumulative H_2 production for the eight combinations evaluated. This was particularly evident for the pretreated substrates.

The combination g (5% - 80°C - 35 min) was observed to provide the highest H_2 production in both OF+LF samples and OF samples. In the control substrates, H_2 production started after 8 h and went on increasing for up to 5 days. These trends were consistent with previously reported ones obtained from pretreated lignocellulosic and organic waste (Cappai et al., 2014; Slezak et al., 2017). In the studies by Cappai et al. (2014) and Slezak et al. (2017), the lag phase in the dark fermentation process of organic waste ranged from 1.5 to 8 h, depending on the pH, inoculum and medium characteristics. The application of the pretreatment before the dark fermentation test generally contributed to reduce the period of acclimation of the microorganisms, to accelerate bacterial growth and consequently to enhance the hydrolysis of the substrates, maximizing H_2 production. The addition of the heat-shocked sludge and the adjustment of the initial substrate to biomass ratio of the medium for the BHP tests resulted in moderating lag phase duration (Cappai et al., 2014; Ghimire et al., 2015b) and also allowed the optimization of the process kinetics (Ghimire et al., 2018).

The Gompertz curves (Figure 7-13 and Figure 7-14) showed that the application of the pretreatment resulted in H_2 production trends evolving consistently with the theoretical ones. Analysing the formed gas it can be noted that the most intensive H_2 production occurred from the 3th to the 20th hour of the process. The highest rate of gas production of the OF substrates continued for about 3 days, while in the OF+LF substrate the maximum H_2 production was reached after 22 h.

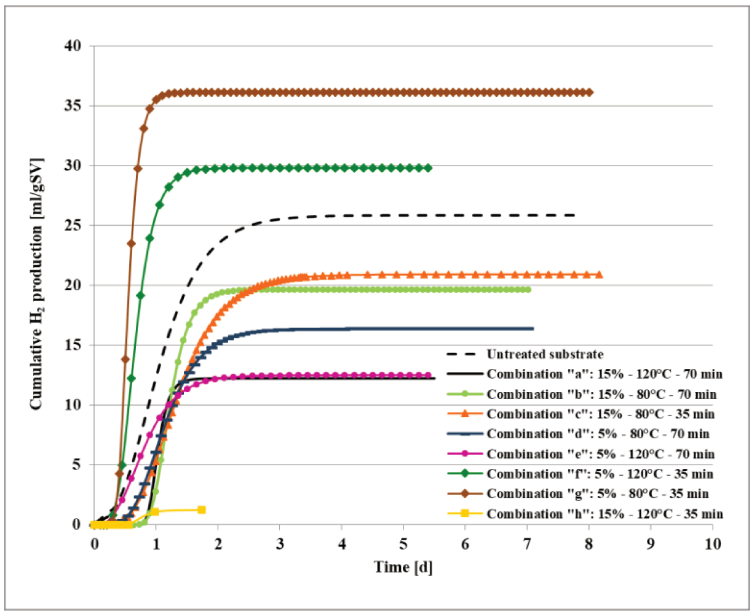


Figure 7-13: Specific cumulative H_2 production yields in the OF+LF substrate (solid lines indicate Gompertz curves)

Table 7-3: Measured and theoretical H_2 ratio in the OF+LF substrate.

Combination	h = H_2 measured/H_2 theoretical
Raw material	89%
15%	35%
5%	47%
a: 15% - 120°C - 70 min	71%
b: 15% - 80°C - 70 min	75%
c: 15% - 80°C - 35 min	73%
d: 5% - 80°C - 70 min	77%
e: 5% - 120°C - 80 min	58%
f: 5% - 120°C - 35 min	68%
g: 5% - 80°C - 35 min	93%
h: 15%- 120°C - 35 min	49%

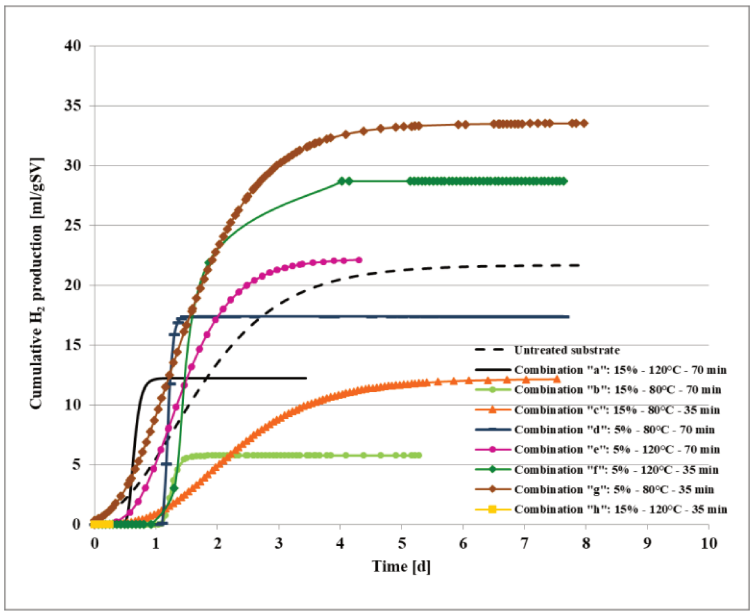


Figure 7-14: Specific cumulative H_2 production yields in the OF+LF substrate (solid lines indicate Gompertz curves)

Table 7-4: Measured and theoretical H_2 ratio in the OF substrate.

Combination	$h = H_2^{measured}/H_2^{theoretical}$
Raw material	85%
15%	42%
5%	40%
a: 15% - 120°C - 70 min	41%
b: 15% - 80°C - 70 min	60%
c: 15% - 80°C - 35 min	71%
d: 5% - 80°C - 70 min	74%
e: 5% - 120°C - 80 min	77%
f: 5% - 120°C - 35 min	68%
g: 5% - 80°C - 35 min	89%
h: 15%- 120°C - 35 min	37%

To determine more detailed information about the contribution of acetate or butyrate formation to the H_2 production yield, as well as the effect of propionic fermentation, theoretical H_2 and experimental H_2 ratios of the different pretreatment combinations were compared. The estimated ratios confirmed the H_2 production trends (Figure 7-15). The results indicated a good ratio (> 85%) for the raw material substrates and

for the substrates pretreated under operating conditions g (5% - 80°C - 35 min). These substrates indeed showed a significant H_2 production leading to optimal acetate/butyrate ratio, as already observed by Cappai et al. (2015). For the substrates pretreated under high autoclave temperature, the ratio fell in the range 60-70%, indicating that the metabolic processes together with fermentation took place. An exception was found for the combination h (15% - 120°C - 35 min), characterized by a very low ratio (35-45%). When this combination was applied to the OF sample, the content of acetic acid was not that expected with reference to the produced H_2, due to likely homoacetogenic fermentation or other pathways associated to the consumption of H_2 (Cappai et al., 2018).

Biohydrogen production is typically accompanied by the generation of VFAs and ethanol during dark fermentation processes. Hence, the produced soluble metabolites are useful indicators for explain the biohydrogen production and the possible use of the products.

The total production of metabolites varied depending on the substrate as well as on the process parameters. The pretreatment, under the combination h (15% - 120°C-35 min) allowed to achieve 4.1 gCOD/l and 4.0 gCOD/l in the OF+LF substrate and OF substrate, respectively. The dark fermentation tests further enhanced these concentrations up to 4.6 gCOD/l and 5.2 gCOD/l.

This study proved that the organic solvent pretreatment promoted the production of metabolites similar to that obtained after dark fermentation processes by different substrates (Cappai et al., 2014; Kim et al., 2013; Nissilä et al., 2011; Slezak et al., 2017). Significant metabolite concentrations were obtained in this study on pretreated organic waste. Nissilä et al. (2011) showed a total VFA production of 4.5 g/l during pure cellulose and starch biomass fermentation. A study published using pretreated lignocellulosic biomass showed a VFA yield of 5.3 g/l (Kim et al., 2013). In the study of Cappai et al. (2014) during dark fermentation of food waste the concentration of VFA reached the value of 14.2 g/l. The VFA production from kitchen waste was analyzed and the concentration of VFA was 3.16 g/L (Slezak et al., 2017).

Experimental results highlighted that different metabolites pathways occurred and some of their combination influenced those of H_2 generation. Substrate conversion decreased and biohydrogen production was significantly lowered when the initial acid concentration increased, as already showed in Figure 7-11 and in Figure 7-12. A simultaneous shift of the metabolic pathways was observed at the higher formic acid concentration. The presence of proprionate and ethanol are not generally indicated as precursors of H_2 production (Kim et al., 2013). However, in the OF+LF substrates pretreated under the combination c (5% - 120°C - 70min), the excessive propionic acid concentration promoted by the pretreatment did not inhibit the H_2

production during the BHP tests in the presence of significant ethanol concentration (1.9 g_{COD}/l).

The results also indicate that the acetic and butyric acids were dominant in the substrates which showed significant H_2 production during DF process (Figure 7-15 and Figure 7-16). Garcia-Aguirre et al. (2017b) and Oshoma and Obueh, (2018) showed that at slightly acidic conditions both the butyric and acetic acids are the main products of dark fermentation. Similarly, in this study, it was observed that the increase of VFAs production at low concentration of formic acid resulted in the increase in the content of butyric acid (combination d: 5% - 80 °C - 70 min for OF+LF substrate and combination e: 5% - 120°C - 70 min for the OF substrate), which caused a drop of the pH.

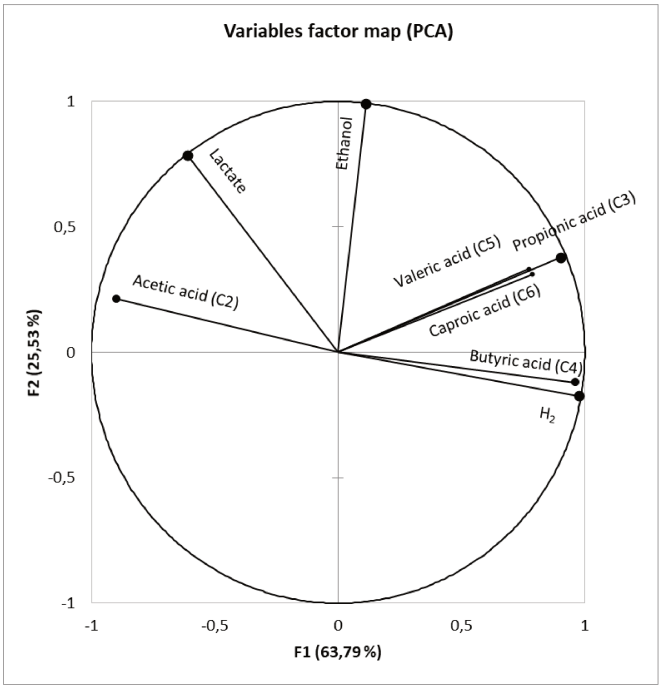


Figure 7-15: Principal component analysis correlation circle plots to hydrogen and major metabolic by-products production after pretreatment under high value of formic acid (15%) of the OF+LF substrate

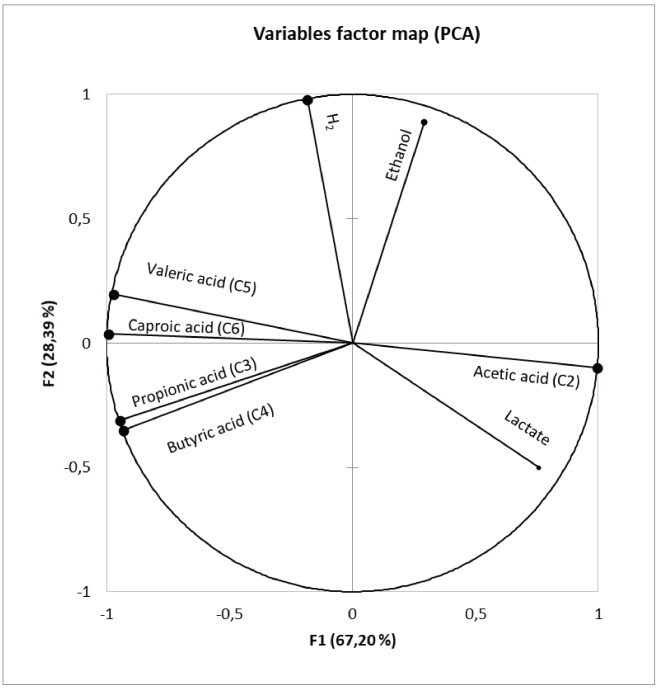


Figure 7-16: Principal component analysis correlation circle plots to hydrogen and major metabolic by-products production after pretreatment under high value of formic acid (15%) of the OF substrate

A proper composition in terms of metabolites promotes the best H_2 yield, which in this study was achieved under the combination g (5% - 80°C - 35 min). During the BHP test of the OF+LF substrates (Figure 7-19), 0.28 g_{COD}/l butyric acid and 0.21 g_{COD}/l acetic acid accumulated, resulting in a butyric acid/acetic acid ratio (1.33 Bu/Ac ratio) in the optimal range to enhance the H_2 production (Ghimire et al., 2016). The Bu/Ac ratio might not always give a relevant indication for high H_2 production. Guo et al. (2014) reported that the homoacetogenesis activities can influence the concentration of end-metabolites due to acetate production from H_2. The presence of acetate at the higher concentrations after DF process (combinations a: 15% - 120°C - 70 min and h: 15% - 120 °C - 35 min) might indicate the predominance of the homoacetogenesis activity responsible of lower H_2 yields (Figure 7-18, Figure 7-19, Figure 7-20, Figure 7-21). The high acid concentration at high temperature applied for the pretreatment step promoted the accumulation of the lactate, confirming an inhibition effect of the substrate conversion and explaining the low H_2 production. The residual effluent of the OF substrates pretreated with 15% of acid concentration at 120°C contained more than 2.8 g_{COD}/l of lactic acid

(Figure 7-20 and Figure 7-21). The elevate temperature helped to solubilize more sugars through better hydrolysis process and deactivate the native unwanted microbes, like as lactic acid producing bacteria, which could affect hydrogen yields (Parthiba Karthikeyan et al., 2018).

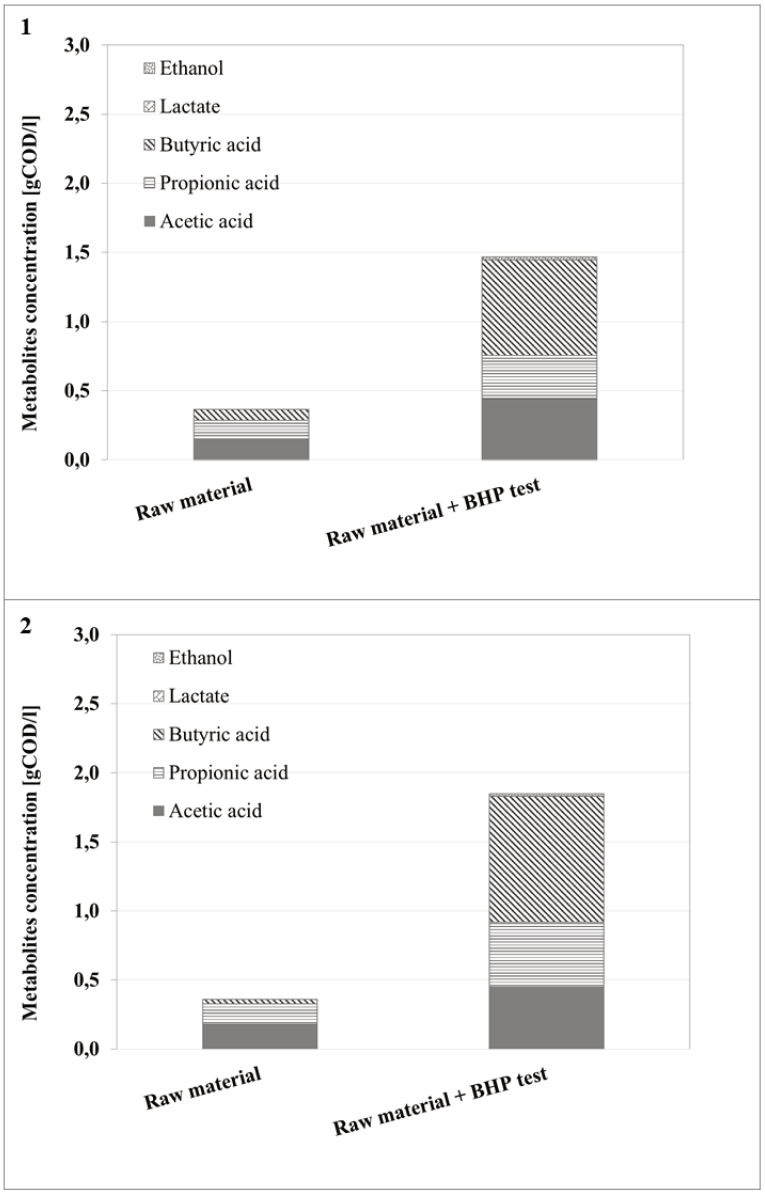


Figure 7-17: Accumulation of end metabolites after BHP test in the OF+LF substrate (1) and OF substrate (2)

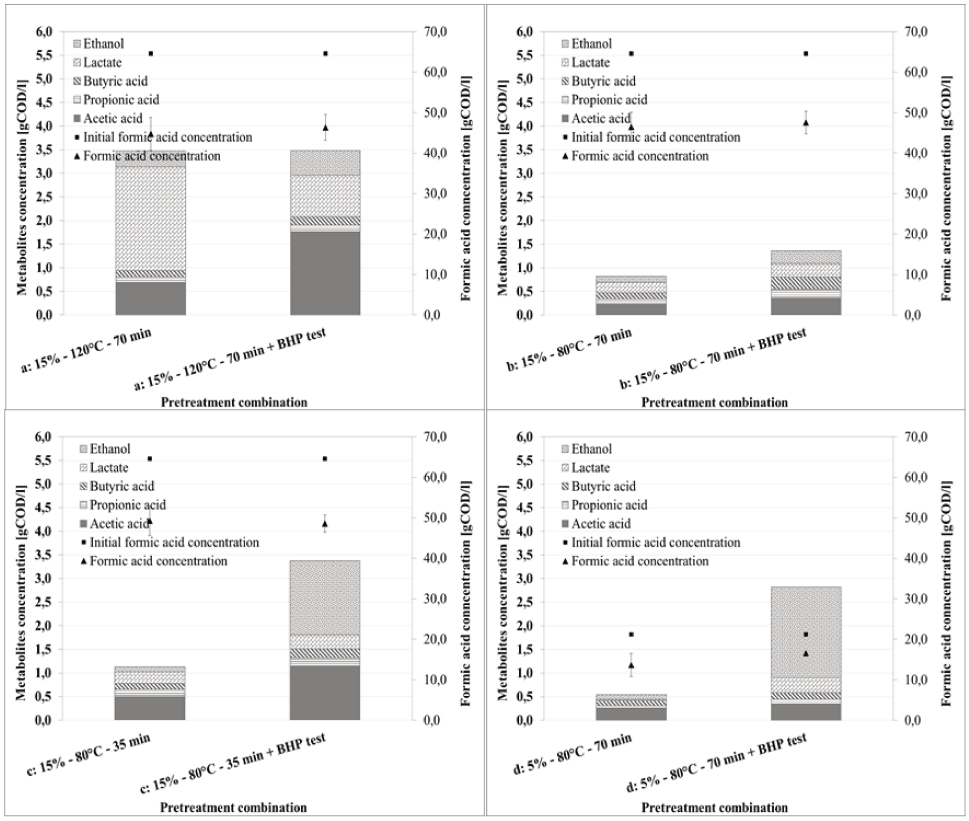


Figure 7-18: Accumulation of end metabolites after pretreatment (combination a, b,c and d) and after BHP test in the OF+LF substrate

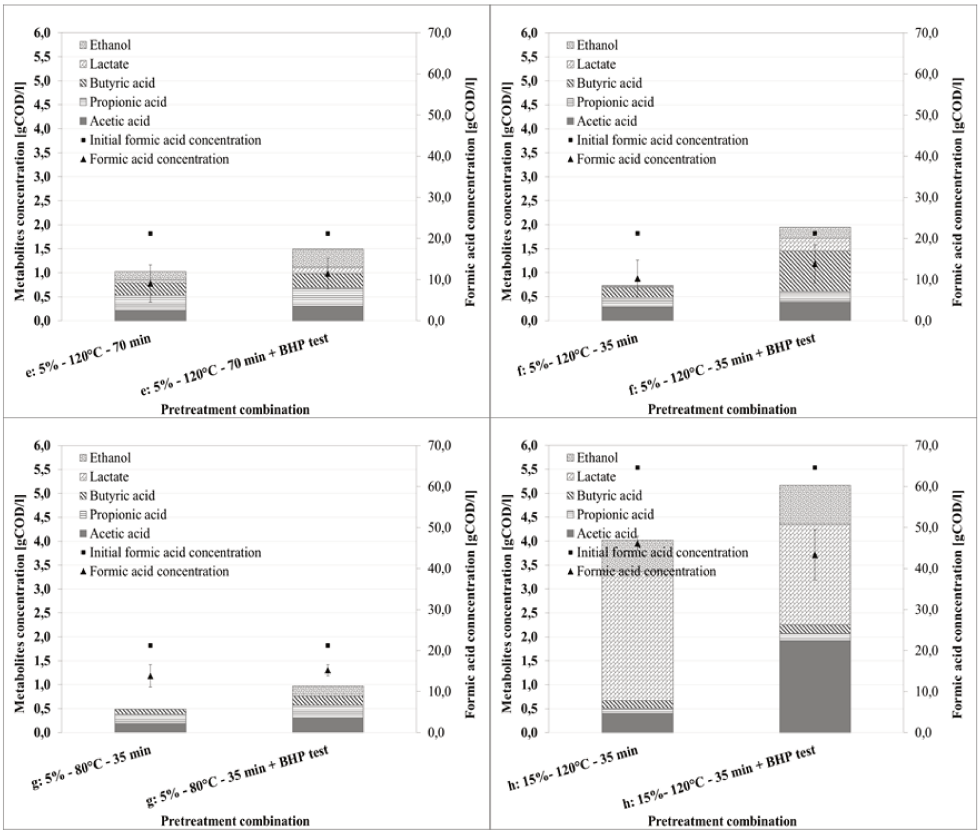


Figure 7-19: Accumulation of end metabolites after pretreatment (combination e, f, g and h) and after BHP test in the OF+LF substrate

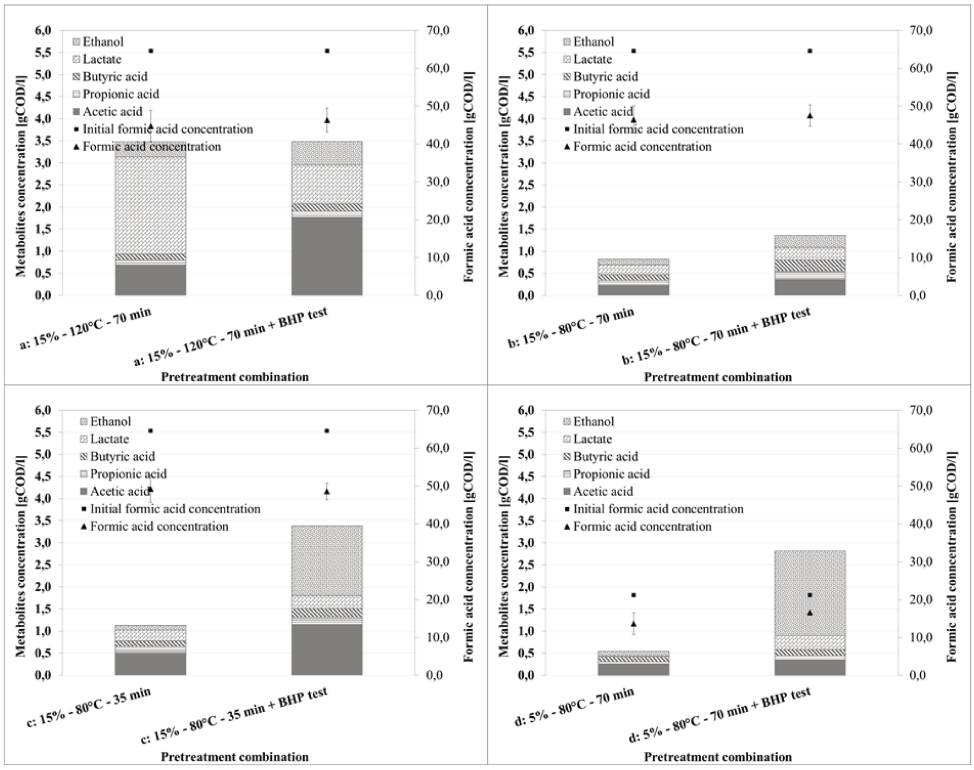


Figure 7-20: Accumulation of end metabolites after pretreatment (combination a, b,c and d) and after BHP test in the OF substrate

The lactate is an intermediate metabolic product, which at low concentrations is converted to other products, such as acetate and propionate (Alexandropoulou et al., 2016). During the DF process of the OF substrate pretreated under the combination h (15% - 120°C - 35 min), the lactic acid was efficiently converted into acetic acid, which is then further consumed to produce H_2 leading a homoacetogenic process, in accordance with what reported by (Ren et al., 2018).

Therefore, the acidic conditions are crucial to pursue the valorization of the pretreatment effluents potentially able to generate lactate. As the lactic acid production is not desired in DF due to its inhibition potential for the H_2 production, it could be extracted from the DF medium and destined to recovery (Moscoviz et al., 2018).

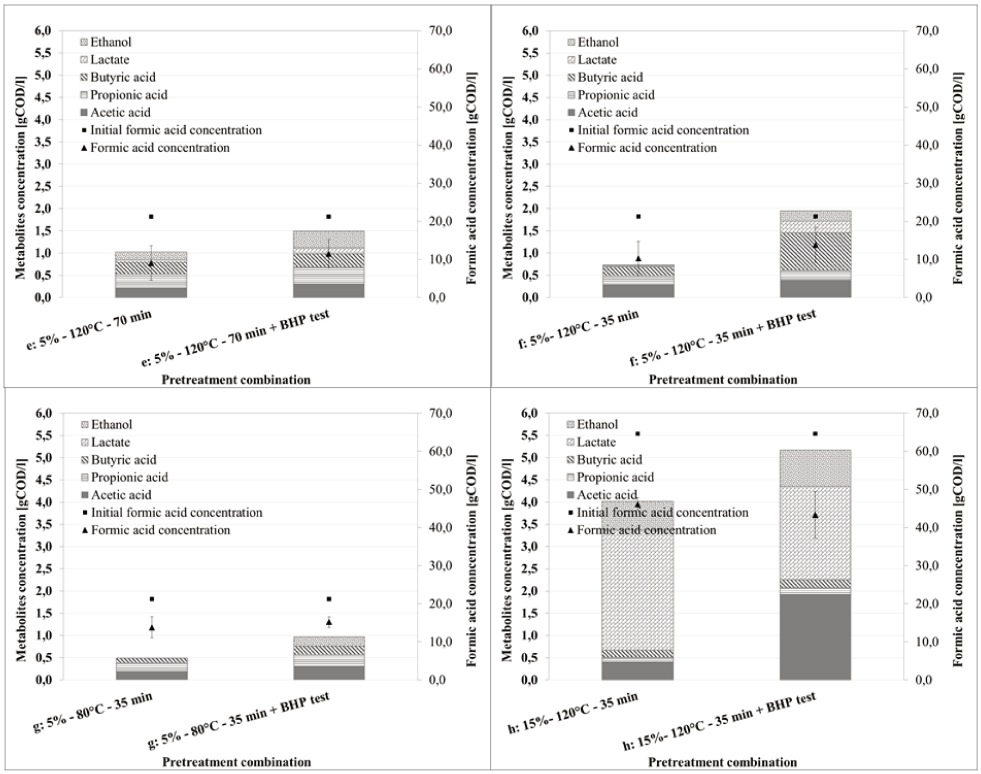


Figure 7-21: Accumulation of end metabolites after pretreatment (combination e, f, g and h) and after BHP test in the OF substrate

However, through acidogenic fermentation, VFA-rich organic waste could be valorised in order to obtain several intermediates to be used for bio-plastic production. Some investigations have been done (Rodriguez-Perez et al., 2018) but further efforts are needed for the scale up. To this end, the identification of the most suitable valorization scheme of the pretreated organic waste should take into amount of the VFA composition that can be obtained. In this view, different pretreatment operating conditions can be set up to address the production of specific metabolites, so as to fulfil market needs.

7.3 The feasibility of the formic acid pretreatment as option for OFMSW management: scale-up opportunity

The results of the experimental activity showed that the formic acid pretreatment can positively affect anaerobic digestion yield of the OF substrate, which was considered as the target sample. However, as the operating conditions of the pretreatment proved to play a fundamental role, different consideration raised for the OF substrate, if the feasibility of formic acid treatment application prior to anaerobic digestion or prior to dark fermentation is assessed for its possible scale up.

In a biorefinery approach for the organic waste management, specific operating conditions of the formic acid pretreatment could differently address the production of value-added biochemicals as well as energy carriers.

The experimental result of this research showed that, generally, the production of great methane volume is related to the solubilisation effects provided by the formic acid pretreatment, which in turn determined the optimal biochemical characteristics of the solid fraction of the substrates.

The solubilisation effects, which were evaluated by means of sCOD increment and VS reduction of the solid fraction of the substrates, were not only affected by the acid concentration, but also by the operating time.

Among the pretreatment combinations in which the formic acid and the temperature have promoted a relevant solubilisation effect (a: 15%- 120°C - 70 min, b: 15% - 80°C - 70 min and h: 15% - 120°C - 35 min), the best performance was that provided when the substrate was pretreated under the combination a (15%- 120°C - 70 min), which promoted a cumulative CH_4 production of 510 mlCH_4/gVS (Figure 7-1), 20% higher than the untreated OF substrate.

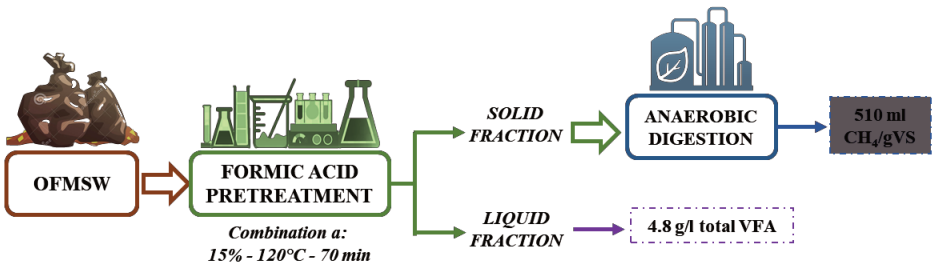


Figura 7-1: Process scheme for the application of the pretreatment to enhance methane production

The same combination of the formic acid pretreatment parameters promoted in the liquid fraction a concentration of total VFA (purified by formic acid concentration) of 4.8 g/l. This concentration did not inhibit the anaerobic digestion of the solid fraction from which had been separated. Thus, the liquid fraction of the substrate, which contain also the residual formic acid concentration, could be reused for a following step of pretreatment.

So, in order to enhance the methane production from solid fraction of the OF substrate, the optimization of the formic acid concentration and the operating time should be pursued.

For the fractionation of the organic biomass, the formic acid concentration and the pretreatment time as well as the pretreatment temperature were found to be relevant. In this case, further studies at larger scale should be directed towards an optimization of all pretreatment parameters is needed.

The highest concentration of total VFA (5.3 g/l) in the OF substrate was found in the liquid substrates pretreated under the combination h (15% - 120°C - 35 min), which promoted the best performances in terms of VFA concentration together with the combination h (15% - 120°C - 35 min).

However, the metabolic pathway occurred in the liquid substrate after the combination h resulted more attractive in order to recovery value-added products. The liquid fraction of the pretreated OF substrates contained, in particular, 0.41 g_{COD}/l of acetic acid, 0.6 g_{COD}/l of ethanol and 2.7 g_{COD}/l of lactic acid (Figure 7-2). These products represent emerging value-added chemicals with an increasing world market interest. The combination h (15% - 120°C - 35 min) also promoted the production of 474 ml CH_4/gVS, 15% higher than the untreated OF substrate, although 10% lower than that obtained from the substrate pretreated under the combination a.

So, the pretreatment combination h (15% - 120°C - 35 min) could represent a treatment strategy of the OF samples in order to recovery valuable biochemical from liquid fraction of the substrate.

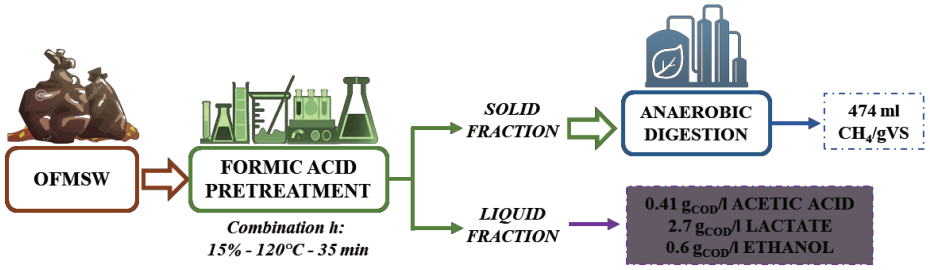


Figura 7-2: Process scheme for the application of the pretreatment to enhance metabolites production without anaerobic processes

Though, as shown in the discussion of the variance analysis, the formic acid concentration and the pretreatment time resulted particularly significant for the VFA increment. Since the combination a (15% - 120°C - 70 min) and the combination h (15% - 120°C - 35 min) differ for the value of the pretreatment time, this pretreatment parameters can represent the best starting condition for future studies aiming at identifying the optimized pretreatment.

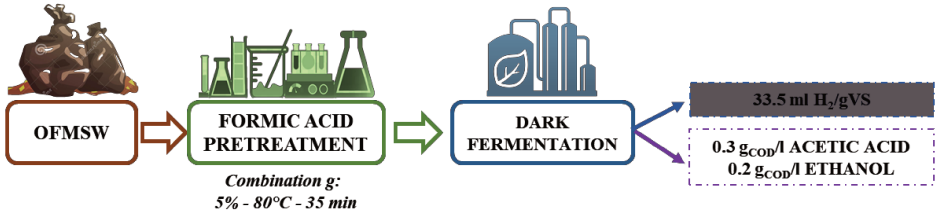


Figure 7-3: Process scheme for the application of the pretreatment to enhance H_2 production by means dark fermentation

As regard the BHP test, the highest H_2 production (33.5 mlH_2/gVS) for the OF substrate occurred under operating conditions g (5% - 80°C - 35 min). This result can be considered to be reliable because, for this combination, a good ratio between the theoretical and the experimental H_2 (> 85%) and an optimal acetic/butyric acid ratio had been verified. In the valorization contest of the OF substrates, the combination g (5% - 80°C - 35 min) could be, then, used to enhance the energy recovery (Figure 7-4). However, this pretreatment combination is characterized by the lower values of the investigated operating parameters. The implementation of the pretreatment combination g (5% - 80°C - 35 min) as a preliminary step to the dark fermentation of the OF substrates could promote a great H_2 production, minimizing the energy consumption if compared to the other pretreatment combinations.

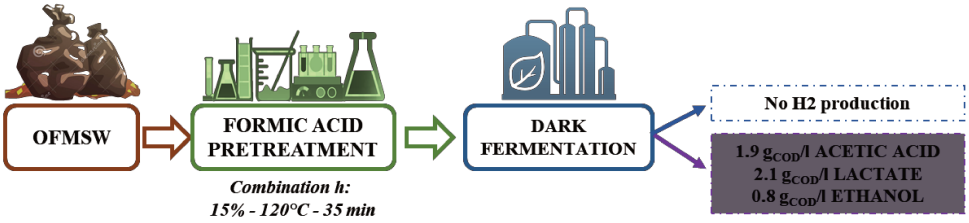


Figura 7-4: Process scheme for the application of the pretreatment to enhance metabolites production by means dark fermentation

As shown in the discussion of the results, when the pretreatment was carried out at the higher operating temperature values, the ratio between the theoretical and the experimental H_2 was found to be in the range 60-70%, indicating that the metabolic processes together with fermentation took place. The best pretreatment combination for the recovery of the value added products from fermentative substrate was the combination h (15% - 120°C - 35 min) as in the case of the valorization of the liquid fraction of the pretreated substrate in terms of the total VFA increment. The pretreated OF substrate under this condition (h: 15% - 120°C - 35 min) contained a great amount of lactic acid (2.7 g_{COD}/l). During the DF process of the OF substrate the lactic acid was converted into acetic acid, which is then further consumed to produce H_2 leading a homoacetogenic process. Thus, the dark fermentation process inhibited the H_2 production and promoted a great metabolites pathway. At the end of BHP test the substrate contained 1.9 g_{COD}/l of acetic acid, 2.1 g_{COD}/l of lactic acid and 0.8 g_{COD}/l of ethanol (Figure 7-5).

As in the case of the pretreated liquid fraction of the OF substrate, the effluent of the dark fermentation of the OF substrate after the pretreatment combination h (15% - 120°C - 35 min) could be used to recovery valuable metabolites, especially lactic acid and ethanol, which are characterized by an increasing world market. However, compared with the valorization of the liquid fraction to obtain great metabolites pathway, in this case the optimization of the pretreatment temperature is needed.

The different schemes confirm the opportunity of managing the organic fraction of municipal solid waste as a fraction in a circular economy approach, involving conventional biological processes, such as AD and DF, and the formic acid treatment previously applied on the different type of waste. The best performances of the pretreatment combination have been identified. However, the optimization of the specific parameters must be studied in order to support the results which have been obtained in this study.

8. Conclusion and future perspectives

The bioconversion of the organic fraction of municipal solid waste (OFMSW) into a wide range of competitive value added bio-chemicals is a circular economy strategy for the management of this kind of waste.
The research has focused on agricultural crops, lignocellulosic biomasses and algae which are defined as the first, the second and the third generation biomass for the conversion respectively. A fourth generation biomass could be the organic solid waste which can be applied in the biotechnological plants to increase its additional value producing multiple target products.
The organic waste is a mixture of different compounds, as sugars, proteins, amino-acids and fatty acids, which are already produced from non-renewable resources. Several examples of new biochemical platform products from organic solid waste are ethanol, lactic acid, and polylactic acid (PLA), polyhydroxyalkanoates (PHAs), succinic acid, 1,4-butanediol (BDO), farnesene, isobutene, acrylic acid, adipic acid, ethylene, and polyethylene.
The production of the multiple targets requires a highly efficient conversion process of the organic waste. In the waste management field, the anaerobic digestion (AD) is even one of the preferred treatments for the intensive biodegradation of organic fraction of municipal solid waste (OFMSW). It enhances the biomass conversion into methane and residual digestate. The methane from organic waste is a gaseous biofuel obtained from a stand-alone methanization process or by means a two-stage process, with a first stage that can be addressed towards hydrogen production (dark fermentation). Currently, the AD of OFMSW has gained further interest for the production of VFAs, which looks more attracting than that of energy carriers, due to the high added value of these products.
In recent years, the ability of the dark fermentation (DF) to synthesize hydrogen has raised great scientific attention. The hydrogen is another promising carbon-free clean fuel, due to its sustainable production and storage.
Nevertheless, the DF appears also as an important sustainable synthesis process of valuable chemicals, which serve as precursors of ubiquitous petrochemical derived products.
Suitable pretreatments of the substrate are necessary in order to improve the efficiency of the bioconversion during the anaerobic processes.

The fractionation of feedstocks substrate by means of appropriate pretreatment is a necessary process in order to improve the efficiency of bioconversion. Chemical, physical and biological methods can be used as pretreatment of organic waste.
The organic solvent pretreatment, which has already been widely used for lignocellulosic substrates, seems to be a promising method to enhance the conversion of organic waste into more amenable bio-products.
The organic solvent pretreatment of biomasses has been studied in order to produce high-quality intermediates, demonstrating higher efficiency for biomass fractionation and readily recovery solvent by means distillation process. As available organic solvent, formic acid has showed an interesting potential as active agent for dissociation of hydrogen ions, acceleration of biomass hydrolysis, increase the carboxyl content.
The research objective was the evaluation of the formic acid pretreatment as the treatment option of the OFMSW for its management in a biorefinery context, in order to propose multiple and alternative uses of the treated samples.
To this end, the influence of the main operating parameters of the pretreatment to improve the quality of useful components of the substrate was studied in order to address the assessment of the technical and economic feasibility of the pretreatment for the anaerobic processes; the production of the building blocks of the value added chemicals was evaluated as well.
The research activity was structured in two main phases. In the first phase, different combination of the pretreatment operating parameters were investigated according to a factorial design. The statistical significance of this parameters on the physicochemical characteristics, the biochemical characteristics of the substrates and the fractionation effects of the biomass were evaluated by means of the analysis of variance. The Biochemical Methane Potential (BMP) after all the considered pretreatment operating conditions was estimated as well. In the second phase, the research activity focused on the evaluation of the effects of the same pretreatment combinations on the dark fermentation yields by means Biochemical Hydrogen Potential (BHP) tests. To this end, the estimation of the hydrogen and soluble metabolites production was carried out.
The suitable combination between pretreatment and anaerobic process was evaluated in order to promote the alternative uses of the pretreated substrate in the waste management.
According to the results of experimental activity, the formic acid pretreatment can positively affect the valorization of the pretreated OF substrates destined to anaerobic processes. Experimental results pointed out that specific operating conditions of the formic acid pretreatment could promote different bioconversion

routes, thus resulting in the prevailing production of either energy carriers or value-added bio-chemicals.

The formic acid concentration of the pretreatment which was studied plays a key role in the conversion of the investigated substrates into a variety of value added products such as hydrogen, ethanol, lactic acid and organic acids. Varying the other pretreatment parameters, such as autoclave temperature and operating duration, the subsequent biological process can be targeted towards the production of either building blocks or energy carriers.

The effectiveness of operating parameters of the formic acid pretreatment on the OF substrate was successfully investigated.

The formic acid concentration and the operating time of the pretreatment are statistically significant for the solubilisation of the OF substrate, which was investigated in terms of sCOD increment and VS reduction.

These pretreatment parameters are also relevant for the fractionation of the organic biomass which was resulted in terms of total VFA increment.

However, the formic acid concentration and the operating time result more statistical significant when the VFA concentration is considered rather than the sCOD increment.

The operating temperature, which is statistically significant for the VFA, is not relevant for the biochemical properties of the OF substrate.

The 15% of formic acid concentration promote a relevant solubilisation effect in the pretreated OF substrate, which induce the CH_4 a production of 510 ml CH_4/gVS, 20% higher than the untreated OF substrate.

A valuable metabolic pathway also occurs when 15% of formic acid concentration is used at 120°C. These operating parameters promote the production of acetic acid, and ethanol, 83% and 74 % higher than those obtained with 5% of formic acid concentration at 80°C. A great amount of lactate is also produced after a pretreatment of 35 minutes with the addition of 15% of formic acid concentration.

On the other hand, the 5% of formic acid concentration promote the H_2 production of 33.5 mlH_2/gVS, 35% higher than the untreated OF substrate.

Moreover, future perspectives could be addressed towards the develop of device design and installation feasibility assessment of the combination of the organic acid pretreatment and anaerobic process, which could to contribute to the biobased society, reducing organic residues and increasing the efficiency of the biomass-based production of energy and valuable commodities, while addressing the biorefinery concept in waste management.

References

Adekunle, K.F., and Okolie, J.A. (2015). A Review of Biochemical Process of Anaerobic Digestion. Adv. Biosci. Biotechnol. *06*, 205–212.

Agler, M.T., Wrenn, B.A., Zinder, S.H., and Angenent, L.T. (2011). Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. Trends Biotechnol. *29*, 70–78.

Agneessens, L.M., Ottosen, L.D.M., Andersen, M., Berg Olesen, C., Feilberg, A., and Kofoed, M.V.W. (2018). Parameters affecting acetate concentrations during in-situ biological hydrogen methanation. Bioresour. Technol. *258*, 33–40.

Alam, F., Mobin, S., and Chowdhury, H. (2015). Third Generation Biofuel from Algae. Procedia Eng. *105*, 763–768.

Albuquerque, M.G.E., Eiroa, M., Torres, C., Nunes, B.R., and Reis, M.A.M. (2007). Strategies for the development of a side stream process for polyhydroxyalkanoate (PHA) production from sugar cane molasses. J. Biotechnol. *130*, 411–421.

Alexandropoulou, M., Antonopoulou, G., Trably, E., Carrere, H., and Lyberatos, G. (2018). Continuous biohydrogen production from a food industry waste: Influence of operational parameters and microbial community analysis. J. Clean. Prod. *174*, 1054–1063.

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

Alibardi, L., and Cossu, R. (2016). Effects of carbohydrate, protein and lipid content of organic waste on hydrogen production and fermentation products. Waste Manag. *47*, 69–77.

Almeida, J.R.M., Fávaro, L.C.L., and Quirino, B.F. (2012). Biodiesel biorefinery: opportunities and challenges for microbial production of fuels and chemicals from glycerol waste. Biotechnol. Biofuels *5*, 48.

Alvarez-Gallego, C., Fdez-Güelfo, L., Romero Aguilar, M. de los A., and Romero García, L. (2015). Thermochemical Pretreatments of Organic Fraction of Municipal Solid Waste from a Mechanical-Biological Treatment Plant. Int. J. Mol. Sci. *16*, 3769–3782.

Alves, H.J., Bley Junior, C., Niklevicz, R.R., Frigo, E.P., Frigo, M.S., and Coimbra-Araújo, C.H. (2013). Overview of hydrogen production technologies from biogas and the applications in fuel cells. Int. J. Hydrog. Energy *38*, 5215–5225.

Alvira, P., Tomás-Pejó, E., Ballesteros, M., and Negro, M.J. (2010). Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. Bioresour. Technol. *101*, 4851–4861.

Alzate-Gaviria, L.M., Sebastian, P.J., Pérez-Hernández, A., and Eapen, D. (2007). Comparison of two anaerobic systems for hydrogen production from the organic fraction of municipal solid waste and synthetic wastewater. Int. J. Hydrog. Energy *32*, 3141–3146.

Amani, T., Nosrati, M., and Sreekrishnan, T.R. (2010). Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects — a review. Environ. Rev. *18*, 255–278.

Amin, F.R., Khalid, H., Zhang, H., Rahman, S. u, Zhang, R., Liu, G., and Chen, C. (2017). Pretreatment methods of lignocellulosic biomass for anaerobic digestion. AMB Express *7*.

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

Amulya, K., Jukuri, S., and Venkata Mohan, S. (2015). Sustainable multistage process for enhanced productivity of bioplastics from waste remediation through aerobic dynamic feeding strategy: Process integration for up-scaling. Bioresour. Technol. *188*, 231–239.

Angelidaki, I., and Ahring, B.K. (2000). Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. Water Sci. Technol. J. Int. Assoc. Water Pollut. Res. *41*, 189–194.

Angeriz-Campoy, R., Fdez-Güelfo, L.A., Tyagi, V.K., Álvarez-Gallego, C.J., and Romero-García, L.I. (2018). New criteria to determine the destabilization of the acidogenic anaerobic co-digestion of organic fraction of municipal solid waste (OFMSW) with mixed sludge (MS). Bioresour. Technol. *248*, 174–179.

Ardolino, F., Parrillo, F., and Arena, U. (2018). Biowaste-to-biomethane or biowaste-to-energy? An LCA study on anaerobic digestion of organic waste. J. Clean. Prod. *174*, 462–476.

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

Atasoy, M., Owusu-Agyeman, I., Plaza, E., and Cetecioglu, Z. (2018). Bio-based volatile fatty acid production and recovery from waste streams: Current status and future challenges. Bioresour. Technol. *268*, 773–786.

Baldi, F., Pecorini, I., Albini, E., Bacchi, D., and Lombardi, L. (2017). OPTIMIZATION OF BIOHYDROGEN PRODUCTION USING DIFFERENT TYPES OF INOCULUM. 11.

Barua, V.B., Rathore, V., and Kalamdhad, A.S. (2018). Comparative evaluation of anaerobic co-digestion of water hyacinth and cooked food waste with and without pretreatment. Bioresour. Technol. Rep. *4*, 202–208.

Bastidas-Oyanedel, J.-R., Bonk, F., Thomsen, M.H., and Schmidt, J.E. (2015). Dark fermentation biorefinery in the present and future (bio)chemical industry. Rev. Environ. Sci. Biotechnol. *14*, 473–498.

Bengtsson, S., Hallquist, J., Werker, A., and Welander, T. (2008). Acidogenic fermentation of industrial wastewaters: Effects of chemostat retention time and pH on volatile fatty acids production. Biochem. Eng. J. *40*, 492–499.

den Boer, E., Łukaszewska, A., Kluczkiewicz, W., Lewandowska, D., King, K., Reijonen, T., Kuhmonen, T., Suhonen, A., Jääskeläinen, A., Heitto, A., et al. (2016). Volatile fatty acids as an added value from biowaste. Waste Manag. *58*, 62–69.

Bolado-Rodríguez, S., Toquero, C., Martín-Juárez, J., Travaini, R., and 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. Bioresour. Technol. *201*, 182–190.

Bolzonella, D., Battistoni, P., Susini, C., and Cecchi, F. (2006). Anaerobic codigestion of waste activated sludge and OFMSW: the experiences of Viareggio and Treviso plants (Italy). Water Sci. Technol. *53*, 203–211.

Bong, C.P.C., Lim, L.Y., Lee, C.T., Klemeš, J.J., Ho, C.S., and Ho, W.S. (2018). The characterisation and treatment of food waste for improvement of biogas production during anaerobic digestion – A review. J. Clean. Prod. *172*, 1545–1558.

Calt, E.A. (2015). Products Produced from Organic Waste Using Managed Ecosystem Fermentation. J. Sustain. Dev. *8*.

Cappai, G., De Gioannis, G., Friargiu, M., Massi, E., Muntoni, A., Polettini, A., Pomi, R., and Spiga, D. (2014). An experimental study on fermentative H2 production from food waste as affected by pH. Waste Manag. *34*, 1510–1519.

Cappai, G., DE GIOANNIS, G., Muntoni, A., Polettini, A., Pomi, R., and Spiga, D. (2015). Effect of inoculum to substrate ratio (ISR) on hydrogen production through dark fermentation of food waste. In Atti Del "Sardinia 2015 15th International Waste Management and Landfill Symposium, pp. 5–9.

Cappai, G., Gioannis, G.D., Muntoni, A., Spiga, D., Boni, M.R., Polettini, A., Pomi, R., and Rossi, A. (2018). Biohydrogen Production from Food Waste: Influence of the Inoculum-To-Substrate Ratio. Sustainability *10*, 4506.

Carmona-Cabello, M., Garcia, I.L., Leiva-Candia, D., and Dorado, M.P. (2018). Valorization of food waste based on its composition through the concept of biorefinery. Curr. Opin. Green Sustain. Chem. *14*, 67–79.

Carrère, H., Dumas, C., Battimelli, A., Batstone, D.J., Delgenès, J.P., Steyer, J.P., and Ferrer, I. (2010). Pretreatment methods to improve sludge anaerobic degradability: A review. J. Hazard. Mater. *183*, 1–15.

Carrere, H., Antonopoulou, G., Affes, R., Passos, F., Battimelli, A., Lyberatos, G., and Ferrer, I. (2016). Review of feedstock pretreatment strategies for improved anaerobic digestion: From lab-scale research to full-scale application. Bioresour. Technol. *199*, 386–397.

Carriquiry, M.A., Du, X., and Timilsina, G.R. (2011). Second generation biofuels: Economics and policies. Energy Policy *39*, 4222–4234.

Cavdar, P., Yilmaz, E., Tugtas, A.E., and Calli, B. (2011). Acidogenic fermentation of municipal solid waste and its application to bio-electricity production via microbial fuel cells (MFCs). Water Sci. Technol. *64*, 789–795.

Cesaro, A., and Belgiorno, V. (2013). Sonolysis and ozonation as pretreatment for anaerobic digestion of solid organic waste. Ultrason. Sonochem. *20*, 931–936.

Cesaro, A., and Belgiorno, V. (2014). Pretreatment methods to improve anaerobic biodegradability of organic municipal solid waste fractions. Chem. Eng. J. *240*, 24–37.

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

Chen, C.-C., Chuang, Y.-S., Lin, C.-Y., Lay, C.-H., and Sen, B. (2012). Thermophilic dark fermentation of untreated rice straw using mixed cultures for hydrogen production. Int. J. Hydrog. Energy *37*, 15540–15546.

Chen, H., Liu, J., Chang, X., Chen, D., Xue, Y., Liu, P., Lin, H., and Han, S. (2017a). A review on the pretreatment of lignocellulose for high-value chemicals. Fuel Process. Technol. *160*, 196–206.

Chen, P., Anderson, E., Addy, M., Zhang, R., Cheng, Y., Peng, P., Ma, Y., Fan, L., Zhang, Y., Lu, Q., et al. (2018). Breakthrough Technologies for the Biorefining of Organic Solid and Liquid Wastes. Engineering *4*, 574–580.

Chen, W.-S., Strik, D.P.B.T.B., Buisman, C.J.N., and Kroeze, C. (2017b). Production of Caproic Acid from Mixed Organic Waste: An Environmental Life Cycle Perspective. Environ. Sci. Technol. *51*, 7159–7168.

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

Chen, Y., Jiang, X., Xiao, K., Shen, N., Zeng, R.J., and Zhou, Y. (2017c). Enhanced volatile fatty acids (VFAs) production in a thermophilic fermenter with stepwise pH increase – Investigation on dissolved organic matter transformation and microbial community shift. Water Res. *112*, 261–268.

Cherubini, F. (2010). The biorefinery concept: Using biomass instead of oil for producing energy and chemicals. Energy Convers. Manag. *51*, 1412–1421.

Ciaciuch, A., Gaca, J., and Lelewer, K. (2017). Effect of the two-stage thermal disintegration and anaerobic digestion of sewage sludge on the COD fractions. Pol. J. Chem. Technol. *19*, 130–135.

Clancy, J.M., Curtis, J., and Ó'Gallachóir, B. (2018). Modelling national policy making to promote bioenergy in heat, transport and electricity to 2030 – Interactions, impacts and conflicts. Energy Policy *123*, 579–593.

Coma, M., Martinez-Hernandez, E., Abeln, F., Raikova, S., Donnelly, J., Arnot, T.C., Allen, M.J., Hong, D.D., and Chuck, C.J. (2017a). Organic waste as a sustainable feedstock for platform chemicals. Faraday Discuss. *202*, 175–195.

Coma, M., Martinez-Hernandez, E., Abeln, F., Raikova, S., Donnelly, J., Arnot, T.C., Allen, M.J., Hong, D.D., and Chuck, C.J. (2017b). Organic waste as a sustainable feedstock for platform chemicals. Faraday Discuss. *202*, 175–195.

D., G., and Grilc, V. (2012). Anaerobic Treatment and Biogas Production from Organic Waste. In Management of Organic Waste, S. Kumar, ed. (InTech), p.

Dagnino, E.P., Chamorro, E.R., Romano, S.D., Felissia, F.E., and Area, M.C. (2013). Optimization of the acid pretreatment of rice hulls to obtain fermentable sugars for bioethanol production. Ind. Crops Prod. *42*, 363–368.

Dahiya, S., Kumar, A.N., Shanthi Sravan, J., Chatterjee, S., Sarkar, O., and Mohan, S.V. (2018a). Food waste biorefinery: Sustainable strategy for circular bioeconomy. Bioresour. Technol. *248*, 2–12.

Dahiya, S., Kumar, A.N., Shanthi Sravan, J., Chatterjee, S., Sarkar, O., and Mohan, S.V. (2018b). Food waste biorefinery: Sustainable strategy for circular bioeconomy. Bioresour. Technol. *248*, 2–12.

Dai, L., Long, Z., Lv, Y., and Feng, Q.C. (2014). The role of formic acid pretreatment in improving the carboxyl content of TEMPO-oxidized cellulose. Cell Chem Technol *48*, 469–475.

DapÃa, S., Santos, V., and ParajoÃ, J.C. (2002). Study of formic acid as an agent for biomass fractionation. Biomass Bioenergy 9.

De Gioannis, G., Muntoni, A., Polettini, A., and Pomi, R. (2013). A review of dark fermentative hydrogen production from biodegradable municipal waste fractions. Waste Manag. *33*, 1345–1361.

Demirbaş, A. (2004). Ethanol from Cellulosic Biomass Resources. Int. J. Green Energy *1*, 79–87.

Deng, W.-J. (2016). Turning Food Waste into Biofuel. In Recycling of Solid Waste for Biofuels and Bio-Chemicals, O.P. Karthikeyan, K. Heimann, and S.S. Muthu, eds. (Singapore: Springer Singapore), pp. 357–379.

Dessie, W., Zhang, W., Xin, F., Dong, W., Zhang, M., Ma, J., and Jiang, M. (2018). Succinic acid production from fruit and vegetable wastes hydrolyzed by on-site enzyme mixtures through solid state fermentation. Bioresour. Technol. *247*, 1177–1180.

Ding, K., Le, Y., Yao, G., Ma, Z., Jin, B., Wang, J., and Jin, F. (2018). A rapid and efficient hydrothermal conversion of coconut husk into formic acid and acetic acid. Process Biochem. *68*, 131–135.

Doğan, E., and Demirer, G.N. (2009). Volatile Fatty Acid Production from Organic Fraction of Municipal Solid Waste Through Anaerobic Acidogenic Digestion. Environ. Eng. Sci. *26*, 1443–1450.

Dong, L., Zhenhong, Y., Yongming, S., Xiaoying, K., and Yu, Z. (2009). Hydrogen production characteristics of the organic fraction of municipal solid wastes by anaerobic mixed culture fermentation. Int. J. Hydrog. Energy *34*, 812–820.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P. t, and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. *28*, 350–356.

Dutta, K., Daverey, A., and Lin, J.-G. (2014). Evolution retrospective for alternative fuels: First to fourth generation. Renew. Energy *69*, 114–122.

Elbeshbishy, E., Hafez, H., Dhar, B.R., and Nakhla, G. (2011). Single and combined effect of various pretreatment methods for biohydrogen production from food waste. Int. J. Hydrog. Energy *36*, 11379–11387.

Elbeshbishy, E., Dhar, B.R., Nakhla, G., and Lee, H.-S. (2017). A critical review on inhibition of dark biohydrogen fermentation. Renew. Sustain. Energy Rev. *79*, 656–668.

Esposito, G. (2012). Bio-Methane Potential Tests To Measure The Biogas Production From The Digestion and Co-Digestion of Complex Organic Substrates. Open Environ. Eng. J. *5*, 1–8.

Esposito, G., Frunzo, L., Panico, A., and Pirozzi, F. (2011). Modelling the effect of the OLR and OFMSW particle size on the performances of an anaerobic co-digestion reactor. Process Biochem. *46*, 557–565.

Favaro, L., Alibardi, L., Lavagnolo, M.C., Casella, S., and Basaglia, M. (2013). Effects of inoculum and indigenous microflora on hydrogen production from the organic fraction of municipal solid waste. Int. J. Hydrog. Energy *38*, 11774–11779.

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

Fisgativa, H., Tremier, A., and Dabert, P. (2016). Characterizing the variability of food waste quality: A need for efficient valorisation through anaerobic digestion. Waste Manag. *50*, 264–274.

Franke-Whittle, I.H., Walter, A., Ebner, C., and Insam, H. (2014). Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities. Waste Manag. *34*, 2080–2089.

Fr/olund, B., Griebe, T., and Nielsen, P.H. (1995). Enzymatic activity in the activated-sludge floc matrix. Appl. Microbiol. Biotechnol. *43*, 755–761.

Gao, C., Ma, C., and Xu, P. (2011). Biotechnological routes based on lactic acid production from biomass. Biotechnol. Adv. *29*, 930–939.

Garcia-Aguirre, J., Aymerich, E., González-Mtnez. de Goñi, J., and Esteban-Gutiérrez, M. (2017a). Selective VFA production potential from organic waste streams: Assessing temperature and pH influence. Bioresour. Technol. *244*, 1081–1088.

Garcia-Aguirre, J., Aymerich, E., González-Mtnez. de Goñi, J., and Esteban-Gutiérrez, M. (2017b). Selective VFA production potential from organic waste streams: Assessing temperature and pH influence. Bioresour. Technol. *244*, 1081–1088.

Gerardi, M.H. (2003). The Microbiology of Anaerobic Digesters (Hoboken, NJ, USA: John Wiley & Sons, Inc.).

Gerssen-Gondelach, S.J., Saygin, D., Wicke, B., Patel, M.K., and Faaij, A.P.C. (2014). Competing uses of biomass: Assessment and comparison of the performance of bio-based heat, power, fuels and materials. Renew. Sustain. Energy Rev. *40*, 964–998.

Ghimire, A., Frunzo, L., Pirozzi, F., Trably, E., Escudie, R., Lens, P.N.L., and Esposito, G. (2015a). A review on dark fermentative biohydrogen production from organic biomass: Process parameters and use of by-products. Appl. Energy *144*, 73–95.

Ghimire, A., Frunzob, L., Salzanoc, E., Panicod, A., Espositoa, G., Lense, P.N., and Pirozzif, F. (2015b). Biomass enrichment and scale-up implications for dark fermentation hydrogen production with mixed cultures. Chem. Eng. *43*.

Ghimire, A., Sposito, F., Frunzo, L., Trably, E., Escudié, R., Pirozzi, F., Lens, P.N.L., and Esposito, G. (2016). Effects of operational parameters on dark fermentative hydrogen production from biodegradable complex waste biomass. Waste Manag. *50*, 55–64.

Ghimire, A., Trably, E., Frunzo, L., Pirozzi, F., Lens, P.N.L., Esposito, G., Cazier, E.A., and Escudié, R. (2018). Effect of total solids content on biohydrogen production and lactic acid accumulation during dark fermentation of organic waste biomass. Bioresour. Technol. *248*, 180–186.

Gilarranz, M.A., Oliet, M., Rodriguez, F., and Tijero, J. (1999). Methanol-based pulping of *eucalyptus globulus*. Can. J. Chem. Eng. *77*, 515–521.

Giuliana, D., and Fabrizio, A. (2006). The contribution of water soluble and water insoluble organic fractions to oxygen uptake rate during high rate composting. Biodegradation *18*, 103–113.

Gomes, B.C., Rosa, P.R.F., Etchebehere, C., Silva, E.L., and AmâncioVaresche, M.B. (2015). Role of homo-and heterofermentative lactic acid bacteria on hydrogen-producing reactors operated with cheese whey wastewater. Int. J. Hydrog. Energy *40*, 8650–8660.

Gonzalez-Garcia, S., Gullón, B., and Moreira, M.T. (2018). Environmental assessment of biorefinery processes for the valorization of lignocellulosic wastes into oligosaccharides. J. Clean. Prod. *172*, 4066–4073.

Guo, M., Song, W., and Buhain, J. (2015). Bioenergy and biofuels: History, status, and perspective. Renew. Sustain. Energy Rev. *42*, 712–725.

Guo, W., Wu, Q., Yang, S., Luo, H., Peng, S., and Ren, N. (2014). Optimization of ultrasonic pretreatment and substrate/inoculum ratio to enhance hydrolysis and volatile fatty acid production from food waste. RSC Adv *4*, 53321–53326.

Guo, X.M., Trably, E., Latrille, E., Carrère, H., and Steyer, J.-P. (2010). Hydrogen production from agricultural waste by dark fermentation: A review. Int. J. Hydrog. Energy *35*, 10660–10673.

Haandel, A. van, and Lubbe, J. van der (2012). Handbook of Biological Wastewater Treatment (IWA Publishing).

Hafid, H.S., Rahman, N.A.A., Shah, U.K.M., Baharuddin, A.S., and Ariff, A.B. (2017). Feasibility of using kitchen waste as future substrate for bioethanol production: A review. Renew. Sustain. Energy Rev. *74*, 671–686.

Hagman, L., Blumenthal, A., Eklund, M., and Svensson, N. (2018). The role of biogas solutions in sustainable biorefineries. J. Clean. Prod. *172*, 3982–3989.

Hawkes, F., Hussy, I., Kyazze, G., Dinsdale, R., and Hawkes, D. (2007). Continuous dark fermentative hydrogen production by mesophilic microflora: Principles and progress. Int. J. Hydrog. Energy *32*, 172–184.

Hellsmark, H., and Söderholm, P. (2017). Innovation policies for advanced biorefinery development: key considerations and lessons from Sweden: Innovation Policies for Advanced Biorefinery Development. Biofuels Bioprod. Biorefining *11*, 28–40.

Hidajat, M.J., Riaz, A., Park, J., Insyani, R., Verma, D., and Kim, J. (2017). Depolymerization of concentrated sulfuric acid hydrolysis lignin to high-yield aromatic monomers in basic sub- and supercritical fluids. Chem. Eng. J. *317*, 9–19.

Holliger, C., Alves, M., Andrade, D., Angelidaki, I., Astals, S., Baier, U., Bougrier, C., Buffière, P., Carballa, M., de Wilde, V., et al. (2016). Towards a standardization of biomethane potential tests. Water Sci. Technol. *74*, 2515–2522.

Huang, X., Shen, Y., Luo, H., Liu, J., and Liu, J. (2018). Enhancement of extracellular lipid production by oleaginous yeast through preculture and sequencing batch culture strategy with acetic acid. Bioresour. Technol. *247*, 395–401.

Huijgen, W.J.J., Reith, J.H., and den Uil, H. (2010). Pretreatment and Fractionation of Wheat Straw by an Acetone-Based Organosolv Process. Ind. Eng. Chem. Res. *49*, 10132–10140.

Im, W.-T., Kim, D.-H., Kim, K.-H., and Kim, M.-S. (2012). Bacterial community analyses by pyrosequencing in dark fermentative H2-producing reactor using organic wastes as a feedstock. Int. J. Hydrog. Energy *37*, 8330–8337.

Islam, M.N., Park, K.-J., and Yoon, H.-S. (2012). Methane Production Potential of Food Waste and Food Waste Mixture with Swine Manure in Anaerobic Digestion. J. Biosyst. Eng. *37*, 100–105.

Izumi, K., Okishio, Y., Nagao, N., Niwa, C., Yamamoto, S., and Toda, T. (2010). Effects of particle size on anaerobic digestion of food waste. Int. Biodeterior. Biodegrad. *64*, 601–608.

Jankowska, E., Chwialkowska, J., Stodolny, M., and Oleskowicz-Popiel, P. (2017). Volatile fatty acids production during mixed culture fermentation – The impact of substrate complexity and pH. Chem. Eng. J. *326*, 901–910.

Jha, A.K., Li, J., Yuan, Y., Baral, N., and Ai, B. (2014). A Review on Bio-butyric Acid Production and its Optimization. Int J Agric Biol *16*, 7.

Jiang, L., Fu, H., Yang, H.K., Xu, W., Wang, J., and Yang, S.-T. (2018). Butyric acid: Applications and recent advances in its bioproduction. Biotechnol. Adv. *36*, 2101–2117.

Jin, Q., Yang, L., Poe, N., and Huang, H. (2018). Integrated processing of plant-derived waste to produce value-added products based on the biorefinery concept. Trends Food Sci. Technol. *74*, 119–131.

Jonker, J.G.G., and Faaij, A.P.C. (2013). Techno-economic assessment of micro-algae as feedstock for renewable bio-energy production. Appl. Energy *102*, 461–475.

Kaparaju, P., and Rintala, J. (2005). Anaerobic co-digestion of potato tuber and its industrial by-products with pig manure. Resour. Conserv. Recycl. *43*, 175–188.

Karimi, K., and Taherzadeh, M.J. (2016). A critical review on analysis in pretreatment of lignocelluloses: Degree of polymerization, adsorption/desorption, and accessibility. Bioresour. Technol. *203*, 348–356.

Karthikeyan, O.P., Balasubramanian, R., and Wong, J.W.C. (2017). 7 - Pretreatment of Organic Solid Substrates for Bioenergy and Biofuel Recovery. In Current Developments in Biotechnology and Bioengineering, J.W.-C. Wong, R.D. Tyagi, and A. Pandey, eds. (Elsevier), pp. 135–156.

Kaur, G., Uisan, K., Ong, K.L., and Ki Lin, C.S. (2018). Recent Trends in Green and Sustainable Chemistry & Waste Valorisation: Rethinking Plastics in a circular economy. Curr. Opin. Green Sustain. Chem. *9*, 30–39.

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

Khan, M.A., Ngo, H.H., Guo, W.S., Liu, Y., Nghiem, L.D., Hai, F.I., Deng, L.J., Wang, J., and Wu, Y. (2016). Optimization of process parameters for production of volatile fatty acid, biohydrogen and methane from anaerobic digestion. Bioresour. Technol. *219*, 738–748.

Khanal, S.K. (2008). Microbiology and Biochemistry of Anaerobic Biotechnology. In Anaerobic Biotechnology for Bioenergy Production, S.K. Khanal, ed. (Oxford, UK: Wiley-Blackwell), pp. 29–41.

Kim, D.-H., Kim, S.-H., and Shin, H.-S. (2009). Hydrogen fermentation of food waste without inoculum addition. Enzyme Microb. Technol. *45*, 181–187.

Kim, N.-J., Park, G.W., Kang, J., Kim, Y.-C., and Chang, H.N. (2013). Volatile fatty acid production from lignocellulosic biomass by lime pretreatment and its applications to industrial biotechnology. Biotechnol. Bioprocess Eng. *18*, 1163–1168.

Kim, S.-H., Han, S.-K., and Shin, H.-S. (2006). Effect of substrate concentration on hydrogen production and 16S rDNA-based analysis of the microbial community in a continuous fermenter. Process Biochem. *41*, 199–207.

Komemoto, K., Lim, Y.G., Nagao, N., Onoue, Y., Niwa, C., and Toda, T. (2009). Effect of temperature on VFA's and biogas production in anaerobic solubilization of food waste. Waste Manag. *29*, 2950–2955.

Kothari, R., Singh, D.P., Tyagi, V.V., and Tyagi, S.K. (2012). Fermentative hydrogen production – An alternative clean energy source. Renew. Sustain. Energy Rev. *16*, 2337–2346.

Kumar, A.K., and Sharma, S. (2017). Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. Bioresour. Bioprocess. *4*.

Kumar, G., Cho, S.-K., Sivagurunathan, P., Anburajan, P., Mahapatra, D.M., Park, J.-H., and Pugazhendhi, A. (2018a). Insights into evolutionary trends in molecular biology tools in microbial screening for biohydrogen production through dark fermentation. Int. J. Hydrog. Energy *43*, 19885–19901.

Kumar, G., Shobana, S., Nagarajan, D., Lee, D.-J., Lee, K.-S., Lin, C.-Y., Chen, C.-Y., and Chang, J.-S. (2018b). Biomass based hydrogen production by dark fermentation — recent trends and opportunities for greener processes. Curr. Opin. Biotechnol. *50*, 136–145.

Kumar, P., Barrett, D.M., Delwiche, M.J., and Stroeve, P. (2009). Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. Ind. Eng. Chem. Res. *48*, 3713–3729.

Kuo, W., and Cheng, K. (2007). Use of respirometer in evaluation of process and toxicity of thermophilic anaerobic digestion for treating kitchen waste. Bioresour. Technol. *98*, 1805–1811.

Kuruti, K., Gangagni Rao, A., Gandu, B., Kiran, G., Mohammad, S., Sailaja, S., and Swamy, Y.V. (2015). Generation of bioethanol and VFA through anaerobic acidogenic fermentation route with press mud obtained from sugar mill as a feedstock. Bioresour. Technol. *192*, 646–653.

Lee, J.-H., Lee, H., Yun, S.-M., Jung, K.H., Jeong, Y., Yan, H.H., Hong, S., and Hong, S.-S. (2013). IPD-196, a novel phosphatidylinositol 3-kinase inhibitor with potent anticancer activity against hepatocellular carcinoma. Cancer Lett. *329*, 99–108.

Lee, M.-J., Song, J.-H., and Hwang, S.-J. (2009). Effects of acid pre-treatment on bio-hydrogen production and microbial communities during dark fermentation. Bioresour. Technol. *100*, 1491–1493.

Lee, W.S., Chua, A.S.M., Yeoh, H.K., and Ngoh, G.C. (2014). A review of the production and applications of waste-derived volatile fatty acids. Chem. Eng. J. *235*, 83–99.

Lemire, J.A., Harrison, J.J., and Turner, R.J. (2013). Antimicrobial activity of metals: mechanisms, molecular targets and applications. Nat. Rev. Microbiol. *11*, 371–384.

Leung, C.C.J., Cheung, A.S.Y., Zhang, A.Y.-Z., Lam, K.F., and Lin, C.S.K. (2012). Utilisation of waste bread for fermentative succinic acid production. Biochem. Eng. J. *65*, 10–15.

Leung, D.Y.C., Wu, X., and Leung, M.K.H. (2010). A review on biodiesel production using catalyzed transesterification. Appl. Energy *87*, 1083–1095.

Li, C., and Fang, H.H.P. (2007). Fermentative Hydrogen Production From Wastewater and Solid Wastes by Mixed Cultures. Crit. Rev. Environ. Sci. Technol. *37*, 1–39.

Li, W., Khalid, H., Zhu, Z., Zhang, R., Liu, G., Chen, C., and Thorin, E. (2018). Methane production through anaerobic digestion: Participation and digestion characteristics of cellulose, hemicellulose and lignin. Appl. Energy *226*, 1219–1228.

Li, Y., Park, S.Y., and Zhu, J. (2011a). Solid-state anaerobic digestion for methane production from organic waste. Renew. Sustain. Energy Rev. *15*, 821–826.

Li, Y., Park, S.Y., and Zhu, J. (2011b). Solid-state anaerobic digestion for methane production from organic waste. Renew. Sustain. Energy Rev. *15*, 821–826.

Li, Y., Jin, Y., Borrion, A., Li, H., and Li, J. (2017). Effects of organic composition on mesophilic anaerobic digestion of food waste. Bioresour. Technol. *244*, 213–224.

Lim, J.W., Ge, T., and Tong, Y.W. (2018). Monitoring of microbial communities in anaerobic digestion sludge for biogas optimisation. Waste Manag. *71*, 334–341.

Lipscomb, G.L., Schut, G.J., Thorgersen, M.P., Nixon, W.J., Kelly, R.M., and Adams, M.W.W. (2014). Engineering Hydrogen Gas Production from Formate in a Hyperthermophile by Heterologous Production of an 18-Subunit Membrane-bound Complex. J. Biol. Chem. *289*, 2873–2879.

Liu, D., Liu, D., Zeng, R.J., and Angelidaki, I. (2006). Hydrogen and methane production from household solid waste in the two-stage fermentation process. Water Res. *40*, 2230–2236.

Liu, D., Zeng, R.J., and Angelidaki, I. (2008). Effects of pH and hydraulic retention time on hydrogen production versus methanogenesis during anaerobic fermentation of organic household solid waste under extreme-thermophilic temperature (70°C). Biotechnol. Bioeng. *100*, 1108–1114.

Łukajtis, R., Hołowacz, I., Kucharska, K., Glinka, M., Rybarczyk, P., Przyjazny, A., and Kamiński, M. (2018). Hydrogen production from biomass using dark fermentation. Renew. Sustain. Energy Rev. *91*, 665–694.

Lundgren, A., and Hjertberg, T. (2010). Ethylene from Renewable Resources. In Surfactants from Renewable Resources, M. Kjellin, and I. Johansson, eds. (Chichester, UK: John Wiley & Sons, Ltd), pp. 109–126.

Luo, G., Talebnia, F., Karakashev, D., Xie, L., Zhou, Q., and Angelidaki, I. (2011). Enhanced bioenergy recovery from rapeseed plant in a biorefinery concept. Bioresour. Technol. *102*, 1433–1439.

Ma, H., Guo, Y., Qin, Y., and Li, Y.-Y. (2018). Nutrient recovery technologies integrated with energy recovery by waste biomass anaerobic digestion. Bioresour. Technol. *269*, 520–531.

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

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

Marques, I.P., Batista, A.P., Coelho, A., and Lopes da Silva, T. (2018). Co-digestion of Rhodosporidium toruloides biorefinery wastes for biogas production. Process Biochem. *64*, 221–227.

Mata-Alvarez, J., Macé, S., and Llabrés, P. (2000). Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresour. Technol. *74*, 3–16.

McDowall, J.S., Murphy, B.J., Haumann, M., Palmer, T., Armstrong, F.A., and Sargent, F. (2014). Bacterial formate hydrogenlyase complex. Proc. Natl. Acad. Sci. *111*, E3948–E3956.

Meherkotay, S., and Das, D. (2008). Biohydrogen as a renewable energy resource—Prospects and potentials. Int. J. Hydrog. Energy *33*, 258–263.

Menon, A., Ren, F., Wang, J.-Y., and Giannis, A. (2016). Effect of pretreatment techniques on food waste solubilization and biogas production during thermophilic batch anaerobic digestion. J. Mater. Cycles Waste Manag. *18*, 222–230.

Momayez, F., Karimi, K., and Taherzadeh, M.J. (2019). Energy recovery from industrial crop wastes by dry anaerobic digestion: A review. Ind. Crops Prod. *129*, 673–687.

Moncada, J., Tamayo, J.A., and Cardona, C.A. (2014). Integrating first, second, and third generation biorefineries: Incorporating microalgae into the sugarcane biorefinery. Chem. Eng. Sci. *118*, 126–140.

Monlau, F., Sambusiti, C., Barakat, A., Guo, X.M., Latrille, E., Trably, E., Steyer, J.-P., and Carrere, H. (2012). Predictive Models of Biohydrogen and Biomethane Production Based on the Compositional and Structural Features of Lignocellulosic Materials. Environ. Sci. Technol. *46*, 12217–12225.

Moscoviz, R., Trably, E., Bernet, N., and Carrère, H. (2018). The environmental biorefinery: state-of-the-art on the production of hydrogen and value-added biomolecules in mixed-culture fermentation. Green Chem. *20*, 3159–3179.

Mshandete, A., Björnsson, L., Kivaisi, A.K., Rubindamayugi, M.S.T., and Mattiasson, B. (2006). Effect of particle size on biogas yield from sisal fibre waste. Renew. Energy *31*, 2385–2392.

Naik, S.N., Goud, V.V., Rout, P.K., and Dalai, A.K. (2010). Production of first and second generation biofuels: A comprehensive review. Renew. Sustain. Energy Rev. *14*, 578–597.

Nath, K., and Das, D. (2004). Improvement of fermentative hydrogen production: various approaches. Appl. Microbiol. Biotechnol. *65*.

Nayak, A., and Bhushan, B. (2019). An overview of the recent trends on the waste valorization techniques for food wastes. J. Environ. Manage. *233*, 352–370.

Nigam, P.S., and Singh, A. (2011). Production of liquid biofuels from renewable resources. Prog. Energy Combust. Sci. *37*, 52–68.

Nissilä, M.E., Tähti, H.P., Rintala, J.A., and Puhakka, J.A. (2011). Thermophilic hydrogen production from cellulose with rumen fluid enrichment cultures: Effects of different heat treatments. Int. J. Hydrog. Energy *36*, 1482–1490.

Nizami, A.S., Rehan, M., Waqas, M., Naqvi, M., Ouda, O.K.., Shahzad, K., Miandad, R., Khan, M.Z., Syamsiro, M., Ismail, I.M.I., et al. (2017). Waste biorefineries: Enabling circular economies in developing countries. Bioresour. Technol. *241*, 1101–1117.

Oshoma, C.E., and Obueh, H.O. (2018). Influence of nitrogen sources on ethanol production by Saccharomyces Spp in the presence of formic acid. Bayero J. Pure Appl. Sci. *11*, 1–7.

Oz, N.A., and Yarimtepe, C.C. (2014). Ultrasound assisted biogas production from landfill leachate. Waste Manag. *34*, 1165–1170.

Özyürek, Ö., and van Heiningen, A. (2018). Formic acid reinforced autohydrolysis of wheat straw for high yield production of monosugars and minimal lignin precipitation. Ind. Crops Prod. *112*, 320–326.

Panagiotopoulos, I.A., Bakker, R.R., Budde, M.A.W., de Vrije, T., Claassen, P.A.M., and Koukios, E.G. (2009). Fermentative hydrogen production from pretreated biomass: A comparative study. Bioresour. Technol. *100*, 6331–6338.

Panda, S.K., Mishra, S.S., Kayitesi, E., and Ray, R.C. (2016). Microbial-processing of fruit and vegetable wastes for production of vital enzymes and organic acids: Biotechnology and scopes. Environ. Res. *146*, 161–172.

Parajuli, R., Dalgaard, T., Jørgensen, U., Adamsen, A.P.S., Knudsen, M.T., Birkved, M., Gylling, M., and Schjørring, J.K. (2015). Biorefining in the prevailing energy and materials crisis: a review of sustainable pathways for biorefinery value chains and sustainability assessment methodologies. Renew. Sustain. Energy Rev. *43*, 244–263.

Parthiba Karthikeyan, O., Trably, E., Mehariya, S., Bernet, N., Wong, J.W.C., and Carrere, H. (2018). Pretreatment of food waste for methane and hydrogen recovery: A review. Bioresour. Technol. *249*, 1025–1039.

Peinemann, J.C., and Pleissner, D. (2018). Material Utilization of Organic Residues. Appl. Biochem. Biotechnol. *184*, 733–745.

Persson, M., Jönsson, O., and Wellinger, A. Biogas Upgrading to Vehicle Fuel Standards and Grid Injection. 19.

Pleissner, D., Lau, K.Y., Schneider, R., Venus, J., and Lin, C.S.K. (2015). Fatty acid feedstock preparation and lactic acid production as integrated processes in mixed restaurant food and bakery wastes treatment. Food Res. Int. *73*, 52–61.

Qureshi, N., Saha, B.C., Hector, R.E., Dien, B., Hughes, S., Liu, S., Iten, L., Bowman, M.J., Sarath, G., and Cotta, M.A. (2010). Production of butanol (a biofuel) from agricultural residues: Part II – Use of corn stover and switchgrass hydrolysates☆. Biomass Bioenergy *34*, 566–571.

Rafieenia, R., Pivato, A., Schievano, A., and Lavagnolo, M.C. (2018). Dark fermentation metabolic models to study strategies for hydrogen consumers inhibition. Bioresour. Technol. *267*, 445–457.

Raposo, F., De la Rubia, M.A., Fernández-Cegrí, V., and Borja, R. (2012). Anaerobic digestion of solid organic substrates in batch mode: An overview relating to methane yields and experimental procedures. Renew. Sustain. Energy Rev. *16*, 861–877.

Razavi, A.S., Hosseini Koupaie, E., Azizi, A., Hafez, H., and Elbeshbishy, E. (2019). Hydrothermal pretreatment of source separated organics for enhanced solubilization and biomethane recovery. Bioresour. Technol. *274*, 502–511.

Ren, N., Guo, W., Liu, B., Cao, G., and Ding, J. (2011). Biological hydrogen production by dark fermentation: challenges and prospects towards scaled-up production. Curr. Opin. Biotechnol. *22*, 365–370.

Ren, Y., Yu, M., Wu, C., Wang, Q., Gao, M., Huang, Q., and Liu, Y. (2018). A comprehensive review on food waste anaerobic digestion: Research updates and tendencies. Bioresour. Technol. *247*, 1069–1076.

Rodriguez-Perez, S., Serrano, A., Pantión, A.A., and Alonso-Fariñas, B. (2018). Challenges of scaling-up PHA production from waste streams. A review. J. Environ. Manage. *205*, 215–230.

Romero-Cedillo, L., Poggi-Varaldo, H.M., Ponce-Noyola, T., Ríos-Leal, E., Ramos-Valdivia, A.C., Cerda-García Rojas, C.M., and Tapia-Ramírez, J. (2017). A review of the potential of pretreated solids to improve gas biofuels production in the context of an OFMSW biorefinery: A review of the potential of pretreated solids. J. Chem. Technol. Biotechnol. *92*, 937–958.

Saha, S., Kurade, M.B., El-Dalatony, M.M., Chatterjee, P.K., Lee, D.S., and Jeon, B.-H. (2016). Improving bioavailability of fruit wastes using organic acid: An exploratory study of biomass pretreatment for fermentation. Energy Convers. Manag. *127*, 256–264.

Saidi, R., Liebgott, P.P., Hamdi, M., Auria, R., and Bouallagui, H. (2018). Enhancement of fermentative hydrogen production by Thermotoga maritima through hyperthermophilic anaerobic co-digestion of fruit-vegetable and fish wastes. Int. J. Hydrog. Energy *43*, 23168–23177.

Saini, J.K., Saini, R., and Tewari, L. (2015). Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. 3 Biotech *5*, 337–353.

Salapa, I., Katsimpouras, C., Topakas, E., and Sidiras, D. (2017). Organosolv pretreatment of wheat straw for efficient ethanol production using various solvents. Biomass Bioenergy *100*, 10–16.

Salihu, A., and Alam, M.Z. (2016). Pretreatment Methods of Organic Wastes for Biogas Production. J. Appl. Sci. *16*, 124–137.

Sánchez, Ó.J., and Cardona, C.A. (2008). Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresour. Technol. *99*, 5270–5295.

Sanchez, N., Ruiz, R.Y., Cifuentes, B., and Cobo, M. (2016). Hydrogen from glucose: A combined study of glucose fermentation, bioethanol purification, and catalytic steam reforming. Int. J. Hydrog. Energy 41, 5640–5651.

Sannigrahi, P., Miller, S.J., and Ragauskas, A.J. (2010). Effects of organosolv pretreatment and enzymatic hydrolysis on cellulose structure and crystallinity in Loblolly pine. Carbohydrate Research 345, 965–970.

Santamaría-Fernández, M., Molinuevo-Salces, B., Lübeck, M., and Uellendahl, H. (2018). Biogas potential of green biomass after protein extraction in an organic biorefinery concept for feed, fuel and fertilizer production. Renew. Energy *129*, 769–775.

Sarkar, O., Kumar, A.N., Dahiya, S., Krishna, K.V., Yeruva, D.K., and Mohan, S.V. (2016). Regulation of acidogenic metabolism towards enhanced short chain fatty acid biosynthesis from waste: metagenomic profiling. RSC Adv. *6*, 18641–18653.

Sárvári Horváth, I., Tabatabaei, M., Karimi, K., and Kumar, R. (2016). Recent updates on biogas production - a review. Biofuel Res. J. *3*, 394–402.

Scarlat, N., Dallemand, J.-F., and Fahl, F. (2018). Biogas: Developments and perspectives in Europe. Renew. Energy *129*, 457–472.

Shen, D., Yin, J., Yu, X., Wang, M., Long, Y., Shentu, J., and Chen, T. (2017). Acidogenic fermentation characteristics of different types of protein-rich substrates in food waste to produce volatile fatty acids. Bioresour. Technol. *227*, 125–132.

Shin, H. (2004). Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis. Int. J. Hydrog. Energy *29*, 1355–1363.

Show, K.Y., Lee, D.J., Tay, J.H., Lin, C.Y., and Chang, J.S. (2012). Biohydrogen production: Current perspectives and the way forward. Int. J. Hydrog. Energy *37*, 15616–15631.

Sidiras, D. (2004). Simulation of acid-catalysed organosolv fractionation of wheat straw. Bioresour. Technol. *94*, 91–98.

Siles, J.A., Martín, M.A., Chica, A.F., and Martín, A. (2010). Anaerobic co-digestion of glycerol and wastewater derived from biodiesel manufacturing. Bioresour. Technol. *101*, 6315–6321.

Silva, F.C., Serafim, L.S., Nadais, H., Arroja, L., and Capela, I. (2013). Acidogenic Fermentation Towards Valorisation of Organic Waste Streams into Volatile Fatty Acids. Chem Biochem Eng Q 10.

Sindhu, R., Binod, P., Satyanagalakshmi, K., Janu, K.U., Sajna, K.V., Kurien, N., Sukumaran, R.K., and Pandey, A. (2010). Formic Acid as a Potential Pretreatment Agent for the Conversion of Sugarcane Bagasse to Bioethanol. Appl. Biochem. Biotechnol. *162*, 2313–2323.

Singh, R., Shukla, A., Tiwari, S., and Srivastava, M. (2014). A review on delignification of lignocellulosic biomass for enhancement of ethanol production potential. Renew. Sustain. Energy Rev. *32*, 713–728.

Singhania, R.R., Patel, A.K., Christophe, G., Fontanille, P., and Larroche, C. (2013). Biological upgrading of volatile fatty acids, key intermediates for the valorization of biowaste through dark anaerobic fermentation. Bioresour. Technol. *145*, 166–174.

Sinha, P., Roy, S., and Das, D. (2015). Role of formate hydrogen lyase complex in hydrogen production in facultative anaerobes. Int. J. Hydrog. Energy *40*, 8806–8815.

Sivagurunathan, P., Kuppam, C., Mudhoo, A., Saratale, G.D., Kadier, A., Zhen, G., Chatellard, L., Trably, E., and Kumar, G. (2018). A comprehensive review on two-stage integrative schemes for the valorization of dark fermentative effluents. Crit. Rev. Biotechnol. *38*, 868–882.

Skaggs, R.L., Coleman, A.M., Seiple, T.E., and Milbrandt, A.R. (2018). Waste-to-Energy biofuel production potential for selected feedstocks in the conterminous United States. Renew. Sustain. Energy Rev. *82*, 2640–2651.

Slezak, R., Grzelak, J., Krzystek, L., and Ledakowicz, S. (2017). The effect of initial organic load of the kitchen waste on the production of VFA and H 2 in dark fermentation. Waste Manag. *68*, 610–617.

Snelders, J., Dornez, E., Benjelloun-Mlayah, B., Huijgen, W.J.J., de Wild, P.J., Gosselink, R.J.A., Gerritsma, J., and Courtin, C.M. (2014). Biorefining of wheat

straw using an acetic and formic acid based organosolv fractionation process. Bioresour. Technol. *156*, 275–282.

Song, W., Cheng, J., Zhou, J., Xie, B., Su, H., and Cen, K. (2010). Cogeneration of hydrogen and methane from protein-mixed food waste by two-phase anaerobic process. Int. J. Hydrog. Energy *35*, 3141–3146.

Srikanth, S., and Venkata Mohan, S. (2014). Regulating feedback inhibition caused by the accumulated acid intermediates during acidogenic hydrogen production through feed replacement. Int. J. Hydrog. Energy *39*, 10028–10040.

Stephen, J.L., and Periyasamy, B. (2018). Innovative developments in biofuels production from organic waste materials: A review. Fuel *214*, 623–633.

Strazzera, G., Battista, F., Garcia, N.H., Frison, N., and Bolzonella, D. (2018). Volatile fatty acids production from food wastes for biorefinery platforms: A review. J. Environ. Manage. *226*, 278–288.

Sun, F.F., Wang, L., Hong, J., Ren, J., Du, F., Hu, J., Zhang, Z., and Zhou, B. (2015). The impact of glycerol organosolv pretreatment on the chemistry and enzymatic hydrolyzability of wheat straw. Bioresour. Technol. *187*, 354–361.

Surendra, K.C., Sawatdeenarunat, C., Shrestha, S., Sung, S., and Khanal, S.K. (2015). Anaerobic Digestion-Based Biorefinery for Bioenergy and Biobased Products. Ind. Biotechnol. *11*, 103–112.

Tapia-Venegas, E., Ramirez, J.E., Donoso-Bravo, A., Jorquera, L., Steyer, J.-P., and Ruiz-Filippi, G. (2013). Bio-hydrogen production during acidogenic fermentation in a multistage stirred tank reactor. Int. J. Hydrog. Energy *38*, 2185–2190.

Tawfik, A., and El-Qelish, M. (2012). Continuous hydrogen production from co-digestion of municipal food waste and kitchen wastewater in mesophilic anaerobic baffled reactor. Bioresour. Technol. *114*, 270–274.

Tomás-Pejó, E., Alvira, P., Ballesteros, M., and Negro, M.J. (2011). Chapter 7 - Pretreatment Technologies for Lignocellulose-to-Bioethanol Conversion. In Biofuels, A. Pandey, C. Larroche, S.C. Ricke, C.-G. Dussap, and E. Gnansounou, eds. (Amsterdam: Academic Press), pp. 149–176.

Trchounian, K., Sawers, R.G., and Trchounian, A. (2017). Improving biohydrogen productivity by microbial dark- and photo-fermentations: Novel data and future approaches. Renew. Sustain. Energy Rev. *80*, 1201–1216.

Tuck, C.O., Pérez, E., Horváth, I.T., Sheldon, R.A., and Poliakoff, M. (2012). Valorization of Biomass: Deriving More Value from Waste. Science *337*, 695–699.

Valdezvazquez, I., Riosleal, E., Esparzagarcia, F., Cecchi, F., and Poggivaraldo, H. (2005). Semi-continuous solid substrate anaerobic reactors for H production from organic waste: Mesophilic versus thermophilic regime. Int. J. Hydrog. Energy *30*, 1383–1391.

Vavouraki, A.I., Volioti, V., and Kornaros, M.E. (2014). Optimization of thermo-chemical pretreatment and enzymatic hydrolysis of kitchen wastes. Waste Manag. *34*, 167–173.

Venkata Mohan, S., Nikhil, G.N., Chiranjeevi, P., Nagendranatha Reddy, C., Rohit, M.V., Kumar, A.N., and Sarkar, O. (2016a). Waste biorefinery models

towards sustainable circular bioeconomy: Critical review and future perspectives. Bioresour. Technol. *215*, 2–12.

Venkata Mohan, S., Nikhil, G.N., Chiranjeevi, P., Nagendranatha Reddy, C., Rohit, M.V., Kumar, A.N., and Sarkar, O. (2016b). Waste biorefinery models towards sustainable circular bioeconomy: Critical review and future perspectives. Bioresour. Technol. *215*, 2–12.

Venkateswar Reddy, M., and Venkata Mohan, S. (2012). Influence of aerobic and anoxic microenvironments on polyhydroxyalkanoates (PHA) production from food waste and acidogenic effluents using aerobic consortia. Bioresour. Technol. *103*, 313–321.

Wainaina, S., Horváth, I.S., and Taherzadeh, M.J. (2018). Biochemicals from food waste and recalcitrant biomass via syngas fermentation: A review. Bioresour. Technol. *248*, 113–121.

Wang, J., and Wan, W. (2008). Comparison of different pretreatment methods for enriching hydrogen-producing bacteria from digested sludge. Int. J. Hydrog. Energy *33*, 2934–2941.

Wang, D., Huang, Y., Xu, Q., Liu, X., Yang, Q., and Li, X. (2019). Free ammonia aids ultrasound pretreatment to enhance short-chain fatty acids production from waste activated sludge. Bioresour. Technol. *275*, 163–171.

Wang, K., Yin, J., Shen, D., and Li, N. (2014a). Anaerobic digestion of food waste for volatile fatty acids (VFAs) production with different types of inoculum: Effect of pH. Bioresour. Technol. *161*, 395–401.

Wang, X., Lu, X., Li, F., and Yang, G. (2014b). Effects of Temperature and Carbon-Nitrogen (C/N) Ratio on the Performance of Anaerobic Co-Digestion of Dairy Manure, Chicken Manure and Rice Straw: Focusing on Ammonia Inhibition. PLoS ONE *9*, e97265.

Wang, X., Meng, Q., Gao, L., Jin, Z., Ge, J., Liu, C., and Xing, W. (2018). Recent progress in hydrogen production from formic acid decomposition. Int. J. Hydrog. Energy *43*, 7055–7071.

Wang, Y., Zhang, Y., Wang, J., and Meng, L. (2009). Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. Biomass Bioenergy *33*, 848–853.

Weimer, P.J., Nerdahl, M., and Brandl, D.J. (2015). Production of medium-chain volatile fatty acids by mixed ruminal microorganisms is enhanced by ethanol in co-culture with Clostridium kluyveri. Bioresour. Technol. *175*, 97–101.

Wonglertarak, W., and Wichitsathian, B. (2014). Alkaline Pretreatment of Waste Activated Sludge in Anaerobic Digestion. J. Clean Energy Technol. 118–121.

Wu, L.-J., Kobayashi, T., Kuramochi, H., Li, Y.-Y., Xu, K.-Q., and Lv, Y. (2018). High loading anaerobic co-digestion of food waste and grease trap waste: Determination of the limit and lipid/long chain fatty acid conversion. Chem. Eng. J. *338*, 422–431.

Xiong, H., Chen, J., Wang, H., and Shi, H. (2012). Influences of volatile solid concentration, temperature and solid retention time for the hydrolysis of waste activated sludge to recover volatile fatty acids. Bioresour. Technol. *119*, 285–292.

Xu, F., Wang, F., Lin, L., and Li, Y. (2016). Comparison of digestate from solid anaerobic digesters and dewatered effluent from liquid anaerobic digesters as inocula for solid state anaerobic digestion of yard trimmings. Bioresour. Technol. *200*, 753–760.

Yang, X., Choi, H.S., Park, C., and Kim, S.W. (2015). Current states and prospects of organic waste utilization for biorefineries. Renewable and Sustainable Energy Reviews 49, 335–349.

Yang, X., Choi, H.S., Park, C., and Kim, S.W. (2015). Current states and prospects of organic waste utilization for biorefineries. Renew. Sustain. Energy Rev. *49*, 335–349.

Yasvanthrajan, N., Nabera, A., Salike, S., Valan, D.T., Sivakumar, P., Muthukumar, K., and Arunagiri, A. (2018). An overview on the process intensification of microchannel reactors for biodiesel production. Chem. Eng. Process. - Process Intensif.

Yeshanew, M.M., Frunzo, L., Lens, P.N.L., Pirozzi, F., and Esposito, G. (2016). Mass Loss Controlled Thermal Pretreatment System to Assess the Effects of Pretreatment Temperature on Organic Matter Solubilization and Methane Yield from Food Waste. Front. Environ. Sci. *4*.

Yokoyama, H., Moriya, N., Ohmori, H., Waki, M., Ogino, A., and Tanaka, Y. (2007). Community analysis of hydrogen-producing extreme thermophilic anaerobic microflora enriched from cow manure with five substrates. Appl. Microbiol. Biotechnol. *77*, 213–222.

Yong, Z., Dong, Y., Zhang, X., and Tan, T. (2015). Anaerobic co-digestion of food waste and straw for biogas production. Renew. Energy *78*, 527–530.

Yun, Y.-M., Lee, M.-K., Im, S.-W., Marone, A., Trably, E., Shin, S.-R., Kim, M.-G., Cho, S.-K., and Kim, D.-H. (2018). Biohydrogen production from food waste: Current status, limitations, and future perspectives. Bioresour. Technol. *248*, 79–87.

Zacharof, M.-P., and Lovitt, R.W. (2013). Complex Effluent Streams as a Potential Source of Volatile Fatty Acids. Waste Biomass Valorization *4*, 557–581.

Zahedi, S., Sales, D., Romero, L.I., and Solera, R. (2013). Hydrogen production from the organic fraction of municipal solid waste in anaerobic thermophilic acidogenesis: Influence of organic loading rate and microbial content of the solid waste. Bioresour. Technol. *129*, 85–91.

Zeshan, Karthikeyan, O.P., and Visvanathan, C. (2012). Effect of C/N ratio and ammonia-N accumulation in a pilot-scale thermophilic dry anaerobic digester. Bioresour. Technol. *113*, 294–302.

Zhang, K., Pei, Z., and Wang, D. (2016). Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: A review. Bioresour. Technol. *199*, 21–33.

Zhang, L., Loh, K.-C., and Zhang, J. (2018). Enhanced biogas production from anaerobic digestion of solid organic wastes: Current status and prospects. Bioresour. Technol. Rep.

Zhang, W., Wei, Q., Wu, S., Qi, D., Li, W., Zuo, Z., and Dong, R. (2014). Batch anaerobic co-digestion of pig manure with dewatered sewage sludge under mesophilic conditions. Appl. Energy *128*, 175–183.

Zhao, X., and Liu, D. (2012). Fractionating pretreatment of sugarcane bagasse by aqueous formic acid with direct recycle of spent liquor to increase cellulose digestibility–the Formiline process. Bioresour. Technol. *117*, 25–32.

Zhao, J., Wang, D., Liu, Y., Ngo, H.H., Guo, W., Yang, Q., and Li, X. (2018). Novel stepwise pH control strategy to improve short chain fatty acid production from sludge anaerobic fermentation. Bioresour. Technol. *249*, 431–438.

Zhao, X., Cheng, K., and Liu, D. (2009). Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. Appl. Microbiol. Biotechnol. *82*, 815–827.

Zhao, X., Li, S., Wu, R., and Liu, D. (2017). Organosolv fractionating pre-treatment of lignocellulosic biomass for efficient enzymatic saccharification: chemistry, kinetics, and substrate structures. Biofuels Bioprod. Biorefining *11*, 567–590.

Zheng, Y., Zhao, J., Xu, F., and Li, Y. (2014). Pretreatment of lignocellulosic biomass for enhanced biogas production. Prog. Energy Combust. Sci. *42*, 35–53.

Zhou, M., Yan, B., Wong, J.W.C., and Zhang, Y. (2018). Enhanced volatile fatty acids production from anaerobic fermentation of food waste: A mini-review focusing on acidogenic metabolic pathways. Bioresour. Technol. *248*, 68–78.

Zhu, B., Gikas, P., Zhang, R., Lord, J., Jenkins, B., and Li, X. (2009). Characteristics and biogas production potential of municipal solid wastes pretreated with a rotary drum reactor. Bioresour. Technol. *100*, 1122–1129.

Zou, S., Wang, H., Wang, X., Zhou, S., Li, X., and Feng, Y. (2016). Application of experimental design techniques in the optimization of the ultrasonic pretreatment time and enhancement of methane production in anaerobic co-digestion. Appl. Energy *179*, 191–202.