Università degli Studi di Padova

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Ph.D. School, XXX Cycle

Food waste to bio-energy through anaerobic digestion under different management scenarios

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UNIVERSITÀ DEGLI STUDI DI PADOVA

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Dipartimento di Ingegneria Industriale

Scuola di Dottorato di Ricerca: Curriculum Chimica e Ambiente

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Ph.D. thesis, October 2017

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"Ninguém sabe para onde vai, mas sabe para onde não quer ir… E se você consegue chegar, então desfrute de tudo que a natureza lhe dá… E não reclame, partilhe… Porque o melhor que há no mundo são as pessoas que você encontra…"

Meu amigo e escritor **Angelino Pereira**.

"Nessuno sa dove sta andando, ma sappiamo dove non vogliamo andare... E se riesci ad arrivare, allora godi di tutto ciò che la natura ti dà... E non lamentarti, condividi... Perché le cose migliori del mondo sono le persone che incontriamo..."

Mio amico e scrittore **Angelino Pereira**.

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1. BACKGROUND AND AIM OF THE Ph.D. ACTIVITY

The problem of food waste (FW) management is currently on an increase, regarding all steps from collection to disposal.

Discharge of food materials occurs along the entire Food Supply Chain (FSC) and it produces an impact at an environmental, social, and economical level. From an environmental point of view, FW improper management contributes to greenhouse gases emissions during final disposal in landfills (uncontrolled methane release) and during activities associated to food production, processing, manufacturing, transportation, storage, and distribution. Other environmental impacts are natural resource depletion in terms of soil, nutrients, water and energy, and disruption of biogenic cycles due to intensive agricultural activities. Social impacts may be ascribed to ethical and moral dimensions within the general concept of global food security. Economical impacts are due to the costs related to food wastage and their effects on farmers and consumer incomes.

A series of solutions may be implemented for appropriate FW management as substantial steps into the transition toward a bio-based economy. An economy based on innovative and cost-efficient use of biomass, like FW, for the production of both bio-based products and bioenergy should be driven by well-developed integrated biorefining systems which need to be planned after a specific and comprehensive investigation of the state of the art.

Therefore, during the first Ph.D. year, much attention was focused on bibliographic research in order to have a complete vision of the subject. The review article entitled “Food waste generation and industrial uses: A review” was published in *Waste Management*. This preliminary bibliographic activity was necessary to detect the three main critical issues to be addressed during the experimental research, namely ***(1)*** the absence of proper source segregation in several parts of the world, ***(2)*** the search for sustainable strategies for decentralized organic waste management, and ***(3)*** the FW valorisation systems improvement for energy and bio-products production (see Figure 1.1).

(1) An efficient source segregation and collection should be the first step towards FW exploitation as a resource. Without it, organic and not-organic wastes end up being landfilled together. FW landfilling is a practice widespread in many parts of the world, such as in China, with several negative consequences on the environment. This is why, in collaboration with Tsinghua University (China), hybrid landfill bioreactors were used to test the effectiveness of altering between aerobic and anaerobic conditions to fasten up the stabilization of the organics inside the landfilled waste mass. The results were published in *Bioresource Technology* as “Targeted modification of organic components of municipal solid waste by short-term pre-aeration and its enhancement on anaerobic degradation in simulated landfill bioreactors”.

(2) In contrast with huge FW generators, the decentralised integrated management of source segregated FW and domestic wastewater streams was another addressed issue. Resuming the

concepts at the basis of Aquanova Project (dealing with the source separation of domestic wastewater into three main streams), elaboration of data about lab-scale co-digestion of brown water and FW was performed. That activity enabled the publication of "Lab-scale co-digestion of kitchen waste and brown water for a preliminary performance evaluation of a decentralized waste and wastewater management" in *Waste Management.*

Following up with small realities, the case-study of a small-medium size Company located in the North of Italy was addressed with the final goal of valorising its putrescible waste. Every Company, according to the type of activity, size, and number of employees has a specific quantity and characterization of generated waste. Specifically for our investigation, an interesting finding was to learn that nearly half of the organic waste composition was represented by spent coffee grounds (SCGs). Coffee production is increasing year by year as a consequence of the increasing demand. The production of residues from its brewing is, therefore, more and more abundant. SCGs, a waste that is not yet enough valorised, is instead a versatile resource and it could be a very good bio-energy feedstock. Therefore, their bio-methane production potential through anaerobic digestion was assessed. "The broad spectrum of possibilities for Spent Coffee Grounds valorisation" was published in *Journal of Material Cycles and Waste Management*. With the aim of finding a way to enhance their methane yield, further batch-scale bio-methane potential production (BMP) tests were then performed on SCGs after alkaline pre-treatment using NaOH. The outcomes were published as "Spent Coffee Grounds alkaline pre-treatment as biorefinery option to enhance their anaerobic digestion yield" in *Waste Biomass and Valorisation.*

(3) In Europe the efforts spent into source segregated FW valorisation are quite high. The preferred ways to deal with FW are anaerobic digestion (AD) and composting. Therefore, it became interesting to go deeper inside the AD process and to investigate how to improve it in order to increase the bioenergy yield. In particular, the possibility to shift the same positive effects of pre-aeration prior anaerobic landfilling to AD process was experimented at first.

Hydrolysis, crucial first step for the degradation of the organic substances, is known to be somehow the most challenging gap to overcome due to the complexity of organic material. Limited aeration was shown to enhance hydrolysis of complex organic matter, which was likely to improve methane yield. Through literature search, the studied effects deriving from the combination of aeration and anaerobic digestion were collected and compared in order to write a review paper entitled "Combination between aeration and anaerobic digestion – a review" published in *Waste and Biomass Valorisation.*

Subsequently, a series of laboratory BMP tests to assess the effects of pre-aeration prior AD of FW were performed. The results were not significant for a single-stage AD process of FW, instead

interesting outcomes were reported when performing pre-aeration of carbohydrate-rich, protein-rich, and lipid-rich substrates in a two-stage AD process. “Effect of aerobic pre-treatment on hydrogen and methane production in a two-stage anaerobic digestion process using food waste with different compositions” was published in *Waste Management*.

Elaboration of data concerning batch two-stage AD tests performed on FW under different substrate to inoculum (S/I) ratio and pH combinations, was also useful to add scientific knowledge about the topic and to compare the yields with those of one-stage AD. The paper “Two-stage anaerobic digestion of the organic fraction of municipal solid waste – Effects of process conditions during batch tests” was submitted to *Renewable Energy.*

In order to go deeper inside the possibility to recover energy and bio-products from putrescible discarded matter, the third year research activity was focused on the acidogenic fermentation processes with a focus on the generation of precursors (ethanol, lactic acid, and volatile fatty acids) exploitable for bioplastic production.

Through acidogenic fermentation, organic waste can be valorised to recover hydrogen but also alcohols and organic acids that, once separated from the fermented broth and purified, can be converted into other compounds such as bioplastic (polylactic acid, polyhydroxyalkanoates, polyethylene, and polyvinyl acetate). The aim of the last experimental activity was to assess what are the best conditions in terms of various combinations of S/I ratio and pH to recover the highest amount of fermentation products when treating food waste and cheese whey. Results were published as “Acidogenic fermentation of the organic fraction of municipal solid waste and cheese whey for bio-plastic precursors recovery - Effects of process conditions during batch tests” in *Waste Management*.

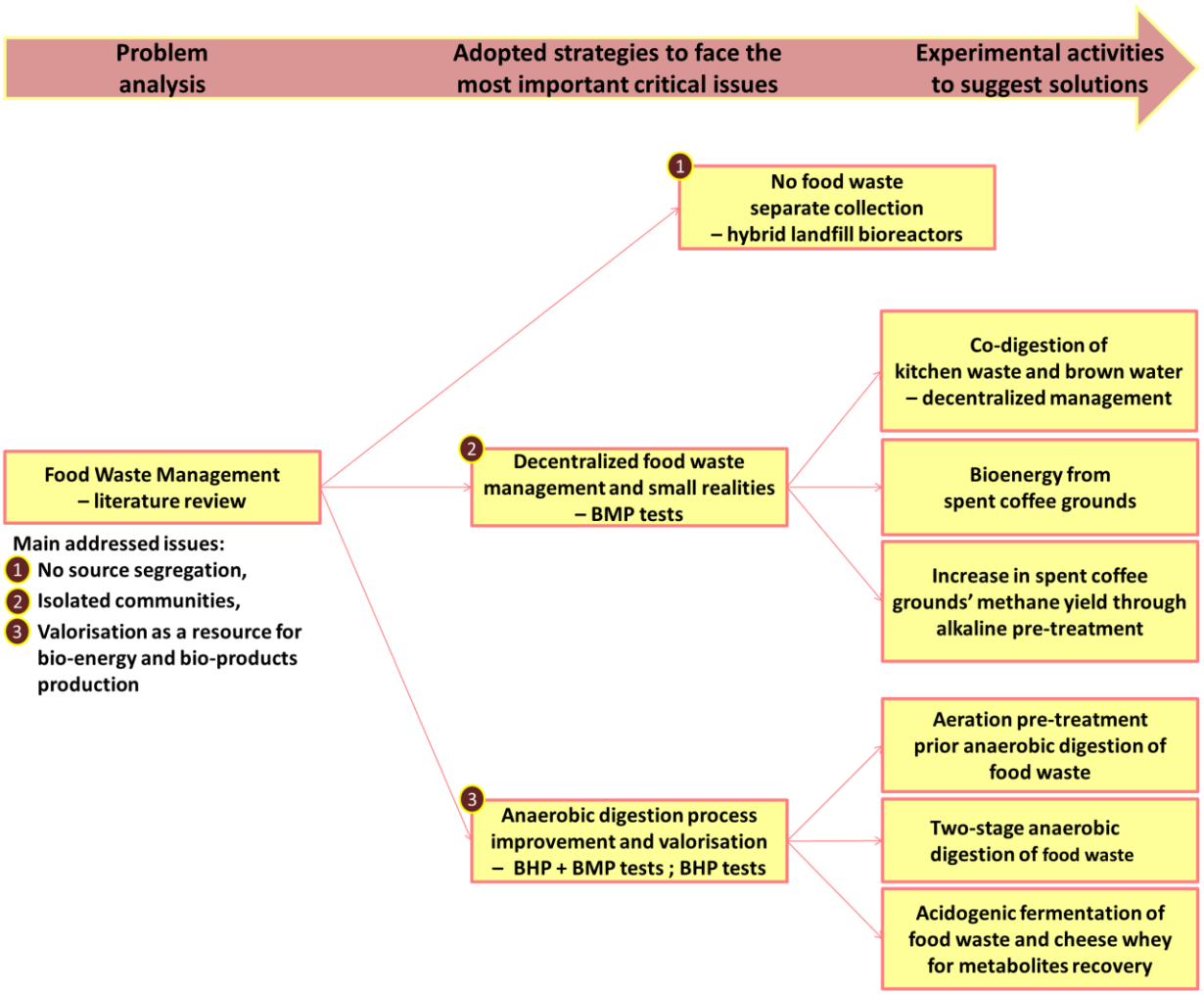

Figure 1.1: Schematic representation of the activity carried out during the three years of Ph.D.

1.1 SCIENTIFIC METHODOLOGY

For each investigated issue discussed above and represented in Figure 1.1, the following methodologies were used, respectively.

(1) *No food waste separate collection – Hybrid landfill bioreactors*

Six laboratory-scale Plexiglas columns were used as bioreactors to compare the effects of different short-term pre-aeration durations on anaerobic landfilling. Another column without pre-aeration was arranged as control (AN bioreactor). Each reactor had an internal diameter of 180 mm and a height of 320 mm. A perforated plate was fixed approximately 50 mm above the bottom of each reactor to support the waste and facilitate aeration. The upper end of each column was equipped with two valves to allow gas extraction and leachate recirculation. The lower end was equipped with a valve for leachate collection. The ventilation rate was regulated by a LZB-10 flowmeter (Shanghai Instrument Co.). A thermo-regulated insulation system was designed to cover all the reactor lateral surfaces and to maintain a constant temperature at 35 ± 1ºC. The waste mass temperature was monitored and recorded using a Pt100 temperature sensor installed in the centre of each reactor, and the data were logged with a two-minute interval using a data collector (Meikong CO.). The reactor sketch is illustrated in Figure 1.2.

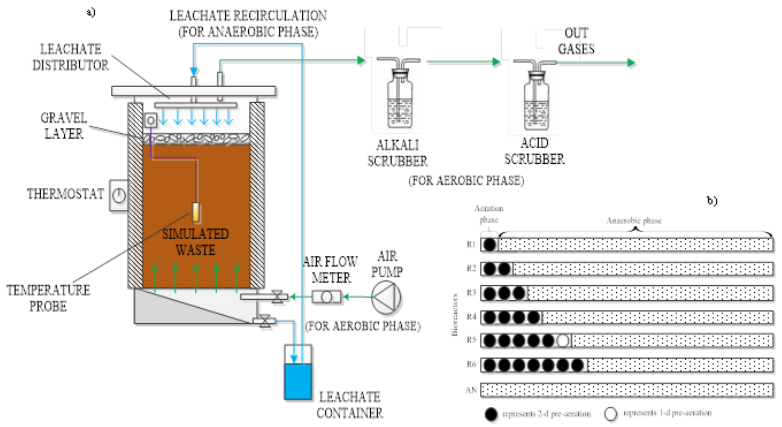

Figure 1.2: Reactor sketch (a) and operative conditions over time in the different reactors (b).

Three kilos of MSW, containing the 63% of food waste, were filled into each column.

Synthetic MSW is preferred to real MSW in order to better understand the reactor inputs and to ensure a reliable comparison between the outputs of short pre-aeration intensities specially arranged in this study. The MSW was manually shredded and well mixed with a uniform size of 20–40 mm. While loading, approximately 150 g of sludge compost (5% inoculation rate) was mixed in the waste to enhance the aerobic degradation process. The short-term pre-aeration duration was the sole variable. Each bioreactor had its own pre-aeration duration: 2, 4, 6, 8, 11 and 14 days (i.e. R1–R6 bioreactors, respectively), in order to evaluate its effects. The initial airflow rate was fixed at 0.5

L/min/kg waste at room temperature in all bioreactors except the AN, with a frequency of 10 minutes every 20 minutes. The headspace gas composition before and after ventilation were investigated: an optimum oxygen level of 5–15% was maintained through regulating ventilation frequency during the whole pre-aeration phase (Ruggieri et al., 2008). There was no leachate recirculation applied in the pre-aeration phase and the leachate produced from individual bioreactors was collected and weighed daily. Exhaust air was consecutively bubbled through a 500 mL 5N NaOH solution, and a 200 mL 2N H_3BO_4 solution, to capture CO_2 and NH_3, respectively.

Destructive sampling was used after completing each pre-aeration term. Approximately, one tenth (w/w) of pre-treated refuses were sampled using sequential quartering procedure to guarantee homogeneity. The samples were firstly dried at 105 ºC, grinded to pass through a 1 mm sieve and stored in a desiccator for later analyses. The remaining refuses were re-filled into the bioreactors and subjected to the anaerobic condition. For AN bioreactor, only 2.5 kg of raw MSW were filled, and no aerobic inoculum and pre-aeration were applied.

An inoculum of anaerobically digested sludge from a full-scale anaerobic digestion plant was added to all bioreactors according to a percentage of 10% (w/w) of the loaded waste to ensure the presence of methanogenic bacteria (Siddiqui et al., 2012).

The generated biogas was collected from the top of each bioreactor using 5 L aluminium sampling bags and tested every two days. Moreover, the modified Gompertz model was used for describing the accumulative methane production during anaerobic degradation phase:

$$P = P_0 \cdot \exp\{-\exp\cdot[\tfrac{R_m \cdot e}{P_0}(\lambda - t) + 1]\}$$

where P is the accumulative methane yield (L/kg_{DM} – DM=dry waste), P_0 is methane production potential (L/kg_{DM}), t is the cumulated time (d), R_m is the maximum methane production rate ($L/kg_{DM}/d$), λ is the lag-phase time (d), and e is Euler's constant (=2.718).

Additionally, leachate was recirculated through a disperser in the top of the bioreactor body every two days and sampled for analysis every four days throughout the entire anaerobic phase. The leachate withdrawn for analysis at each sampling (25–30 mL) was replaced by an equivalent volume of deionized water.

(2) *&* ***(3)*** *Decentralized management & anaerobic digestion process improvement and valorisation – BMP tests, BHP+BMP tests and BHP tests*

Bio-methane potential (BMP) tests were implemented to get significant information about the bio-methanation of specific organic substrates. Stopping AD before methanogenesis, biological production of hydrogen and metabolites from the fermentation of different substrates can also be examined in batch tests using heat-shocked mixed cultures (BHP tests).

There are not fixed protocols for BMP and BHP tests, therefore, according to the specific substrate and the specific needs to be accomplished during the experimentation, a clear setting of all those parameters that can affect significantly the experimental results, such as temperature, pH, physical and chemical characteristics of substrates, and substrate to inoculum (S/I) ratio must be carefully chosen and declared.

Batch scale tests (Table 1.1) performed using various organic substrates and process conditions were several. They were simulating one or two-stage AD process:

- one-stage AD process: BMP tests to assess the methane potential production;
- two-stage AD process: BHP+BMP tests to assess the hydrogen and methane potential productions, respectively;
- acidogenic fermentation: BHP tests to evaluate the amount of fermentation products generation (alcohols, organic acids).

Table 1.1: Summary of all batch scale AD tests. (FW = food waste; CW = cheese whey; BW = brown water)

	Substrate						
	FW+BW	**coffee**	**coffee**	**FW**	**FW**	**FW**	**CW**
Notes	co-digestion of food waste and brown water			three substrates: FW rich in carbohydrates, in proteins, and in lipids		acidogenic fermentation	acidogenic fermentation
type of test	BMP	BMP	BMP	BHP + BMP	BHP + BMP	BHP	BHP
pre-treatment			24h alkaline treatment (NaOH: 2 , 4, 6, 8 %w/w)	pre-aeration (air flow rate fixed at 5 L/h using a flow meter - 24h)			
S/I ratio (gVS/gVS)	0.5	0.5, 1, 2	2	0.3	0.5, 1, 2, 4, 6	2, 4, 6	2, 4, 6
pH	7	7.5	between 8 and 12	6	5.5, 7, 9	5, 7, 9	5, 7, 9
T	35 & 55 °C	35 °C	35 °C	35 °C	35 °C	35 °C	35 °C

The inoculum chosen for each batch scale test was granular sludge from a full-scale Upflow Anaerobic Sludge Blanket (UASB) digester of a brewery factory located in Padova, Italy.

When performing BHP tests, heat treatment was carried out on the granular sludge in a rotary water-bath incubator at a fixed temperature of 80 °C for 15 minutes in order to suppress methanogenic bacteria.

Batch tests were carried out using 1 L or 500 mL glass bottles which were subsequently sealed with silicon plugs. Substrate concentration was 5 or 10 gVS/L (according to the bioavailability of the substrate). Bottles were flushed with N_2 gas for 3 minutes to ensure anaerobic conditions and incubated at a temperature of 35±1ºC using a water bath. All tests were performed in triplicate.

The gas volume generated during the tests was measured by means of the water displacement method (see Figure 1.3). When performing BHP tests, the produced gas composition was analysed

using a micro-GC (Varian 490-GC) equipped with an MS5A column to measure H_2 and CH_4, and a PPU column for CO_2 and two Thermal Conductivity Detectors. When performing BMP tests, CH_4 and CO_2 amounts in the biogas were monitored using a portable measuring instrument (LFG20). Hydrogen, methane and carbon dioxide volumes produced during the first and second stages of AD were calculated according to the following equation:

$V_{c,t} = C_{c,t} * V_{b,t} + V_H * (C_{c,t} - C_{c,t-1})$

where $V_{c,t}$= Volume of H_2, CH_4 or CO_2 produced in the interval between t and t-1; $V_{b,t}$= Volume of total biogas produced in the interval between t and t-1; V_H = Volume of headspace of bottles; $C_{c,t}$= Concentrations of H_2, CH_4 or CO_2 measured at time t; $C_{c,t-1}$= Concentrations of H_2, CH_4 or CO_2 measured at time t-1.

Figure 1.3: Lab tests experimental equipment. Mesophilic water bath containing the BHP/BMP bottles (a) and manual measurement of the biogas produces through water displacement (b).

At the end of acidogenic fermentation tests, liquid samples were taken for ethanol, VFAs (acetic, propionic, butyric, iso-valeric, caproic, and heptanoic acids), lactate and TOC analyses. Samples were centrifuged at 6000 rpm for 10 min, the supernatants were filtered using 0.45 µm Phenex-RC filters (Phenomenex, Castel Maggiore, Italy), and stored at −20 °C until analysis. Ethanol, VFAs, and lactate concentrations were analysed by injection into a high-performance liquid chromatography system complete with an LC 9A Shimadzu pump, a SIL 10A auto-sampler, and a RID-model Shimadzu 10A detector (Shimadzu, Tokyo, Japan).

2. FOOD WASTE GENERATION AND INDUSTRIAL USES: A REVIEW

Authors: Francesca Girotto, Luca Alibardi, Raffaello Cossu

Food waste is made up of materials intended for human consumption that are subsequently discharged, lost, degraded or contaminated. The problem of food waste is currently on an increase, involving all sectors of waste management from collection to disposal; the identifying of sustainable solutions extends to all contributors to the food supply chains, agricultural and industrial sectors, as well as retailers and final consumers. A series of solutions may be implemented in the appropriate management of food waste, and prioritised in a similar way to waste management hierarchy. The most sought-after solutions are represented by avoidance and donation of edible fractions to social services. Food waste is also employed in industrial processes for the production of biofuels or biopolymers. Further steps foresee the recovery of nutrients and fixation of carbon by composting. Final and less desirable options are incineration and landfilling. A considerable amount of research has been carried out on food waste with a view to the recovery of energy or related products. The present review aims to provide an overview of current debate on food waste definitions, generation and reduction strategies, and conversion technologies emerging from the biorefinery concept.

2.1 INTRODUCTION

Food loss and food waste are often used in scientific literature to identify materials intended for human consumption that are subsequently discharged, lost, degraded or contaminated. The Food and Agriculture Organization of the United Nations (FAO) defined food loss (FL) as any change in the availability, edibility, wholesomeness or quality of edible material that prevents it from being consumed by people. This definition was provided for the post-harvest period of food ending when it comes into the possession of the final consumer (FAO, 1981). Gustavsson et al. (2011) reported a similar definition of FL but included also the production stage of a food supply chain (FSC) and not only postharvest and processing stages. Parfitt et al. (2010) defined food waste (FW) as the food loss occurring at the retail and final consumption stages and its generation is related to retailers' and consumers' behaviour. Recently the European Project FUSIONS (Östergren et al., 2014) defined FW by using the resource flows of the agri–food system. FW was defined as "*any food, and inedible parts of food, removed from (lost to or diverted from) the food supply chain to be recovered or disposed (including composted, crops ploughed in/not harvested, anaerobic digestion, bio-energy production, co-generation, incineration, disposal to sewer, landfill or discarded to sea).*" Any food being produced for human consumption, but which leaves the food supply chain, is considered FW while organic materials produced for the non-food production chain are not

considered FW (Östergren et al., 2014). The definitions of FL and FW therefore overlap. These terms are used in literature for material discharged at both the manufacturing and retail stages and the consumption or household levels, highlighting the need for commonly-agreed and improved definitions (Williams et al., 2015).

Discharge of food material occurs along the entire Food Supply Chain (FSC) and it involves all sectors of waste management from collection to disposal. Detailed analysis of a FSC system will highlight how the generation of waste material (food losses, organic waste or food waste) affects all sectors involved in the production, distribution and consumption of food (Parfitt et al., 2010; Pfaltzgraff et al., 2013). A FSC starts with the production of food from the agricultural sector where both farming and husbandry produce waste or sub-products that may be either organic waste (i.e. cornstalk, manure), food waste or food loss (i.e. low quality fruits or vegetable, damaged productions left in the field, good products or co-products with a low or absent commercial value). The food processing and manufacturing industry produces food losses and food waste throughout the entire production phase due to reasons such as: damage during transport or non-appropriate transport systems, problems during storage, losses during processing or contamination, inappropriate packaging. The retail system and markets also generate FL and FW, largely due to problems in conservation or handling, and lack of cooling/cold storage (Parfitt et al., 2010).

The generation of FW by the end consumer is caused by over- or non-appropriate purchasing, bad storage conditions, over-preparation, portioning and cooking as well as confusion between the terms "best before" or "use by" dates (Papargyropoulou et al., 2014). The generation of FW at household level is influenced by a series of interconnected factors, mainly socio-demographic characters of the household, consumption behaviour and food patterns (Glanz and Schneider, 2009).

FL and FW generation produces an impact at an environmental, social and economical level. From an environmental point of view, FL and FW contributes to Green House Gas (GHG) emissions during final disposal in landfills (uncontrolled methane release) and during activities associated to food production, processing, manufacturing, transportation, storage and distribution. Other environmental impacts associated to FL and FW are natural resource depletion in terms of soil, nutrients, water and energy, disruption of biogenic cycles due to intensive agricultural activities and all other characteristic impacts at any step of the FSC. Social impacts of FL and FW may be ascribed to ethical and moral dimension within the general concept of global food security. Economical impacts are due to the costs related to food wastage and their effects on farmers and consumer incomes (Lipinski et al., 2013; Papargyropoulou et al., 2014).

Similar to the Waste Management Hierarchy introduced in Europe, based on a hierarchy of solutions of distinct steps (waste prevention, reuse, recovery and recycling of materials, energy

recovery and safe landfilling of residues) and often graphically represented by a reverse triangle (Cossu, 2009), the Environmental Protection Agency (EPA, 2014) defined the following hierarchy concept in relation to FW management: source reduction, feed hungry people, feed animals, industrial uses, composting, incineration or landfilling.

The first steps to be taken in reducing FW generation should commence by tackling the undesirable food surplus, and preventing over-production and over-supply of food (Papargyropoulou et al., 2014; Smil, 2004). The subsequent steps in the hierarchy foresee the utilisation of FW as animal feed or in the industrial sector. Several options are available for an industrial-scale use, ranging from the use of food waste for energy production by means of anaerobic digestion (e.g. bio-hydrogen or bio-methane productions) to the production of specific chemical compounds as precursors for plastic material production, chemical or pharmaceutical applications. Composting can be applied to recover nutrients or as a carbon sequestration process, through the formation of humic substances. Composting can be used to treat FW or residues from industrial processes (e.g. digestate). Landfilling or incineration represents the last and least desirable option. It is an acknowledged fact that biodegradable organic material is the main source of adverse environmental impacts and risks in traditional landfilling (odours, fires, VOC's, groundwater contamination by leachate, global climate changes, etc.) (see also Manfredi et al., 2010; Thomsen et al., 2012; Beylot et al., 2013) while thermal treatment, although providing for energy recovery, is limited by the low heating values of organic waste (Nelles et al., 2010). Accordingly, these options are not highly sought after (Papargyropoulou et al., 2014; Vandermeersch et al., 2014).

This paper reviews the data available on the magnitude of food waste generation, the strategies for food waste reduction and the possibilities reported and discussed in scientific literature for industrial uses of food waste.

2.2 GENERATION OF FOOD WASTE

The Food and Agriculture Organization of the United Nations estimated that 32% of all food produced in the world was lost or wasted in 2009 (Gustavson et al., 2011; Buzby and Hyman, 2012). While 870 million people are reported as being chronically undernourished, approximately 1.3 billion tons/year, i.e. one third of the food produced for human consumption, is wasted globally (Kojima and Ishikawa, 2013). In United States nearly 61 million tons of food waste are generated every year (GMA, 2012). Dee (2013) reported a food waste generation rate of 4 million tons per year in Australia. Other food waste generation data regards South Korea with 6,24 million tons per year (Hou, 2013), China with 92,4 million tons per year (Lin et al., 2011) and Japan where about 21 million tons of food waste were generated in 2010 (Kojima and Ishikawa, 2013). In Europe, food

waste generation is estimated at 90 million tons annually (EC, 2013). Studies indicate the United Kingdom (UK) as the Country with the highest FW generation rate in Europe, reaching more than 14 million tons in 2013 (WRAP, 2013; Thi et al., 2015; Youngs et al., 1983). Quested et al., (2013) reported a generation of food waste at household level of 160 kg per year in UK, representing 12% of the food and drink entering a home and 30% of the general waste stream from UK household. Nellman et al., (2009) reported that a percentage ranging between 25% and 50% of food produced is wasted through the supply chain.

The order of magnitude of food waste generation is consistent and is not limited to developed Countries. Gustavson at al. (2011) reported data on FW generation from different parts of the world, indicating that FW generation displays a similar order of magnitude in both industrialised Countries and developing Countries (DCs). Nevertheless, industrialized and developing Countries differ substantially. In the latter, more than 40% of food losses occur at the postharvest and processing stages, while in the former, about 40% of losses occur at the retail and consumer levels and, on a per-capita basis, much more food is wasted in the industrialized World than in DCs (Gustavson et al., 2011).

The causes of food losses and waste in low-income Countries are mainly linked to financial, managerial and technical limitations in harvesting techniques, storage and cooling facilities in difficult climatic conditions, infrastructure, packaging and marketing systems. Many smallholder farmers in DCs live on the margins of food insecurity, and a reduction in food losses could have an immediate and significant impact on their livelihoods. Food supply chains in DCs should be strengthened, encouraging small farmers to organize, diversify and upscale their production and marketing. Investments in infrastructure, transportation, food industries and packaging industries should also be boosted, with both the public and private sectors playing an important role in achieving this.

The causes of food losses and waste in medium/high-income Countries relate mainly to consumer behaviour as well as to a lack of coordination between the various actors in the supply chain. Farmer-buyer sales agreements may contribute towards the wastage of farm crops. Food may also be wasted due to quality standards, with food items that do not fit with the required shape or appearance being rejected. On a consumer level, inadequate planning and expiry of "best before dates" likewise lead to large amounts of waste, combined with the at-times careless attitude of consumers. Food waste in industrialized Countries can be reduced by raising awareness amongst the food industries, retailers and consumers. This inevitably implies the unnecessary use of huge amounts of resources used in food production, and consequent increase in GHG emissions (Gustavsson et al., 2011).

In terms of wasted investments, Naham and de Lange (2013) estimated costs of edible food waste throughout the value chain in South Africa at approximately € 7.3 billion per annum, equivalent to 2.1% of annual gross domestic product. In the Unites States € 85 billion worth of food was estimated to be thrown away every year (Parfitt et al., 2010), € 28 billion in China (Zhou, 2013), € 27,8 billion in Australia (Dee, 2013), € 40 billion in Europe and nearly € 17 billion in the UK (WRAP, 2015).

Two aspects are in connection with the FW generation problem: prevention upstream and source segregation downstream. The primary action to be implemented in a successful FW management strategy is prevention of generation. The unavoidable generated FW amount needs, then, to be properly source segregated.

Prevention can be achieved either attempting to reduce losses and, therefore, decreasing the demand for food production, or diverting food losses, exceeds, and still safe and edible FW to other end-consumers. FW prevention campaigns have been promoted by advisory and environmental groups, and by media focus. Several papers have analysed the behaviour of companies and the population in developed Countries at different levels (household, restaurant, retail) to assess the governing factors influencing wastage of food products (Glanz and Schneider, 2009; Schneider and Lebersorger, 2009; Silvennoinen et al., 2012; Quested et al., 2013; Katajajuuri et al., 2014; Garrone et al., 2014; Graham-Rowe et al., 2014; Mena et al., 2014).

The focus of measures implemented will vary from Country to Country as highlighted by the work of Gustavsson et al. (2011). In developed Countries, food waste prevention should focus on the consumer's behaviours at household level, while in developing Countries it should focus increasingly on the retail and distribution system. The issues of food security and utilisation of food surplus to satisfy the nutritional needs of the poor represent indirect measures of FW prevention.

BIO Intelligence Service carried out a survey about FW generation across EU27 (EC, 2010) which resulted in a technical report where three priority options are highlighted: data reporting requirements, date labeling coherence, and targeted awareness campaigns.

The retail system may result in the generation of FW throughout various stages of food distribution and purchase: damage during transport or non-appropriate transport systems, problems during intermediate storage, losses during processing or contamination, inappropriate packaging, problems in conservation or handling, lack of cooling/cold storage. The food supply chain is also affected by loss of products nearing their expiry date (Aiello et al., 2014).

Other potential influences discussed in the literature can be divided into production and distribution level, and consumer level. Prevention strategies related to the first point are: development of markets for ‘sub-standard’ products, development of contract farming linkages between processors

and farmer, marketing cooperatives and improved market facilities (Gustavson et al., 2011) together with studies targeted in finding the optimal turnover frequency and wholesale pack size (Eriksson et al., 2014). Wasted investments should provide an incentive to push the food industry to reduce food waste generation in order to gain benefits on both the financial and environmental fronts. More specifically, interventions should first and foremost be targeted at the processing and packaging stages of the fruit and vegetable value chain, which alone accounts for the 13% of the total; as well as the distribution stage of the fruit and vegetable value chain, and the agricultural production and distribution stages of the meat value chain (Naham and de Lange, 2013).

Betz et al., (2015) reported several actions geared at FW prevention and reduction, and indicated award schemes including incentives for food industries reducing FW generation. Future preventive measures should focus on the return of fresh products (shifting responsibilities from local shops to retail companies), internal optimization (benchmarking among retail outlets within a company and application of best practices), training, information and education of employees, and amending the display at the end of the day when stocks are decreasing (Lebersorger and Schneider, 2014; Scherhaufer and Schneider, 2011). If food losses have to be discarded at the retailer's expense, this will act as an incentive for the outlet to minimize losses by optimizing planning and ordering according to demand. Lebersorger and Schneider (2014) reported how, in the bread & pastry market, shifting the responsibility for unsold products from bakeries to the retail company would provide an incentive for retail outlets to reduce high quantities of wasted bread, for example by optimizing demand planning and ordering and providing specific information to the supermarket customers.

On a household level, consumer behaviour may produce a huge impact on FW generation. Since the past Century, a wide range of factors influencing FW generation has been identified (Youngs et al., 1983) such as poor selection of food items, overbuying, poor food storage, excessive preparation losses, inability to use by-products, poor cooking/holding techniques, shortage of labour and equipment, excessive portion sizes, inability of the eater to remove all edible material, service method. Nowadays, over- or inappropriate purchasing, bad storage conditions, over-preparation, portioning and cooking as well as confusion between the terms "best before" or "use by" dates are still some of the main factors affecting food loss. This behaviour is influenced by a series of interconnected factors, mainly socio-demographic characteristics of the household, consumption behaviour and food patterns. Moreover, the barriers to surpass in achieving food loss minimisation at household level may also involve emotional or psychological aspects. The householder may wish to be a "good provider" in terms of supplying an abundance of healthy food for the family. A lack of food may produce a sense of inability to take care of the needs of the family, in this way driving

the purchase of additional goods unnecessarily. Another example is avoidance of frequent trips to shops, resulting in the purchase of more food products to avoid running out. A general lack of awareness of the amount of FW generated at household level may exert a strong impact on food waste generation, due to the fact that small quantities thrown away a bit at a time with other waste does not provide the proper order of magnitude of the problem to consumers (Graham-Rowe et al., 2014). At consumer level a wide range of optimal behaviours can be listed: planning meals in advance, checking levels of food in cupboards and fridge prior to shopping, making a shopping list, storing meat and cheese in appropriate packaging or wrapping, storing vegetables and fruit in the fridge, using the freezer to extend the shelf-life of food, portioning rice and pasta, using up leftovers, using date-labels on food (Quested et al., 2013; Eriksson et al., 2014).

Additional measures should be considered, including the raising of customer awareness and information (Scherhaufer and Schneider, 2011). Whitehair et al., (2012), for example, found out how to simply reach a 15% reduction in FW generation from Universities' canteens by using written messages such as *'Eat what you take. Don't waste food'* or *'All taste, no waste'*.

With regards to the set of activities aimed at addressing lost, exceeding and safe wasted food to alternative end-consumers, donation of food constitutes a specific application of urban mining in view of the fact that food is recovered for its original purpose – human intake (Schneider, 2013a) and it is a valid alternative to minimise FW generation. Donation is a well-established food waste prevention measure implemented worldwide. The largest domestic hunger-relief organisation in the United States of America is Feeding America, a national network of more than 200 food banks operating within all 50 states, as well as the District of Columbia and Puerto Rico. It coordinates the distribution of edible food and grocery products with the help of 61,000 agencies, which supply 37 million people in the US. Three billion pounds of food were collected and distributed to people in need in 2009 (Echevarria et al., 2011).

The European Federation of Food Banks was established in 1984 and more than 30 years later there are 247 food banks operating in 21 European Countries. According to the reports published on their website, a total of 401,000 tons of food were collected and distributed to 31,000 social welfare organisations in 2011. It is estimated that these products are worth several hundred million € and approximately 5.2 million people are supported by these goods (FEBA, n.a.).

The largest contribution to the amount donated, with a share of 36% in 2006, was made by dairy products; the next largest product group was biscuits, cereals and starchy food with a share of 31%, and the third largest group was fruits and vegetables at 15% (European Food Banks, 2007; Schneider, 2013a). The majority of the products (55%) distributed by the European Food Banks are donated by the European Union or by member states ~~(4%)~~. Part of these products has been subject

to a withdrawal or intervention approach used to stabilise market prices.

A central piece of legislation related to food donation is the General Food Law EC/178/2002. The aim of this Regulation is to provide a framework to ensure a coherent approach in the development of food legislation. It lays down definitions, principles and obligations covering all stages of food/feed production and distribution. According to Article 3.8, food donation falls under "placing on the market" operations, which are holdings of food or feed for the purpose of sale, including offering for sale or any other form of transfer, whether free of charge or not, and the sale, distribution, and other forms of transfer (EESC, 2014). This definition essentially points out that all food donations have to comply with the EU General Food Law. In other words, a food business operator has to comply with the same rules whether he is selling or donating food. Food banks and charities are considered “food business operators” (EESC, 2014). According to Article 17 “food and feed business operators at all stages of production, processing and distribution within the businesses under their control shall ensure that foods or feeds satisfy the requirements of food law which are relevant to their activities and shall verify that such requirements are met” (EESC, 2014). The food business operator is held responsible for any hygiene problem occurring only in the part of the food chain under its own control.

Donations to social services are beneficial from a series of different points of view. They reduce waste quantities and waste management costs (Alexander and Smaje, 2008; Schneider, 2013b) and may contribute towards promoting a positive image for the company together, and producing social benefits for the clients of these services. An example of how this can be implemented is represented by SOMA, a social supermarket set up in Linz, Upper Austria. This is a private initiative set up by food wholesalers with the intention of helping people in need by selling them low priced food products (Schneider, 2013a).

In Europe a central piece of legislation related to food donation is the General Food Law EC/178/2002. The aim of this Regulation is to provide a framework to ensure a coherent approach in the development of food legislation. It lays down definitions, principles and obligations covering all stages of food/feed production and distribution. According to Article 3.8, food donation falls under "placing on the market" operations, which are holdings of food or feed for the purpose of sale, including offering for sale or any other form of transfer, whether free of charge or not, sale, distribution or any other forms of transfer (EESC, 2014). This definition essentially points out that all food donations have to comply with the EU General Food Law. In other words, a food business operator has to comply with the same rules whether is selling or donating food. Food banks and charities are considered “food business operators” (EESC, 2014). According to Article 17 *“food and feed business operators at all stages of production, processing and distribution within the*

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Aiello et al., (2014) developed a model to determine the optimal time to withdraw the products from the shelves, and to ascertain the quantities to donate to non-profit organizations and those to be sent to the livestock market maximizing retailer profit. The optimal time is based on the assumption that the residual market demand will not be satisfied. Products near to their expiry date or damaged by improper transportation or production defects are usually scarcely appealing for the consumer in the target market, although maintaining their nutritional properties. In particular, after the optimisation of the variable 'time to withdraw the product from the shelves' the results show that 44.13% of food losses is suitable for donation to non-profit organizations, 28.69% to be sold at the livestock market, and 27.18% is disposed through the usual channel, namely the landfill.

When discussing the considerably large amounts of FW generated by consumers at a household level, it is necessary to point out the basic difficulty in achieving its optimal source segregation as the first step for the following most appropriate management strategy.

A series of factors that influence an active participation in source separation of food waste have been reported in literature, although the studies performed to date have reported varying results. Wan et al., (2013) conducted a questionnaire survey in Malaysia indicating education as the main factor affecting positive behaviour of consumers towards FW separation rather than convenience. The results of another study performed by Parizeau et al., (2014), indicated multiple relationships between FW segregation and household shopping practices, food preparation behaviours, household waste management practices and food-related attitudes, beliefs and lifestyles. The Authors observed that food and waste awareness, family and convenience lifestyles were related to FW generation, and concluded that convenience is a major issue when asking families to implement source segregation of FW at their house. Rousta et al., (2015) concluded that convenience and information go together. In fact, they concluded that information stickers about food waste sorting and property close location of drop-off point reduced the miss-sorted fraction by more than 70%.

Similarly, several studies showed that convenience in sorting, storage space at home, availability of sorting facilities, access to a curbside collection system and distance to collection points are important influential factors that can increase the recycling rate (Rousta et al., 2015; Ando and Gosselin, 2005; Barr and Gilg, 2005).

Bernstad (2014) highlighted how the need for a practical solution to improve FW separation was more important than providing appropriate information to consumers. Two different strategies

aimed at increasing household source-separation of FW were evaluated in a Swedish residential area: the study involved the use of written information, distributed as leaflets amongst households, and installation of equipment for source-segregation of waste, aimed at increasing convenience FW sorting in kitchens. On the basis of the results obtained, distribution of written information amongst households failed to result in either an increased source-separation ratio, or a statistically significant and long-term increase of the amount of separately collected household FW. Conversely, following the installation of sorting equipment in all households in the area, both the source separation ratio and the amount of separately collected FW increased markedly. Changes remained consistent even months after the installation of the sorting equipment in the area (Bernstad, 2014).

Bernstad and la Cour Jansen (2011) compared composting, anaerobic digestion and incineration of FW within a life cycle approach, highlighting the crucial role of household participation for efficient source-separation of FW. Incorrect sorting reduces process efficiency and causes limitation in the final use of stabilized materials from biological treatments.

Prior to implementing any FW management strategy, probably the best would be to test the opinion of the population in the area of interest, in order to understand if attitude or convenience is the main predictor towards food waste separation. Thus, local Authorities will be guided to design the most meaningful intervention campaign.

2.3 INDUSTRIAL USES

Increasing efforts are currently being focused on defining effective and stable means of obtaining biofuel and bio-products from FW. These options could afford benefits from an environmental point of view due to the reduction of methane gas emissions from landfills and the preservation of natural resources such as coal and fossil fuels, from a social point of view due to the lack of a food vs. fuel competition, and from an economical point of view thanks to costs saving linked to surplus food production and specific investments in establishing non-food crops dedicated to biofuel or bioplastic production.

Biorefineries are the concept underlying industrial FW utilisation. Similarly to the transformation by oil refineries of petroleum into fuels and ingredients for use in a wide variety of consumer products, biorefineries convert organic waste and biomass (corn, sugar cane and other plant-based materials) into a range of ingredients for bio-based fuels or products. FW produced from agriculture and food processing is abundant and concentrated in specific locations. These materials could be less susceptible to deterioration if compared to FW produced at household level at the end of the FSC (Galanakis, 2012). These characteristics highlight the potential to develop industrial utilisation processes based on symbiosis where the wastes from one sector are inputs for other sectors.

Availability of FW and location of potential users define the feasibility of industrial symbiosis (Mirabella et al., 2014). Therefore particular effort will be required from the agricultural and the industrial sectors to define sustainable and innovative processes for residues use and conversion, and from governments to stimulate and support this new vision with specific legislations. Industrial symbiosis within FSC and biorefineries represent possibilities for a complete utilisation of food processing residues, FL or FW in a vision of circular flow of resources, zero waste and final sink of stabilised residues (Cossu, 2012; Curran and Williams, 2012). The potential profitability of chemicals and biofuels produced from FW will stimulate investments on biorefinery chains rather than treatments of FW in traditional waste management processes. Finally legislations have to be developed to stimulate, support, define and control the marketability of chemicals, materials or biofuels obtained from agro-industrial residues or FW depending by their final applications (nutraceutical/pharma or non-feed/nonpharma applications) for a effective management of products traceability, health and safety issues and environmental protection (Lin *et al.*, 2013; Tuck *et al.*, 2012).

Valorisation routes of FW in biorefinery chains include both extraction of high-value components already present in the substrates to be used for nutrition or pharmaceutical applications and conversion into chemicals, materials or biofuels by use of chemical or biological processes. Type, origin, seasonal generation and territorial distribution of FW will affect transport logistic for its utilisation and its compatibility with the transformation process. High and concentrated volumes of FW will be generally required to sustain large production capacities and meet economy of scale. Cost-effectiveness of conversion processes will then be ensured by security of supply at regional scale, low heterogeneity of substrates and large variety of extractable chemicals, biopolymers and biofuels. For these reasons, large fluxes of agro-industrial wastes seem to be more suitable for biorefinery chains where stability of supply and substrate homogeneity are required for extraction or production of specific commodities while source segregated organic waste from household or restaurants would be more indicated for treatment processes where composition variability, origins and contaminations do not represent limits for the selected process (Pfaltzgraff et al., 2013).

Biofuel and bioenergy production

Food waste is characterised by a variable chemical composition depending on its origin of production. FW may therefore comprise a mixture of carbohydrates, lipids and proteins, or, if generated from specific agro-industrial sectors, may be rich in one of these constituents. Different biofuels are therefore produced from FW using bioprocesses or thermo-chemical processes, depending on their chemical composition.

The use of FW for energy production was recently reviewed by Pham et al., 2014 and by Kiran et

al., 2014. FW can be converted into biofuels or energy by means of the following processes:

- transesterification of oils and fats to produce biodiesel;
- fermentation of carbohydrates to produce bioethanol or biobutanol;
- anaerobic digestion to produce biogas (methane rich gas);
- dark fermentation to produce hydrogen;
- pyrolysis and gasification;
- hydrothermal carbonization
- incineration;

Not all the listed processes are currently developed at industrial level for full-scale application. For example, FW is widely studied as a substrate for the biological production of hydrogen by dark fermentation, although no full-scale applications have been realised to date (Alibardi et al., 2014; De Giannis et al., 2013). Incineration is a mature technology applied to reduce waste volumes and produce electrical energy and heat; however, the high moisture contents of FW limits its application together with the concerns of local communities on air emissions (Pham et al., 2014). Anaerobic digestion, on the contrary, is a technology facing growing interests and large applications (Clarke and Alibardi, 2010, Levis et al., 2010). The high biodegradability and moisture content of FW are ideal characteristics for biogas production and digestion residues can be used as soil conditioner or amendment (digestate) or as nutrient source (e.g. ammonia or struvite).

Biodiesel can be defined as fatty acid alkyl esters (methyl/ethyl esters) of short-chain alcohols and long-chain fatty acids derived from natural biological lipid sources such as vegetable oils or animal fats, which have had their viscosity reduced by means of a process known as transesterification, and are suited to use in conventional diesel engines and distributed through existing fuel infrastructure. Any fatty acid source may be used to prepare biodiesel (Refaat, 2012). Thus, any animal or plant lipid should represent a ready substrate for the production of biodiesel. However the use of edible vegetable oils and animal fats for biodiesel production has traditionally been of high concern due to their competing with food materials. The use of non-edible vegetable oils in biodiesel production is likewise questionable, as the production of crops for fuel implies an inappropriate use of land, water, and energy resources vital for the production of food for human consumption; the use of waste oil may therefore represent a more realistic and effective element for use in the production of biodiesel (Gasparatos et al., 2011; Timilsina and Shrestha, 2011; Pirozzi et al., 2012; Refaat, 2012).

The new process technologies developed in recent years have enabled the production of biodiesel from recycled frying oils, resulting in a final quality comparable to that obtained with virgin vegetable oil biodiesel. Canakci (2007) reported that the annual production of oils, greases and animal fats from restaurants in the United States could replace more than 5 million litres of diesel

fuel if collected and converted to biodiesel. Waste cooking oil requires a series of pre-treatment steps to eliminate solid impurities and reduce free fatty acids and water contents. The pre-treatment process may include washing, centrifugation, flash evaporation, and acid esterification. Final ester yield could be up to 80% (Yaakob et al., 2013). These results are expected to encourage the public and private sectors to improve the collection and recycling of used cooking oil to produce biodiesel. Waste oils can be co-treated with animal fats from slaughterhouses and fleshing oils from leather industries to gather cheap biodiesel feedstock (Alptekin et al. 2014). FW can also be used to grow microorganisms, microalgae or insects rich in lipids from which biodiesel can be produced (Ghanavati et al. 2015; Kiran et al., 2014; Li et al., 2011).

First-generation bioethanol can be derived from renewable sources of virgin feedstock; typically starch and sugar crops such as corn, wheat, or sugarcane. Indeed, most of the feedstocks used in first generation biofuel production are food crops. For this reason, biofuel expansion may compete with food production both directly (food crops diverted for biofuel production) and indirectly (competition for land and agricultural labour) (Gasparatos et al., 2011). These barriers can be partly overcome by the utilization of lignocellulosic materials for the production of the so-called second-generation bioethanol. One potential advantage of cellulosic ethanol technologies is that they avoid direct competition for crops used in the food supply chain, as the materials used are not edible; this option however should be limited to cases in which an overt sustainable surplus of crops occurs or where crop wastes and wood wastes are available as feedstock. (Timilsina and Shrestha, 2011; Pirozzi et al., 2012; Refaat, 2012). Cellulosic ethanol has a number of potential benefits over corn grain ethanol, but although the cost of biomass is low, releasing fermentable sugars from these materials remains challenging.

Bioethanol can be produced from FW and agricultural waste, the latter being cost effective, renewable and abundant substrates (Kiran et al., 2014; Sarkar et al., 2011). Pre-treatments are frequently applied to improve carbohydrate saccharification of organic substrates, as yeast cells cannot ferment starch or cellulose directly into bioethanol. Cekmecelioglu and Uncu (2013) demonstrated the feasibility of lowering ethanol production costs using kitchen wastes as substrate, and by excluding the fermentation nutrients traditionally used in fermentation practice. Pre-treatment prior to enzymatic hydrolysis was not required to obtain high glucose levels from the kitchen wastes, and the nutrients present provided sufficient nutritive medium for yeast to produce high ethanol yields (Cekmecelioglu and Uncu, 2013). Kim et al. (2011) reported ethanol yields from FW rich in carbohydrates between 0.3 and 0.4 g ethanol per g total solids. Waste fruits may also represent a substrate for bioethanol production. For example, banana waste or rotten banana, peels and sub-quality fruits have been extensively studied as substrates for bioethanol production (Graefe

et al., 2011; Oberoi et al., 2011; Hossain et al. 2011; Arumugam and Manikandan 2011; Gonçalves Filho et al., 2013; Bello et al., 2014).

Butanol is obtained from food waste by fermentation processes using *Clostridium acetobutylicum* bacteria. This organism features a number of unique properties, including the ability to use a variety of starchy substances and to produce much better yields of acetone and butanol than those obtained using Fernbach's original culture (Stoeberl et al., 2011). Butanol as fuel or blending component has several advantages compared to ethanol, for example a lower vapour pressure, improved combustion efficiency, higher energy density, and it can be dissolved with vegetable oils in any ratio reducing their viscosity. Results for butanol production indicated a potential of 0.3 g of butanol from 1 g carbohydrates from waste whey, a substrate characterised by high lactose content. Whey production worldwide, estimated in approximately $160*10^6$ Mg/year, contains about $8*10^6$ Mg of carbohydrates that could be converted into $2.4*10^6$ Mg solvents or fuels every year (Stoeberl et al., 2011). Similarly, industrial starchy food waste such as inedible dough, bread and batter liquid represent feasible alternative substrates for fermentative production of butanol with butanol yields of approximately 0.3 g butanol per g of FW (Ujor et al. 2014).

Studies indicate therefore the feasibility of alcohol production from specific fractions of FW and these technologies could also contribute to solve the debate on the use of food crops for energetic purposes. Anyway the overall economic viability still has to be evaluated and further studies are required to identify optimal conditions for cost minimisation and market development (Pham et al., 2014).

Anaerobic digestion for biogas production (methane rich gas) is a well established technology perfectly suited for FW management. Interest in anaerobic digestion (AD) has been continuously growing over the last decades, being more and more frequently promoted by national programmes for energy production from renewable resources (Clarke and Alibardi, 2010). Possibilities for biogas production from FW were recently reviewed by Kiran et al. (2014), with Kondusamy and Kalamdhad (2014), Pham et al. (2014) and Zhang et al. (2014) highlighting the potentials for renewable energy production from anaerobic treatment of FW. Anaerobic digestion is a mature technology that can be applied to almost all types of biodegradable substrates as source separated organic fraction of municipal solid waste, agricultural or industrial food waste and food manufacturing residues. The potential of anaerobic digestion process has also recently been evaluated for the biological conversion of hydrogen and carbon dioxide of different origins into methane for energy storage purposes (Burkhardt et al., 2015) and as carbon capture strategy during digestion of FW or sewage sludge (Bajón Fernández et al., 2014). Anaerobic digestion therefore represents a flexible process that can be used as final conversion process in a biorefinery chain for

all those substrates and residual flows not further convertible to high value products. AD processes are also considered to be the best option for the biological production of hydrogen, one of the most interesting and promising biofuels (Guo et al., 2010; Ozkan et al., 2010; De Gioannis et al., 2013).

Several substrates have been evaluated as potentially suitable for biohydrogen generation through dark fermentation. Among these, FW may represent relatively inexpensive and ideal sources of biodegradable organic matter for H_2 production, mainly due to the high carbohydrate content and wide availability. Dark fermentation of FW can also be combined with other bioprocesses to maximise energy conversion (Alibardi et al., 2014; Kiran et al. 2014; De Gioannis et al., 2013). Dark fermentation process performances are affected by several aspects as the type and treatment of inoculum, type of reactor, organic loading rate and hydraulic retention time, process temperature and pH conditions (Wang and Wan, 2009; Guo et al., 2010; Nanqi et al., 2011). Different process conditions and specific aspects of the dark fermentation process have been analysed, although the results remain controversial, at times lacking direct comparability and at times being divergent or even antithetic (De Gioannis et al., 2013). Cappai et al. (2014) recently reported an optimal pH of 6.5 for hydrogen production from FW while at pH of 5.5, commonly assumed as the optimum, minimum hydrogen productions were recorded. Alibardi and Cossu (2015) demonstrated how changes in FW waste composition markedly affect hydrogen potential productions explaining the high variability of data reported in literature on FW. Favaro et al. (2013) reported good capacity of indigenous microflora of FW to produce biohydrogen. De Gioannis et al. (2014) measured significant fermentative biohydrogen productions from different types of cheese whey at pH values between 6.5 and 7.5, with the highest productions up to 170 $mLH_2/kgTOC$.

Pyrolysis and gasification are thermal processes viewed as alternatives to combustion in waste management (Pham et al., 2014). Pyrolysis of food waste, using temperatures between 400 and 800 °C, converts the material from the solid state into liquid products (so-called pyrolysis oil) and / or gas (syngas), which can be used as fuels or raw materials intended to subsequent chemical processes. The solid carbon residues can be further refined by providing products such as activated carbon. The products of pyrolysis are therefore gaseous, liquid and solid and their proportion depends upon the pyrolysis method and the reaction parameters.

Gasification partially oxidises food waste to produce a combustible gas mixture. Temperatures typically range between 800 and 900 °C. The gas produced can be burnt directly or used as a fuel for gas engines and gas turbines or used as a feedstock in the production of chemicals (e.g. methanol) (Pham et al., 2014).

Applicability and feasibility of these processes are strongly dependant on waste characteristics such as elemental composition, heating values, ash, moisture and volatile solids content, presence of

contaminants, bulk density. These characteristics are crucial for process performances and limit the applicability of gasification and pyrolysis to FW. The majority of gasification technologies for example use pre-treated waste as feedstocks and no gasification/pyrolysis processes have been developed using raw food waste (Arena, 2012; Pham et al., 2014). Only few researches were published on gasification or pyrolysis of food waste. Liu et al. (2014) investigated the effectiveness of catalytic pyrolysis of food waste by using microwave power for heating. These Authors reported an energy ratio of production to consumption (ERPC) of 0.91 without the use of catalysts while when $CuCl_2$ or MnO_2 were added as catalysts, ERPC increased to 2.04 and 1.93, respectively. Bio-char (solid product) was in all cases the main energetic product of pyrolysis while bio-oil or gases yields were variable being the conversions into gaseous or liquid products competing processes (Liu et al. 2014). Opatokun et al. (2015) evaluated the pyrolysis of both dry raw FW and digested FW after biological anaerobic treatment and concluded that both substrates demonstrated potential for fast degradation due to high volatile matter content. Energy content was for both cases mainly spread into biochar and bio-oil fractions while gases provided significantly lower energy.

The impact of pre-treatments and drying processes on overall energy production is still not clear thus FW water content seems to remain the limiting characteristic of these processes. Processes not requiring a drying step are hydrothermal water gasification or hydrothermal carbonization as both utilise water as the main reaction medium and reactant. Hydrothermal (subcritical and supercritical water) gasification can generate hydrogen gas from biodegradable wastes. Muangrat et al. (2012) investigated the effect of carbohydrate, protein and lipid proportions in several FW samples for hydrogen production by using subcritical water gasification and reported that carbohydrate-rich samples were preferred for the reaction conditions applied as protein and lipid promoted side reactions of neutralization and saponification, respectively.

Hydrothermal carbonization (HTC) is a thermal treatment technique used to convert food wastes and associated packaging materials to a valuable, energy-rich resource. HTC is attracting increased attention from researchers, especially for waste streams with high moisture content (80– 90%) (Pham et al., 2014; Li et al. 2013). HTC was applied to several organic wastes, at different operating conditions, temperature ranges (200 – 350 °C) and process duration (0.2 to 120 h) (i.a. Pham et al., 2014). Results demonstrated that food waste could be beneficially treated by HTC resulting in the production of hydrochar with high carbon and energy. Lin et al. (2013) reported positive energy balances on HTC treatment of food waste collected from local restaurants. The presence of packaging materials may influence the energy content of the recovered solids. The higher the presence of packaging materials, the lower the energy content of recovered solids due to the low energetic retention associated with the packaging materials (Li et al. 2013).

Biomaterials production

The challenge of finite fossil resources has been addressed by academic and industrial researchers with the development of valuable compounds and polymers based on renewable resources (besides the previously mentioned biofuels). The use of agro-industrial residues for the extraction of high-value chemicals was recently reviewed by Mirabella *et al.* (2014). Biopolymers production is a possibility facing growing interest as it is applicable both to agro-industrial residues and organic waste from household level. The corresponding monomers are accessible either through fermentation of carbohydrate feedstocks by microbes, often genetically modified, or by chemical processing of plant oils (Fuessl et al., 2012). The production of biological metabolites to be used as renewable and biodegradable substitutes for petrochemical products is currently the focus of growing interest. These metabolites are: lactate for the production of polylactate, a plastic constituent; polyhydroxyalkanotes, particularly polyhydroxybutyrate, which are natural storage polymer of many bacterial species with properties similar to polyethylene and polypropylene; succinate, a valuable and flexible precursor for pharmaceutical, plastic and detergent production (Hassan et al., 2013; Sulaiman et al., 2014; Li et al., 2015). As for the biofuel production from virgin feedstocks, considerable debate surrounds the manufacture of bioplastics from natural materials, raising the issue as to whether they produce a negative impact on human food supply. In this context, the opportunity of using waste food in the production of bio-plastics seems a highly feasible option.

Bio-production of optically pure L-lactic acid from food waste has attracted considerable interest due to its ability to treat organic wastes with simultaneous recovery of valuable by-products (Li et al., 2015). A new strategy was reported for effective production of optically pure L-lactic acid from food waste at ambient temperature, regulating key enzyme activity by sewage sludge supplementation and intermittent alkaline fermentation. A production of optically pure L-lactic acid was achieved from food waste at ambient temperature with a yield of 0.52 g/gCOD (Li et al., 2015). Dairy industries generate high amounts of whey from milk processing for various manufactured products. Whey is a by-product discharged by the cheese production process, and its disposal is currently a major pollution problem for the dairy industry (Abdel-Rahman et al. 2013). Whey is a potent and suitable raw material for lactic acid production, consisting in lactose, proteins, fats, water-soluble vitamins, mineral salts, and other essential nutrients for microbial growth (Panesar et al., 2007). Theoretically, 4 mol of lactic acid can be produced from 1 mol of lactose through a homofermentative pathway following the cleavage of lactose to 1 mol of glucose and 1 mol of galactose (Abdel-Rahman et al. 2013). The market for yogurt has also grown rapidly over the past few years. Consequently, damaged or expired yogurts create huge amounts of waste products.

Yogurt is usually sweetened with additional sugars, such as sucrose and glucose, which would result in higher lactic acid production than cheese whey containing fewer sugars.

At present, among the various types of starch-based biodegradable plastics such as polylactic acid (PLA) and polyvinyl acetate (PVA), the group of polyhydroxyalkanoates (PHAs) is one of the most promising. Polyhydroxyalkanoates (PHAs) are linear polyesters of hydroxyacids (hydroxyalkanoate monomers) synthesized by a wide variety of bacteria through bacterial fermentation (Reis et al., 2011). The strength and toughness of PHAs are good, they are completely resistant to moisture and feature a very low oxygen permeability. Accordingly, PHA is suitable for use in the production of bottles and water resistant film (Van Wegen et al., 1998). The simplest type of PHA is polyhydroxybutyrate (PHB). The majority of bacteria synthesizing PHAs can be broadly subdivided into two groups. One group produces short-chain-length PHAs (SCL-PHAs) with monomers ranging from 3 to 5 carbons in length, while a distinct group synthesizes medium-chain-length PHAs (MCL-PHAs) with monomers from 6 to 16 carbons. PHAs accumulate in bacteria cytoplasm as a high molecular weight polymer forming intracellular granules of 0.2–0.7 mm in diameter. Typically, PHAs accumulate to a significant proportion of the cell dry weight when bacteria are grown in a media that is limited in a nutrient essential for growth (typically nitrogen or phosphorus), but with an abundant supply of carbon (for example glucose). Under these conditions, bacteria convert the extracellular carbon into an intracellular storage form, namely PHA. When the limiting nutrient is resupplied, intracellular PHA is degraded and the resulting carbon is used for growth (Reis et al., 2011).

The main limitation in using bacterial PHAs as a source of biodegradable polymers is their production cost. In particular the average cost is by far the most significant contributor to overall PHB price, approximately two and a quarter times greater than the capital cost of equipment (Van Wegen et al., 1998). Using agro-industrial food waste as substrate instead of virgin feedstock of refined sugar such as glucose, sucrose and corn steep liquor could represent a turning point. Sugarcane and beet molasses, cheese whey effluents, plant oils, swine waste liquor, vegetable and fruit wastes, effluents of palm oil mill, olive oil mill, paper mill, pull mill and hydrolysates of starch (e.g., corn and tapioca), cellulose and hemicellulose are all excellent alternatives characterized by a high organic fraction (Reis *et al.*, 2011).

A three-stage biotechnological process proposed by Reis et al. (2011) demonstrated good potential for PHA production from waste/surplus-based feedstocks using enriched mixed cultures. The basic concept was based on an initial acidogenic fermentation phase of the feedstock, a second phase of selection and production of PHA-storing bacterial biomass under dynamic feeding, and the last phase in which PHA was accumulated in batch conditions. It is important to underline the main role

played by the initial acidogenic fermentation stage needed to overcome the weak point of the process represented by the fact that most waste and surplus feedstocks contain several organic compounds that are not equally suitable for PHA production. Carbohydrates in fact are not stored by mixed cultures as PHA, but rather as glycogen. Thus, acidogenic fermentation is an essential stage to increase the potential of producing PHA by mixed cultures from surplus-based feedstocks. Carbohydrates and other compounds are, in this way, transformed into VFA that are readily convertible into PHA (Reis et al., 2011).

As assessed by Koller et al. (2013), PHAs and their follow-up products can be processed to create a broad range of marketable products for a variety of applications. They have potential in agro-industrial applications (carriers and matrices for controlled release of nutrients, fertilizers and pesticides), therapeutic applications (controlled release of active pharmaceutical ingredients), in buildings blocks, in packaging materials and surgical implants. Naranjo et al. (2014) investigated the integrated production of PHB and ethanol from banana residues as agro-industrial waste. PHB production was carried out using the glucose obtained in the hydrolysis stage from banana pulp, while peels were exploited for ethanol production. The theoretical yields of PHB and ethanol were 31.5 and 238 kg/ton bananas, respectively. Other food waste used for PHA production were fruit pomace and waste frying oil (Follonier et al. 2014), spent coffee grounds (Obruca et al., 2014), distillery spent wash (Amulya et al., 2014), and margarine waste (Morais et al., 2014). Zhang et al., (2014) evaluated how PHA composition is influenced by ratio of even-numbered to odd-numbered VFAs from co-treatment of food waste and sewage sludge. The consumption of even-numbered VFAs was correlated with the PHB synthesis, while the consumption of odd-numbered VFAs was correlated with the synthesis of polyhydroxyvalerate (PHV). The relatively constant quality and fermentable sugar content characterise food waste as an ideal substrate for PHAs production.

As already highlighted for dark fermentation, interconnections of biotechnological processes for the co-production of bio-fuels and bio-products therefore represent a key strategy in maximising food waste utilisation and potential income of the entire bioprocess chain (Venkateswar Reddy et al., 2014). Lin (2012) demonstrated for example that microalgae can grow on pure food waste hydrolysate without any negative effects on growth or biomass composition. The outcomes open up for an economically feasible cultivation of heterotrophic microalgae based on mixed food waste hydrolysate and a use of microalgal biomass for a production of biofuels and platform chemicals. In this way it would be possible to weave a complex and elaborate scheme leading at maximizing the yield within the FW biorefinery concept.

2.4 CONCLUSIONS

The development of sustainable solutions for food waste management represents one of the main challenges for society. These solutions should be capable of exploiting the precious resources represented by food waste to achieve social, economical and environmental benefits. The development of sustainable solutions for food waste management represents one of the main challenges for society. These solutions should be capable of exploiting the precious resources represented by food waste to achieve social, economical and environmental benefits. Clear and generally accepted definitions of food waste and related terms are anyway still missing and estimations on generated amounts are not yet consolidated. Avoidance of food waste generation could be ideally obtained by a proper equilibrium between food production and consumption, but such an optimum arranging is still far from being attained. A feasible management of excess production of edible food consists in its redistribution to feed poor people. The practice of food donation needs to find support from governments to facilitate the recovery and redistribution by food banks or social services. Agro-industrial residues and household food waste no longer suitable for human consumption can be used as feedstocks for the production of bio-plastics and bio-fuels together with the extraction of high-value components. This requires active participation from the public as well, in order to end up with a properly segregated FW to be transformed into resource. Practical and convenient solutions hand in hand with proper information campaigns targeted accordingly the area of interest need to be designed. Similar to the production of biofuel from virgin feedstocks, considerable debate surrounds the manufacture of bioplastics from natural materials, raising the issue as to whether they produce a negative impact on human food supply. In this context, the opportunity of using food waste as a feedstock in the production of bio-fuels and bio-plastics seems a feasible option. To conclude therefore, the interconnection of biotechnological processes in the co-production of bio-fuels and bio-products represents a key strategy aimed at maximising the utilisation of food waste and raising the potential income of the entire bioprocess chain.

3. TARGETED MODIFICATION OF ORGANIC COMPONENTS OF MUNICIPAL SOLID WASTE BY SHORT-TERM PRE-AERATION AND ITS ENHANCEMENT ON ANAEROBIC DEGRADATION IN SIMULATED LANDFILL BIOREACTORS

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Pre-aeration is effective on regulating subsequent anaerobic degradation of municipal solid waste (MSW) with high organic fractions during landfilling. The strength of pre-aeration should be optimized to intentionally remove some easily biodegradable fractions while conserve bio-methane potential as much as possible. This study investigates the evolution of organic components in MSW during 2–14 days preaeration process and its impacts on subsequent anaerobic degradation in simulated landfill bioreactors.

Results showed that a 6-day pre-aeration enabled to develop a thermophilic stage, which significantly accelerated biodegradation of organics except lignocelluloses, with removal rates of 42.8%, 76.7% and 25.1% for proteins, carbohydrates and lipids, respectively. Particularly, ammonia from accelerated ammonification in the thermophilic stage neutralized VFAs generated from anaerobic landfilling. As a result, the MSW with 6-day pre-aeration obtained the highest methane yield 123.4 NL/kg dry matter.

Therefore, it is recommended to interrupt pre-aeration before its cooling stage to switch to anaerobic landfilling.

3.1 INTRODUCTION

Municipal solid waste (MSW) generated in Asia developing Countries has been characterized typically by high moisture content and high organic content. Food waste is usually the dominant composition accounting for more than 50% by weight (Norbu et al., 2005; Zheng et al., 2014a). Landfilling is the prevalent method of MSW disposal in developing Countries, especially in China (Zhang et al., 2010). However, due to its physical-chemical characteristics, direct landfilling of raw MSW, which is commonly applied in China, is easy to cause a series of complex environmental problems, e.g. complex leachate generation, odorous charge, fugitive GHG emissions, undesirable land uses and long-term health hazards (Cai et al., 2015; O'Keefe and Chynoweth, 2000; Reinhart et al., 2016).

Under this scenario, aiming at better minimizing adverse environmental impacts, some in-situ landfill modes (generally including anaerobic bioreactor landfill, aerobic bioreactor landfill, hybrid

bioreactor landfill, facultative bioreactor landfill, flushing bioreactor landfill and flooding/draining landfill cells) and multistage landfill modes (e.g. ex-situ pre-treatment prior to anaerobic bioreactor landfill) are of increasing interest in recent landfill management schemes (Clarke et al., 2015; Cossu et al., 2015; Mali et al., 2012; Siddiqui et al., 2012; Slezak et al., 2015; Xu et al., 2014).

Among the multifarious alternatives, aerobic biological pre-treatment has been shown to be effective and became an important landfilling practice, especially in European Countries (Montejo et al., 2013; Scaglia et al., 2010). When applying a long-term aerobic biological pre-treatment, the goal is to produce stabilized residuals that can be safely disposed of to minimize
the pollutant emissions, but almost renouncing the opportunity to collect a good amount of biogas to be converted into energy.

Meanwhile, waste management costs increase significantly when this process is used (Reinhart et al., 2016). After considering a balance between environmental and economic benefits, a short-term aerobic biological pre-treatment (2–4 weeks or less) prior to landfilling was proposed
(Gerassimidou et al., 2013; Salati et al., 2013; Scaglia et al., 2013). This method would enable to obtain semi-stabilized products, allowing, on one hand, landfill pollutant emissions abatement, and, on the other hand, the preservation of a certain part of biogas producible under anaerobic treatment (Tambone et al., 2011). Gerassimidou et al. (2013) reported that 8-day aerobic biological pre-treatment was very effective for improving the performances of landfill bioreactors which accepted MSW with a high content of putrescibles. In this regard, considering the reasonable pre-treatment costs and specific characteristics of generated MSW, this short-term biological pre-aeration followed by anaerobic landfilling is of great economic, environmental and social importance to effectively meet the recent requirements of MSW
disposal in developing Countries.

To determine an appropriately short but effective pre-aeration duration is still challenging. Previous researches have been directed towards the evaluation of the effects of aerobic pretreatment on subsequent anaerobic landfilling, taking into account different aeration modes and aeration time, and many advantages have been already proven (Cossu et al., 2015; Mansour et al., 2012; Xu et al., 2015). Data in the references suggests using VS and carbon losses as criteria to determine the right time to start subsequent anaerobic treatment (Gerassimidou et al., 2013; Mansour et al., 2012). Even so, there are still gaps in understanding what level of pre-aeration is actually required, especially for MSW with high organic fractions, as these parameters does not provide much information about the specific components remaining. It is well known that the types of organic components in waste significantly influence its anaerobic degradation behaviours (Kobayashi et al., 2012; Wang and Barlaz, 2016; Zheng et al., 2014b). This means that, in order to optimize pre-aeration process,

targeted removal of organic components in waste should be extremely considered. However, little attention has been paid to systematically evaluate the temporal change of organic components in MSW (e.g. carbohydrates, proteins, lipids and lignocellulose) during short-term preaeration process and its correlations with subsequent anaerobic treatment. Owing to deficiencies in consistent pre-aeration intensity, previous reports (Gerassimidou et al., 2013; Mansour et al., 2012; Scaglia et al., 2013) achieved yet cannot definitely provide the sound evidences on how short-term pre-aeration could modify he organic components in MSW, consequently failing to present the right time switching between pre-aeration process and subsequent anaerobic landfilling. In this study, a series of short-term pre-aeration durations, namely 2, 4, 6, 8, 11 and 14 days, was applied before anaerobic degradation of MSW in simulated landfill bioreactors. In line with different pre-aeration durations, the associations with leachate emissions and methane production from anaerobic landfilling phase, as well as comprehensive organic components characterization during the whole combined process were investigated. The overall objectives of this study were (1) to evaluate the modification of organic components achieved during short-term preaeration and compare its subsequent impacts on leachate quality and methane yield from anaerobic landfilling; (2) to discuss mechanisms of short-term pre-aeration to enhance anaerobic degradation; and (3) to identify potential process indicators to determine switching point between pre-aeration and subsequent anaerobic treatment.

3.2 MATERIALS AND METHODS

Substrate - Synthetic municipal solid waste

On the basis of the typical MSW composition in Beijing (Sun et al., 2014), a synthetic MSW was prepared using a mixture of food waste (FW), office paper (OFP), plastic (P) and inorganic materials (I) such as stones, glasses and metals. The mixture ratio of these components was fixed at 63% (FW), 13% (OPF), 5% (P) and 19% (I) on a wet weight basis. The initial water content and biodegradable VS content of the synthetic MSW were 57.5% (w/w) and 47.3%TS, respectively. The chemical components are showed in Table 3.1. Synthetic MSW is preferred to real MSW in order to better understand the reactor inputs and to ensure a reliable comparison between the outputs of short pre-aeration intensities specially arranged in this study. The MSW was manually shredded and well mixed with a uniform size of 20–40 mm.

Table 3.1: Chemical components of synthetic MSW used in this study.

Parameter	Values[b]
Carbohydrates (g/g TS)[a]	0.174 ± 0.013
Crude proteins (g/g TS)	0.229 ± 0.003
Crude lipids (g/g TS)	0.155 ± 0.001
Lignocelluloses (g/g TS)	0.363 ± 0.024
Total carbon (g/g TS)	0.454 ± 0.001
Total nitrogen (g/g TS)	0.030 ± 0.001

[a] Results were tested after plastic and inorganic materials removal.

[b] Data are the means of triplicate measurements ± standard deviations.

Experimental equipment

Six laboratory-scale Plexiglas columns were used as bioreactors to compare the effects of different short-term pre-aeration durations on anaerobic landfilling. Another column without preaeration was arranged as control (AN bioreactor). Each reactor had an internal diameter of 180 mm and a height of 320 mm. A perforated plate was fixed approximately 50 mm above the bottom of each reactor to support the waste and facilitate aeration. The upper end of each column was equipped with two valves to allow gas extraction and leachate recirculation. The lower end was equipped with a valve for leachate collection. The ventilation rate was regulated by a LZB-10 flowmeter (Shanghai Instrument Co.). A thermo-regulated insulation system was designed to cover all the reactor lateral surfaces and to maintain a constant temperature at 35 ± 1 °C. The waste mass temperature was monitored and recorded using a Pt100 temperature sensor installed in the centre of each reactor, and the data were logged with a two-minute interval using a data collector (Meikong CO.). The reactor sketch is illustrated in Figure 3.1.

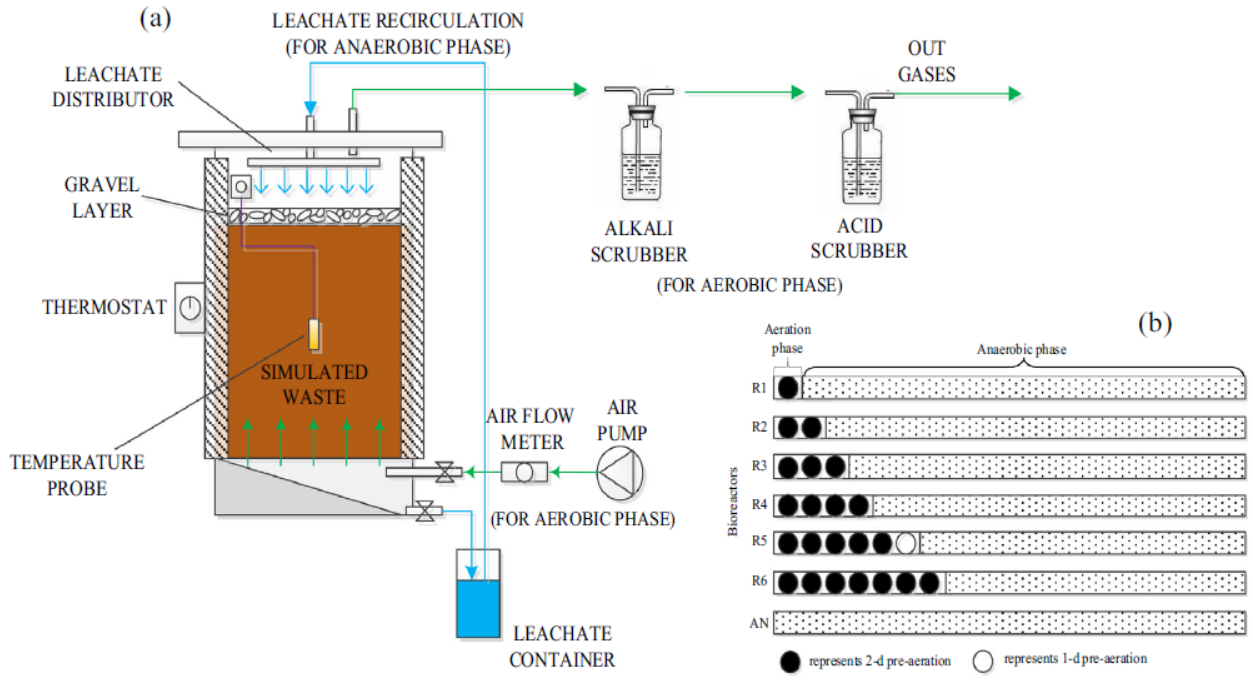


Figure 3.1: Reactor sketch (a) and operative conditions over time in the different reactors (b).

Methodology

Pre-aeration phase

Three kilos of MSW was filled into each column. While loading, approximately 150 g of sludge compost (5% inoculation rate) was mixed in the waste to enhance the aerobic degradation process (Mali et al., 2012). The short-term pre-aeration duration was the sole variable. Each bioreactor had its own pre-aeration duration: 2, 4, 6, 8, 11 and 14 days (i.e. R1–R6 bioreactors, respectively), in order to evaluate its effects. The initial airflow rate was fixed at 0.5 L/min/kg waste at room temperature in all bioreactors except the AN, with a frequency of 10 min every 20 min. The headspace gas composition before and after ventilation were investigated: an optimum oxygen level of 5–15% was maintained through regulating ventilation frequency during the whole pre-aeration phase. The results of temporal evolution of oxygen contents and temperature during pre-aeration phase are shown in Figure 3.2. Typical trends of the aerobic biological treatment were identified during this preaeration stage, showing a satisfactory repeatability compared with previous preliminary test.

There was no leachate recirculation applied in the pre-aeration phase and the leachate produced from individual bioreactors was collected and weighed daily. Exhaust air was consecutively bubbled through a 500 mL 5 N NaOH solution, and a 200 mL 2 N H_3BO_4 solution, to capture CO_2 and NH_3, respectively.

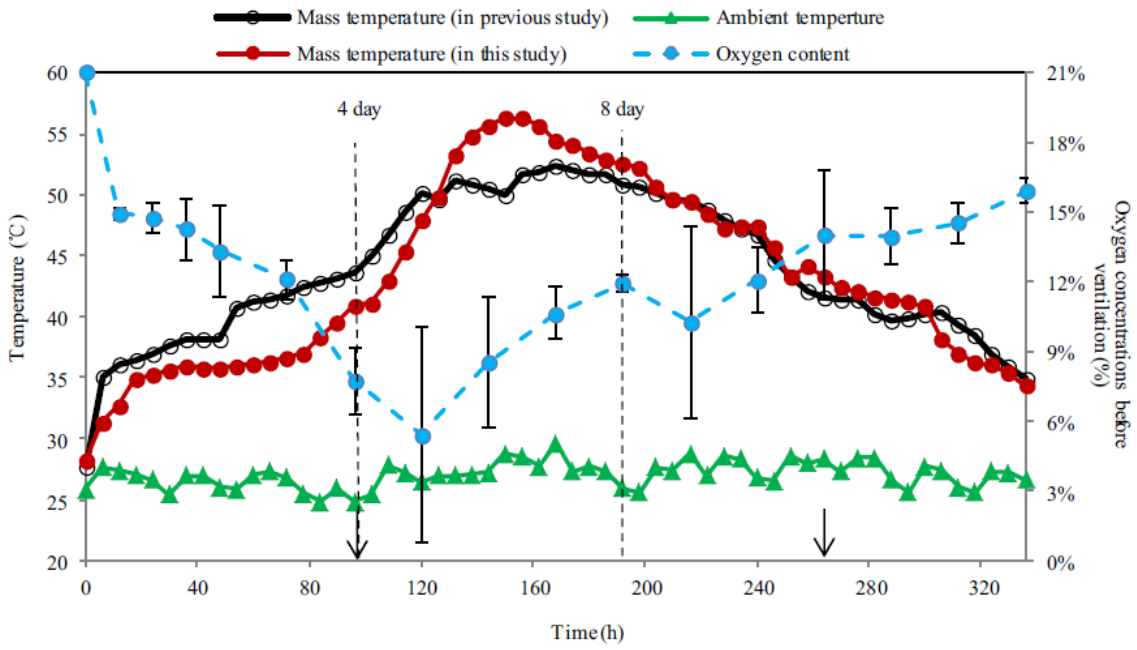


Figure 3.2: Evolution of temperature and oxygen content during the pre-aeration phase (note: arrows indicate aeration rate change to meet oxygen content requirements; error bars represent oxygen content standard deviation; two to three samples from random reactors were used to calculate the standard deviations except for the last three points, which were calculated using the data obtained from sampling of the successive two aeration cycles in R6).

Anaerobic landfilling phase

Destructive sampling was used after completing each preaeration term. Approximately, one tenth (w/w) of pre-treated refuses were sampled using sequential quartering procedure to guarantee homogeneity. The samples were firstly dried at 105 °C, grinded to pass through a 1 mm sieve and stored in a desiccator for later analyses. The remaining refuses were re-filled into the bioreactors and subjected to the anaerobic condition. For AN bioreactor, only 2.5 kg of raw MSW were filled, and no aerobic inoculum and pre-aeration were applied.

An inoculum of anaerobically digested sludge from a full-scale anaerobic digestion plant located in Beijing, was added to all bioreactors according to a percentage of 10% (w/w) of the loaded waste to ensure the presence of methanogenic bacteria (Siddiqui et al., 2012). The anaerobic digestion sludge had the following characteristics: pH=7.8, TS=7.2% (w/w), VS=46.5% TS. To reach 55–60% (w/w) moisture content and to guarantee initial leachate production, deionized water (10% weight of the loaded waste) was also introduced into each bioreactor.

The generated biogas was collected from the top of each bioreactor using 5 L aluminium sampling bags and tested every two days. Moreover, the modified Gompertz model (Equation 1) was used for

describing the accumulative methane production during anaerobic degradation phase (Uçkun Kiran et al., 2015):

$$P = P_0 \cdot exp\left\{-exp \cdot \left[\frac{R_m \cdot e}{P_0}(\lambda - t) + 1)\right]\right\} \quad (1)$$

where P is the accumulative methane yield (L/kg dry waste), P_0 is methane production potential (L/kg dry waste), t is the cumulated time (d), R_m is the maximum methane production rate (L/kg drywaste/d), λ is the lag-phase time (d), and e is Euler's constant (=2.718).

Additionally, leachate was recirculated through a disperser in the top of the bioreactor body every two days and sampled for analysis every four days throughout the entire anaerobic phase.

The leachate withdrawn for analysis at each sampling (25–30 mL) was replaced by an equivalent volume of deionized water.

Analytical methods

The physical and chemical characteristics of the substrate before and after aerobic pre-treatment were measured. Elemental analysis was performed using a carbon-hydrogen-nitrogen analyser (Equipment CE 440; EAI USA). Crude proteins content was determined by multiplying Kjeldahl nitrogen (KN) amount by the factor 6.25 (APHA, 2005). Lipids were extracted with petroleum ether and quantified by measuring the mass loss of the residue after combustion (AOAC, 1990, method 920.39). Crude fibres were analysed according to AOAC (1990) method 962.09. The amount of carbohydrates was calculated by subtracting the sum of crude lipids, crude proteins, and crude fibres contents from VS. The dry matter and VS reductions, achieved during the aerobic phase, were calculated in accordance with the mass conservation principle.

Leachate samples, both from aerobic and anaerobic phase, were tested for the following analytical parameters according to APHA methods (2005): pH, TOC, soluble COD (SCOD), TN, NH_4^+–N and VFA. The concentration of TOC and VFA were measured using a total organic carbon analyser (TOC-Vcph, Shimadzu) and a GC- 2014 gas chromatographer equipped with a flame ionization detector (Shimadzu), respectively.

The O_2 and CO_2 content emitted during the short-term preaeration and the biogas produced in the subsequent anaerobic phase were measured by a GC-2014 gas chromatographer equipped with a thermal conductivity detector (Shimadzu).

All the analysis on solid and liquid samples was conducted in duplicate.

3.3 RESULTS AND DISCUSSION

Leachate

The evolution of the main leachate parameters, such as pH, VFA, SCOD and NH_4^+–N, extracted from each bioreactor during anaerobic landfilling, is presented in Figure 3.3.

Potential accumulation of organic acids and decrease of pH are the major problems in stable methane production and materials stabilization during landfilling operations. The positive effects of pre-aeration on pH increase during the subsequent anaerobic degradation were satisfactory (Figure 3.3a). In particular, an initial pH value of 7.1–7.3, when applying pre-aeration for more than 6 days, showed a significant advantage for the start-up of the steady methanegenesis over AN, R1 and R2, in which the initial pH values were lower than 5.0. The pH values of R3–R6 bioreactors increased slowly and maintained a stable level between 8.1 and 8.3 at the end of study. However, throughout the whole anaerobic period, pH values in AN, R1 and R2 were still below the optimal value for methanogenesis (Cossu et al., 2015; Xu et al., 2014).

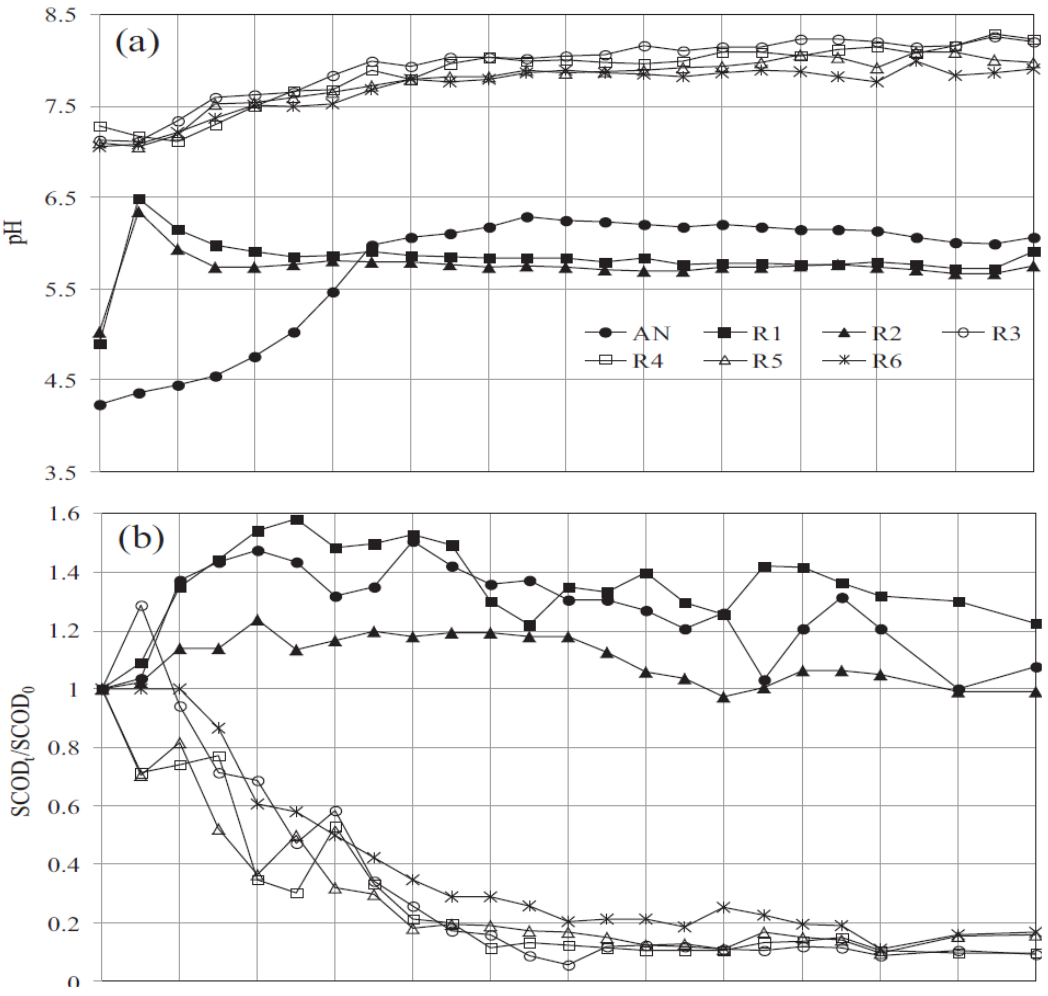
At the beginning of the anaerobic operation the SCOD concentrations in the leachate from each bioreactor were in range of 15,100–62,010 mg/L. The application of various pre-aeration durations resulted in different initial values of SCOD. Higher initial SCOD values, detected in the leachate from AN, R1 and R2, where pre-aeration time was less than 4 days, were possibly induced by an intensive hydrolysis of easily biodegradable organics remained in the pre-treated substrates. In order to compare with the SCOD evolution in each bioreactor, the $SCOD_t/SCOD_0$ ratio was used (Figure 3.3b), where $SCOD_0$ represents the SCOD concentration in the first leachate sample. An obvious increase of $SCOD_t/SCOD_0$ during the early stage of anaerobic operation in AN and R1 was observed, and, later on, these values were still maintained at high level, being 1.16 and 1.33 for AN and R1, respectively, based on the average of the last five samples. Meanwhile, the similar pattern was visible in R2, but with a relatively lower extent than R1. This could be explained by higher $SCOD_0$ concentration was tested in R2 than that in R1, possibly due to intensified hydrolysis of organic matter achieved by 4-day pre-aeration. By contrary, the highest $SCOD_t/SCOD_0$ value in R3 occurred on the 8th day of anaerobic operation (an increase of 28.6%), while in bioreactors R4, R5 and R6, no obvious accumulation of SCOD in the leachate were observed. With the progress of anaerobic operation, SCOD concentrations in R3–R6 decreased rapidly, ranging from 2500 to 2800 mg/L at the end of the study, with SCOD removal efficiencies of 84.2–90.9%, respectively.

The evolution of VFAs concentration with time was consistent with the SCOD evolution (Figure 3.3c). The VFAs concentrations in R3–R6 decreased rapidly after the initial peak; while, only acetic acid was detected in the final samples and the average content was less than 20 mgCOD/L, which implied the maturation of final substrates. Higher accumulated VFAs were observed in leachate from AN, R1 and R2 (~31,000–57,000 mgCOD/L), suggesting that these bioreactors were still in acidogenesis phase and methane production was indeed interrupted. Additionally, the VFAs production in R1 and R2 were obviously higher compared to that from AN.

This discrepancy can be explained by strengthened acidogenesis achieved by pre-aeration. Moreover, butyric acid measured was dominant in accumulated VFAs from AN, R1 and R2, showing a typical butyric-type fermentation in these bioreactors (Feng et al., 2014).

The results regarding NH_4^+–N concentration are pointed out in Figure 3.3d. NH_4^+–N concentrations in AN, R1 and R2 bioreactors showed an increasing trend with time and fluctuated ~3300 mg/L at the end of the experiment; meanwhile, faster accumulation of NH_4^+–N concentrations were observed in the leachates from R1 and R2. By contrast, the initial NH_4^+–N concentrations in R3–R6 were obviously higher (~1300–2900 mg/L), and increased slightly in the subsequent days of anaerobic operation, with the final values of 3018, 2441, 1680, and 1693 mg/L, respectively.

These discrepancies of initial NH_4^+–N concentrations among each bioreactor can be explained by accelerated ammonification process achieved by different pre-aeration durations. Furthermore, these obtained ammonia could provide buffers and neutralize VFAs produced in anaerobic digestion process (Zhai et al., 2015), thus resulting in higher initial pH values in the fermentation liquor(~7.1–7.3).



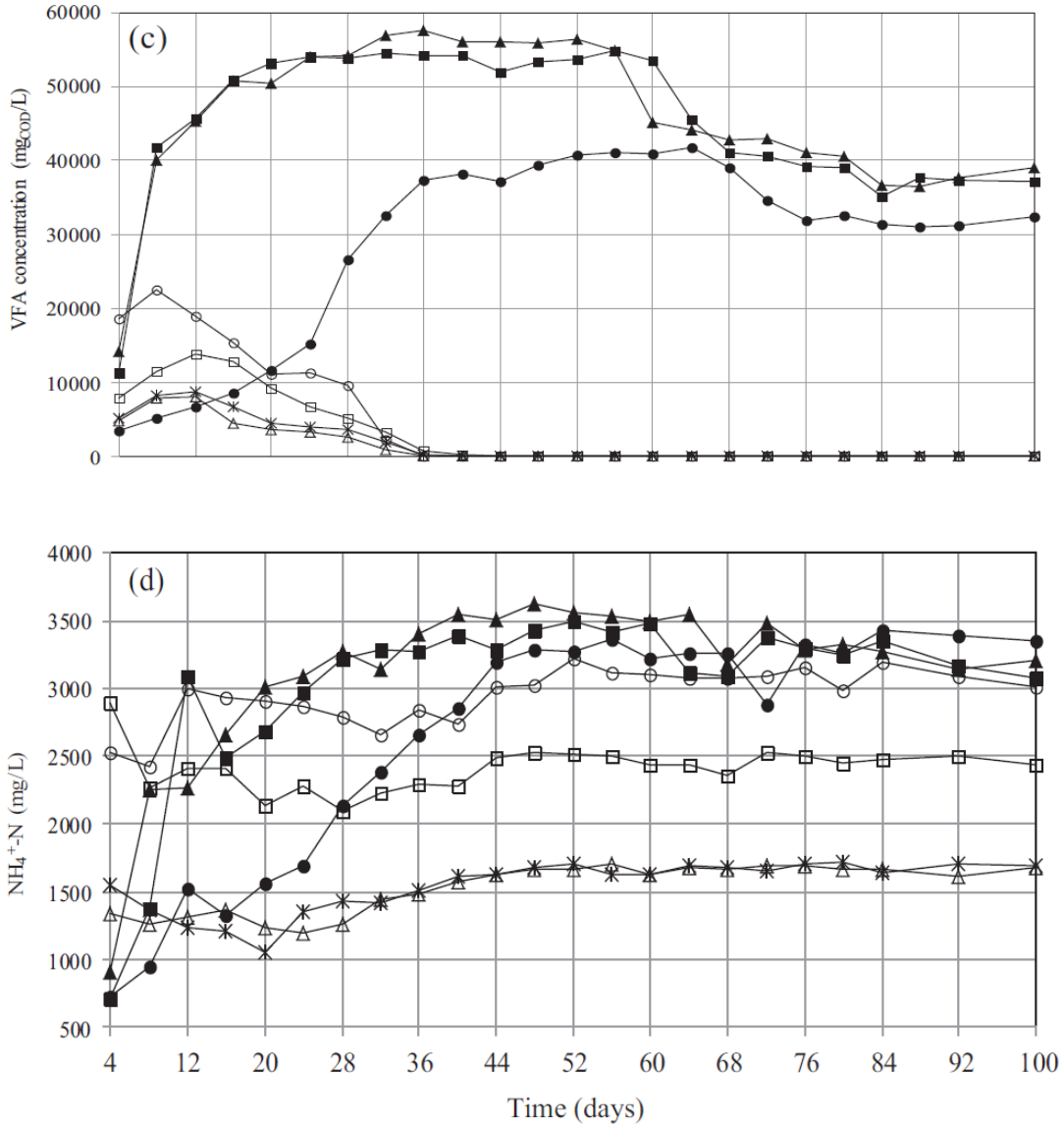


Figure 3.3: Monitoring of parameters in leachate over time during anaerobic phase: (a) pH, (b) $SCOD_t/SCOD_0$, (c) VFAs, (d) NH_4^+–N.

Methane production

The methane cumulative yields from all bioreactors are illustrated in Figure 3.4 in terms of L of methane per kg of dry matter (DM). The start-up of the steady methanegenesis didn't occur in AN bioreactor during 100 days anaerobic operation. This is likely due to the excessive acidification caused by the anaerobic fermentation of easily degradable matters in raw waste, as evidenced by a pH drop to 4.3 in leachate (Figure 3.3a).

Pre-aeration of 2 and 4 days in R1 and R2 slightly altered VS content and the percentage of substrate composition. Nevertheless, these 2–4 days pre-aeration were not long enough to completely relieve the excessive acidification (more than 10,000 mgCOD/L) and low pH value (~5.0) in subsequent anaerobic operation. There was no methane production enhancement from R1

in comparison with AN. A methane yield increase in R2 bioreactor was observed after 54 days of anaerobic cultivation. After 100 days of anaerobic operation, the average methane content of biogas produced in R2 improved from the initial 1.3% (v/v) to ~13.3% (v/v), with an accumulated methane yield of 3.3 L/kg_{DM}, thus generating low quality biogas with only a little amount of methane.

Different methane yield trends were observed from the bioreactors filled with substrates aerobically pre-treated for more than 6 days. The evolutions of cumulative methane production in R3–R6 bioreactors increased rapidly after an initial lag phase and reached a plateau at the end of the study. The methane yields obtained in R3 and R4 bioreactors were 123.4 and 107.7 L/kg_{DM}, with average methane concentrations of 56.6% (v/v) and 56.1% (v/v), respectively. While, prolonging aerobic phase up to 11 and 14 days caused a decrease in methane yields of 88.8 and 83.5 L/kg_{DM} in R5 and R6, respectively. Notwithstanding, the average methane concentrations in the biogas obtained from R5 and R6 still reached 54.9% (v/v) and 55.1% (v/v), respectively. The methane yields obtained from R3–R6 are comparable with the previous studies in Table 3.2, except the results reported by Gerassimidou et al. (2013), who carried out the experiment only using organic fractions of MSW rather than mixed wastes as substrates.

This indicated that pre-aeration of 6–14 days was highly effective in enhancing methane production in MSW anaerobic landfilling; but, with pre-aeration durations prolonged, the methane production gradually decreased.

In order to further assess the overall performances of methane production, the results obtained from bioreactors R3–R6 were simulated using the modified Gompertz model (Equation 1) to determine the kinetic parameters. AN, R1, and R2 failed to produce methane during experimental periods, so they were not considered for simulation.

The modified Gompertz model showed a strong fit correlation ($r^2 > 0.98$) for describing the progress of cumulative methane production in all runs (Table 3.3). Methane production potential (P) and maximum methane production rate (R_m) were distinguished for each pre-aeration time. The highest value of P was obtained for R3, being 9.9%, 36.7% and 44.8% higher than that of R4, R5 and R6, respectively. The lag-phase times (λ) of R3, R5 and R6 were almost the same, ~9 days; however, in R4 a longer lag-phase time of 16 days was observed, possibly attributing to its higher initial NH_4^+–N detected in the fermentation liquor (Figure 3.3d).

Additionally, $T_{90\%}$, which represents the accumulated time needed to reach 90% of biogas production potential, should be taken into consideration for the actual methane collection in order to achieve its highest utilization efficiency under landfilling condition (Ritzkowski and Stegmann, 2013; Zhai et al., 2015). All the four bioreactors completed 90% methane production in 46–64 days,

but complete methane production ($T_{100\%}$) would require up to 260–400 days, averagely 6 times longer than $T_{90\%}$.

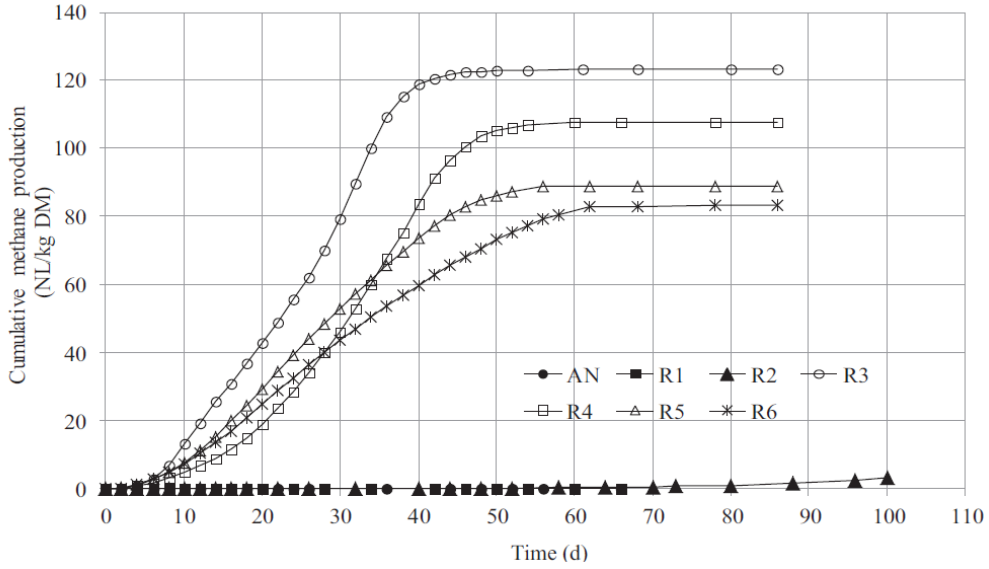


Figure 3.4: Enhancing effects of short-term pre-aeration on methane production.

Table 3.2: Comparison of aerobic pre-treatment methods and effects to enhance methane production in anaerobic bioreactor landfilling.

Substrates	**Aerobic/anaerobic reactor scale/volume**	**Aeration mode**	**Pre-aeration duration**	**Effects on methane production**	**Reference**
Municipal solid waste	68 L simulated bioreactor for pre-aeration and anaerobic landfilling	Composting-like process	Less than 5 d	11 times higher than that of control; 110 L/kg$_{DM}$	Mansour et al. (2012)
Food waste: wasted office paper 2.4:1 (w/w)	200 L reactor for preaeration; Simulated anaerobic bioreactors with total volume of 160 L	2 L/min, continuous aeration	8, 45 and 90 d	54% increase for 8 days pre-aeration; 308 L/kg$_{DM}$ 70% and 85% decrease for 45 and 90 days pre-aeration, respectively; 58 and 28 L/kg$_{DM}$, respectively	Gerassimidou et al. (2013)
Municipal solid waste	MBT full-scale for preaeration; simulated anaerobic landfill bioreactor of 11 L working	Composting-like process	28 d	1.5 times higher than that of control; 105 L/kg$_{DM}$	Scaglia et al. (2013)

	volume				
Synthesized municipal solid waste	Hybrid bioreactors with working volume of 11.5 L	170 mL/min/ kgVS, intermittent aeration	60 and 75 d	Positive impact; 85 and 72 L/kg_{DM}, respectively	Xu et al. (2015)
Municipal solid waste	Hybrid bioreactors with working volume of 36.8 L	0.5 L/d/kgTS, continued and intermittent aeration	35, 55, 81 and 95 d	1.3–2.4 times higher than that of control; 31.7–59.7 L/kg_{DM}	Cossu et al. (2015)
Synthesized municipal solid waste	Simulated bioreactors with working volume of 6.4 L	0.5 L/min/kg waste, intermittent aeration	2–14 d	Positive impact; 83.5–123.4 L/kg_{DM}, respectively	This study

Table 3.3: Calculated parameters and correlation coefficients using the modified Gompertz model.

Bioreactor	**P (L/kg_{DM})**	**λ (d)**	**R_m ($L/kg_{DM}/d$)**	**$T_{90\%}$[a] (d)**	**$T_{100\%}$[b] (d)**	**r^2**
R3	133.4	9.9	4.4	46.3	261	0.989
R4	121.4	16	3.6	56.4	295	0.993
R5	97.6	9	2.6	53.7	400	0.999
R6	92.5	8.2	2.0	63.6	390	0.999

[a] $T_{90\%}$ is the methane production time needed to reach 90% of methane production potential.
[b] $T_{100\%}$ is the methane production time needed to reach methane production potential.

Waste degradation

Comprehensive evaluation of organic components changes

The results of solid organics characterization at the beginning of the test as well as at the end of pre-aeration phase are reported in Figures 3.5 and 3.6.

Pre-aeration of 2 and 4 days conducted in R1 and R2 almost obtained 9% and 17% VS degradation, respectively. The removal of carbohydrates contributed to the largest fraction of VS losses in these stages (>50%), with the maximum removal rate of 46.7% obtained on day 4 (i.e. R2). However, the degradation of proteins from R1 and R2 was only 2.7% and 8.1%, respectively, meaning that a large fraction of easily degradable organic nitrogen was still contained in the substrates, resulting in a potential overload of anaerobic systems (Figures 3.3c and 3.4).

Pre-aeration of 6 and 8 days applied in R3 and R4 bioreactors obviously speeded up the degradation of proteins in waste, with removal rates of 42.8% and 51%, respectively; meanwhile, carbohydrates removal rate reached up to 76.7% on day 6. The remaining contents of organic components were

6.9% DM for proteins, 2.0% DM for carbohydrates, 5.3% DM for lipids and 15.4% DM for lignocelluloses (on dry weight basis) after 6 days pre-aeration, and
slightly decreased further after 8 days pre-aeration. Proteins and carbohydrates are considered as the easiest substrates to be hydrolysed and acidified during common anaerobic digestion process
(Kong et al., 2016). These results indicated that pre-aeration of 6–8 days was capable of achieving high efficient removal of organic components which were easy to cause excessive acidification, thereby improving the subsequent anaerobic behaviours (Figures 3.3 and 3.4).

The prolonged pre-aeration time up to 11 and 14 days caused further degradation of organic matter (R5 and R6). Especially, in the aeration interval of 9–14 days, lignocellulose degradation
(~25.7%) was obviously accelerated, becoming the main carbon source under aerobic condition. Moreover, 11 and 14 days pre-aeration further consumed the proteins content in waste, showing advantages to eliminate the NH_4^+ –N pollution in the anaerobic leachate (Figure 3.3d), which was expected to save leachate handling expenses externally. However, the reduction of leachate treatment costs should be further compared with the increasing cost due to prolonged pre-aeration duration and losses of methane potential, under the actual conditions.

After the pre-aeration phase, the remaining organic components within the pre-aerated waste would continue to degrade under anaerobic conditions. Higher degradation efficiencies of lipids, carbohydrates and lignocellulose were assessed in bioreactors R3–R6, which is consistent with favourable methanogenic conditions in these bioreactors (Figure 3.4). At the end of the study, the remaining chemical components in solid residuals from R3– R6 were almost at the same level, being 4.3–4.7% DM for proteins, 0.3–0.5% DM for lipids, 6.2–7.5% DM for lignocelluloses, and almost no carbohydrates were detected (Figure 3.5). Therefore, there is no evidence
that pre-aeration would influence the final storage quality of MSW in anaerobic landfilling. In AN, R1 and R2, high levels of proteins losses in waste were observed as well, possibly because of proteins hydrolysis. Yu and Fang (2003) concluded that VFAs didn't inhibit proteins degradation under anaerobic condition. This implied that excessive hydrolysis and acidification of organic nitrogen within the tested waste were the main reasons to cause acid (VFAs or amino acids) inhibition; then leading to poor lignocelluloses and carbohydrates degradation under the anaerobic conditions. This finding is consistent with the results of Li et al. (2014), who considered that the accumulation of the tyrosine-like group from degradation of organic nitrogen hampered the hydrolysis of insoluble organic polymers, though the main components of VFAs in leachate were different from those detected in our study.

In conclusion, during the experimental period, the total VS removal rates obtained in R3–R6 were 72%, 76%, 74% and 72%, respectively (Figure 5.5), being approximately 2.4–3.3 times higher than

those obtained in AN, R1 and R2 bioreactors. Additionally, the removed organics in AN, R1 and R2 were mainly detected into their high concentration leachates (Figures 3.3b and 3.3c) rather than being converted to available methane (Figure 3.4), which could explain the complex leachate generated and low methane collection efficiency from actual landfills (Qi et al., 2013; Sun et al., 2014).

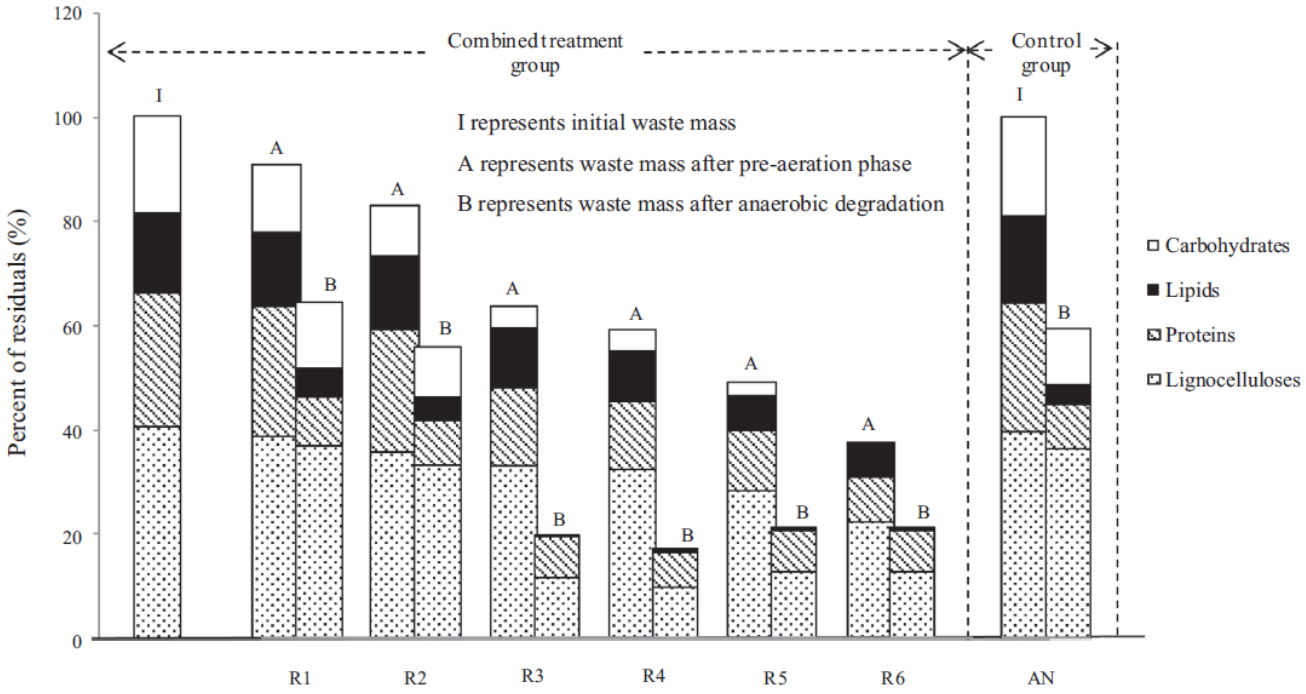


Figure 3.5: Comprehensive evaluation of organic components evolution during the whole experimental period in all bioreactors.

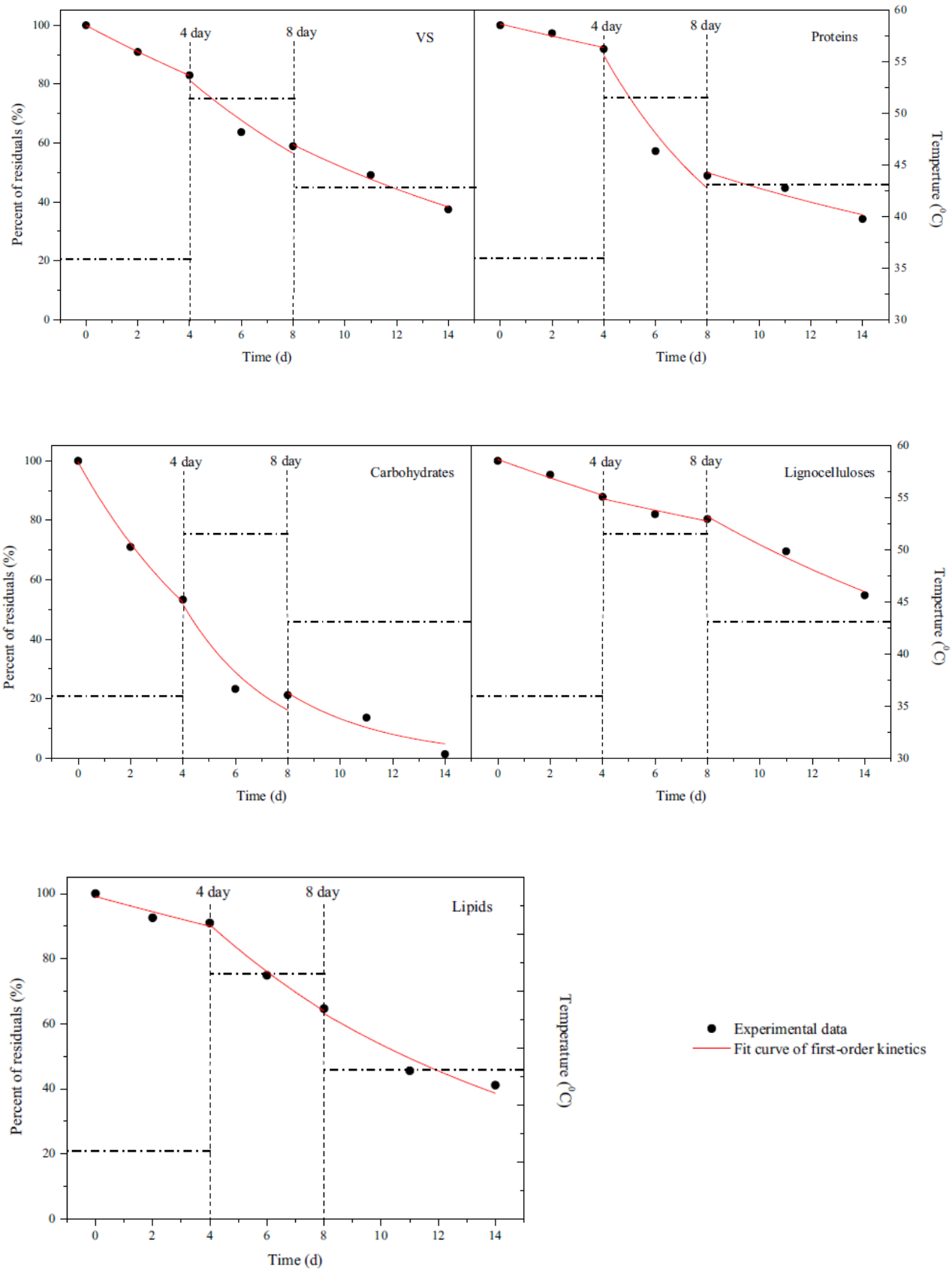


Figure 3.6: Evolution of organic components in solid samples during pre-aeration phase (note: horizontal dash dot line represents the average temperature in different time intervals, i.e. 0–4 day, 36.1 °C; 5–8 day, 51.6 °C; and 9–14 day, 43.1 °C, respectively).

Roles of temperature evolution on organic components

Temperature is the crucial factor affecting aerobic biological degradation of organics. In this study, 0–14 days pre-aeration scheme could develop a complete temperature evolution due to the self-heating generated by the microbial activities from the breakdown of the available organic matter, containing a temperature rise stage, a thermophilic stage and a cooling stage, with average temperature of 36.1 °C, 51.6 °C, and 43.1 °C, respectively (Figure 3.2). In order to present deeper insight into the degradation of organic components over time, the temperature effects are evaluated by first-order kinetic equation (Huiliñir and Villegas, 2014) (Figure 3.6), and the calculated organics degradation rate constants (k values) are shown in Table 3.4. The highest k values of proteins, lipids and carbohydrates were observed during the thermophilic phase (days 5–8); especially, the k value of proteins degradation in this stage was 3 times higher than that in subsequent cooling stage (9–14 day). Conversely, thermophilic temperature inhibited the degradation of lignocelluloses and a higher k value was occurred during the cooling stage of pre-aeration. The above results indicated that, with the help of thermophilic temperature generated by microorganisms metabolism during short-term pre-aeration process, the easily hydrolysable organics, including proteins and carbohydrates, were preferentially and efficiently removed in this stage; meanwhile, the relative fraction content of lignocelluloses was enriched in pre-treated waste intentionally, and thus could be used as the main carbon source for a stable methane production during anaerobic landfilling, subsequently.

While, excessive pre-aeration treatment during cooling stage could accelerate the degradation of lignocelluloses remaining in waste and thus reduce the methane production potential. Based on this reason, it is advisable to interrupt pre-aeration process before waste mass temperature entering into cooling stage to give way to anaerobic landfilling.

Table 3.4: Calculated k values and correlation coefficients using first-order reaction equation.

Chemical components	**Days 0–4**		**Days 5–8**		**Days 9–14**	
	k value	**r2**	**k value**	**r2**	**k value**	**r2**
VS	0.047	0.99	0.091	0.84	0.073	0.98
Proteins	0.021	0.92	0.175	0.88	0.056	0.84
Carbohydrates	0.16	0.99	0.29	0.82	0.252	0.77
Lignocelluloses	0.032	0.95	0.023	0.82	0.062	0.96
Lipids	0.024	0.78	0.087	0.98	0.082	0.86

3.4 CONCLUSIONS

Short-term pre-aeration improved anaerobic degradation of MSW with high organic fractions in simulated landfill bioreactors.

The highest methane yield of 123.4 L/kg_{DM} was obtained from the waste with 6-day pre-aeration. The development of favourable methanogenic conditions is attributed to the removal of excess easily degradable organics and ammonia obtained from the accelerated proteins degradation by pre-aeration. Thermophilic temperature during pre-aeration, besides inhibiting the nitrification reactions, effectively intensified the degradation of proteins and, meanwhile, caused the relative enrichment of lignocelluloses in pre-treated waste. Therefore, a possible strategy is interrupting the pre-aeration process before the mass temperature entering into cooling stage, then switching to anaerobic landfilling.

4. DECENTRALIZED FOOD WASTE MANAGEMENT AND SMALL REALITIES

This chapter deals with the sustainable management of segregated FW streams, either coming from isolated realities or specifically valorised (spent coffee grounds case study) as an energy feedstock.

4.1 LAB-SCALE CO-DIGESTION OF KITCHEN WASTE AND BROWN WATER FOR A PRELIMINARY PERFORMANCE EVALUATION OF A DECENTRALIZED WASTE AND WASTEWATER MANAGEMENT

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An overall interaction is manifested between wastewater and solid waste management schemes. At the Laboratory of Environmental Engineering (LISA) of the University of Padova, Italy, the scientific and technical implications of putting into practice a decentralized waste and wastewater treatment based on the separation of grey water, brown water (BW - faecal matter) and yellow water (YW - urine) are currently undergoing investigation in the *Aquanova Project*. An additional aim of this concept is the source segregation of kitchen waste (KW) for subsequent anaerobic co-digestion with BW. To determine an optimal mixing ratio and temperature for use in the treatment of KW, BW, and eventually YW, by means of anaerobic digestion, a series of lab-scale batch tests were performed. Organic mixtures of KW and BW performed much better (max. 520 m^3CH_4/tVS) in terms of methane yields than the individual substrates alone (max. 220 m^3CH_4/tVS). A small concentration of urine proved to have a positive effect on anaerobic digestion performance, possibly due to the presence of micronutrients in YW. When considering high YW concentrations in the anaerobically digested mixtures, no ammonia inhibition was observed until a 30% and 10% YW content was added under mesophilic and thermophilic conditions, respectively.

4.1.1 INTRODUCTION

An overall interaction is manifested between wastewater and solid waste management schemes. Solid waste treatment generates liquids (such as landfill leachate), which are often sent to wastewater treatment plants. Wastewater treatment plants produce sludge, sand and screened materials requiring landfill disposal. In recent decades, numerous research studies have been performed to close the co-management cycle of the two systems through co-stabilization, by composting and/or anaerobic digestion of the sludge with the organic fraction of municipal solid

waste (Rajagopal et al., 2013; Lim and Wang, 2013; Lim et al., 2014; Rajagopal et al., 2014; Lagerkvist et al., 2015).

In the wastewater sector, there is a tendency to define a systematic approach for the different wastewater fractions and to analyse the effects of different scenarios on the control and source segregation of the different streams, with characterization of the components: black water (urine and faecal matter), grey water, and rainwater (Krebs and Larsen, 1997). The decentralized treatment of municipal wastewater, based on the separation between grey and black waters, and even between brown water (faecal matter) and yellow water (urine), represents a sustainable and future solution for waste (water) treatment (Elmitwalli et al., 2006).

Ongoing discussion surrounding the sustainable co-management of domestic wastewater and organic solid waste is focused on the concepts of avoidance, source separation, recycling and reuse (Henze, 1997). At the Laboratory of Environmental Engineering (LISA) of the University of Padova, Italy, the scientific and technical implications of putting into practice these concepts are currently undergoing investigation as part of the *Aquanova Project* (Figure4.1.1). This project focuses on the source separation of domestic wastewater into three main streams: yellow water (YW - urine), brown water (BW - faecal matter), and grey water (i.e. wastewater coming from the washbasin, shower, bath). The purpose of separate management of these fractions is to facilitate water reuse and minimize energy requirements for wastewater treatment (YW and GW) to be performed in a phytotreatment unit (Lavagnolo et al., 2016). An additional aim of this concept is the source segregation of kitchen waste (KW) for subsequent anaerobic co-digestion with BW and recovery of energy in the form of methane. The *Aquanova Project* establishes that kitchen waste should be shredded in a mill installed in the kitchen sink and sent, together with the faecal stream, to an anaerobic digestion (AD) unit.

In recent decades very few studies have investigated the co-digestion of black water and kitchen waste in anaerobic systems (Kujawa-Roeleveld et al., 2003, 2005, 2006; Elmitwalli et al., 2006; Wendland et al., 2007). To avoid the concerns raised over ammonia accumulation, the addition of brown water alone (without urine) to kitchen waste prior to AD was also investigated (Zeeman et al., 2008; Curry and Pillay, 2012; Rajagopal et al., 2013; Lim et al., 2014). BW is capable of improving stability of the anaerobic digestion process by providing additional nutrients and maintaining buffer capacity (Lim et al., 2014). The benefits of co-digesting BW and KW were described by Rajagopal et al. (2013) - higher biogas production and biodegradation efficiencies were observed when BW was added as a co-substrate to the anaerobic degradation of KW, likely due to the adequate buffering capacity provided by BW to KW digestion (Rajagopal et al., 2013). Production of methane via anaerobic co-digestion of KW and BW not only provides a cheaper and

greener alternative to disposal, but may help to reduce the use of fossil fuel-derived energy and, consequently, the impact on global warming (Abbasi et al., 2012).

The aim of this research was to compare the specific methane yield of single substrates and of their mixtures under mesophilic and thermophilic conditions.

Tests to assess the specific methane yield of kitchen waste (KW), brown waters (BW), yellow waters (YW), and a series of different mixtures, are summarized in Table 4.1.1, and can be grouped into the following three experimental phases:

I. specific bio-methane production batch tests (BMP tests) were performed on the three individual substrates, KW, BW, and YW, under mesophilic (35 ± 1 °C) and thermophilic (55 ± 1 °C) conditions;

II. the three substrates were mixed according to different percentages and BMP tests were performed under mesophilic conditions, varying YW at a maximum concentration of 6% in the mixture;

III. BMP tests were fed with substrate mixtures prepared with increasing percentages of YW up to 50%, to evaluate the anaerobic bacteria inhibition caused by high ammonia content.

Mixture percentages are reported in terms of wet basis.

Table 4.1.1: Sequence of experiments performed.

Experimental batch tests phase	**Feeding**	**Substrates**	**Temperature during the AD process**
I	Single matrix	KW	Mesophilic (35 ± 1 °C)
		BW	
		YW	
		KW	Thermophilic (55 ± 1 °C)
		BW	
		YW	
II	Mixture	KW + BW	Mesophilic (35 ± 1 °C)
		KW + BW +$YW_{(up\ to\ 6\%)}$	
III		KW + BW +$YW_{(up\ to\ 50\%)}$	Mesophilic (35 ± 1 °C)
		KW + BW +$YW_{(up\ to\ 50\%)}$	Thermophilic (55 ± 1 °C)

4.1.2 MATERIALS AND METHODS

On the basis of *Aquanova Project* concepts (Figure 4.1.1), a pilot plant for domestic sewage separation was set up at the LISA Laboratory of the University of Padova, Italy, and a purpose-designed toilet (Otterpol et al., 1999) for the separation of YW and BW was installed.

Grey water from the sink and toilet flows were conveyed separately to three different stainless steel tanks, each with a 100 litre capacity. The tanks were equipped with a sampling system, an agitator and a sensor for level control, connected to a motorized valve for tank emptying. At the entrance to the bathroom two photocells were installed to monitor inputs. The whole system was controlled by

purpose-designed LabView software, which allowed continuous measurement of flow, calculation of pro-capita production, control of mixers and electric valves for discharging to the sewer system.

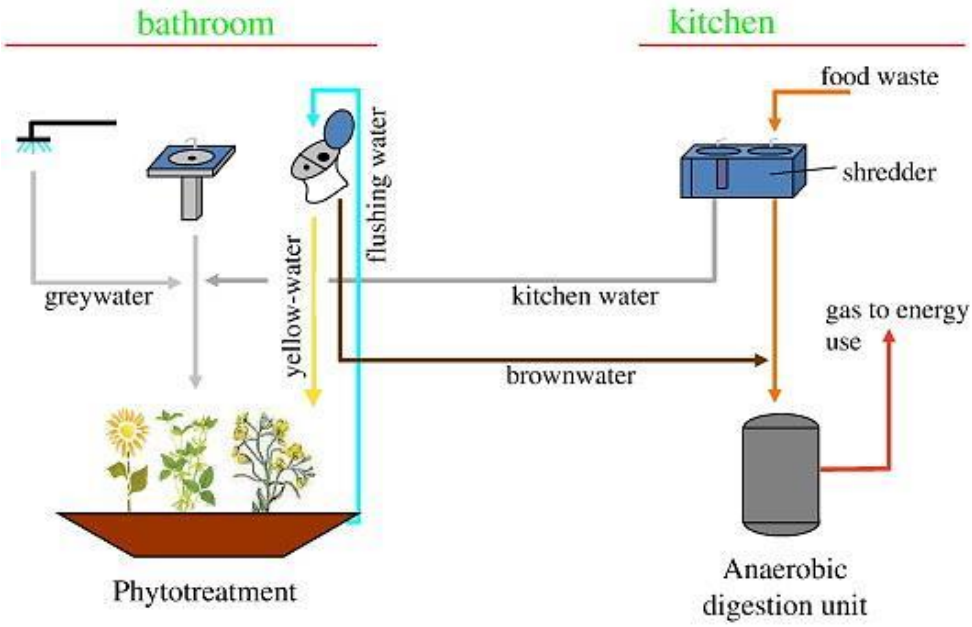


Figure 4.1.1: *Aquanova Project*. Based on the source separation of various domestic sewage components and on the integrated management of domestic solid and liquid wastes (Lavagnolo et al., 2016).

Substrate Characterization

Yellow water (YW) and brown water (BW) obtained from the pilot plant installed at the University of Padova, Italy, were collected from the sampling tanks as substrates to be tested. Kitchen waste (KW) was collected from Padova University canteen and shredded using a kitchen mill.

Prior to anaerobic digestion, the individual substrates (KW, YW, and BW) were tested to measure the following parameters: total solids (TS), volatile solids (VS), pH, alkalinity (Alk), BOD_5, COD, ammonia nitrogen ($N\text{-}NH_3$), TKN, total phosphorus (P_{tot}), volatile fatty acids (VFAs), and heavy metals, as reported in Table 4.1.2. In order to better discuss the results of experimental phase III, free ammonia concentration (mg/L) inside the mixed substrates was calculated using the following equation 1 (Anthonisen et al., 1976):

$$\text{free ammonia} = 1.214 * NH_4^+ * 10^{pH} / [e^{6344/(273+T)} + 10^{pH}] \quad [1]$$

where NH_4^+ is the total ammonia concentration as nitrogen (mgN/L), and T is the temperature (°C).

Anaerobic sludge, used as inoculum, was collected from a full-scale wastewater treatment plant (Ca'Nordio) located in Padova, Italy.

Table 4.1.2: Chemical characteristics of kitchen waste, brown water, and yellow water used as substrates for anaerobic digestion tests.

	KW		BW		YW	
Parameter	**range**	**average**	**range**	**average**	**range**	**average**
TS (mg/L)	106678-19555	168851	2359-4587	3445	4350-8125	6485
VS (mg/L)	87035-154108	119997	1676-3978	2845	755-2380	1450
pH	5.2-5.5	5.3	6.7-8.3	7.5	8.5-8.7	8.6
Alk (mg/L)	-	-	440-720	527	5080-7660	5777
BOD_5 (mg/L)	-	-	267-1135	874	610-1700	1257
COD (mg/L)	122559-18660	154156	5090-7660	5905	2320-4600	3048
N-NH_3 (mg/L)	870-1242	1080	38-132	52	1530-2394	2034
TKN (mg/L)	6778-9544	8830	59-173	125	2296-3458	2766
P_{tot} (mg/L)	-	-	3.2-24.8	12	14.5-96.5	61
VFAs (mg/L)	2755-2992	2802	56-120	78	-	-
Cr (mg/L)	-	-	-	-	0-0.48	0.12
Cu (mg/L)	-	-	0.16-0.31	0.24	0.06-0.15	0.11
Fe (mg/L)	-	-	0.58-2.4	1.15	0.15-0.93	0.53
Mn (mg/L)	-	-	0-0.19	0.07	0-0.05	0.01
Ni (mg/L)	-	-	0.02-0.21	0.09	0.02-0.07	0.04
Pb (mg/L)	-	-	0.02-0.06	0.04	0-0.4	0.02
Zn (mg/L)	-	-	1.17-3.1	2.14	0.42-1.36	0.97

Bio-methane production tests

Lab-scale BMP tests were performed to evaluate the specific bio-methane yield of KW, BW, and YW as individual substrates (I phase) and as mixtures in a series of combinations (see Table 4.1.1) subsequent to anaerobic digestion (II phase).

Throughout experimental phase II the mixtures were characterized by different KW concentrations, namely 7%, 11%, 20%, 27%, and 33%, initially combined with BW alone, and then with a percentage of YW ranging from 4% to 6%.

During phase III, YW concentrations were increased up to 50% to evaluate the effect of ammonia and the mixed substrates compositions can be seen in Tables 4.1.3 and 4.1.4.

In phases I, II, and mesophilic III, tests were carried out in 120 mL serum bottles under both mesophilic (35 ± 1 °C) and thermophilic (55 ± 1 °C) conditions. Reactors were hermetically closed using 20 mm aluminium crimp caps (Agilent). Substrate concentration and substrate to inoculum ratio (S/I) were 5 gVS/L and 0.5 gVS/gVS, respectively. The liquid volume in each reactor, consisting of the substrate, and inoculum, was 80 mL. Each vial was fed with 2.7 g inoculum (characterized by 37% of total solids) and 0.8 g of buffer ($NaHCO_3$) was added to maintain neutral

pH conditions. In phase thermophilic III, larger 12 liter reactors were used and filled with the same substrate to inoculum ratio as the serum bottles.

After preparation, the reactors were flushed with N_2 gas for 3 min and incubated under static conditions in a thermostatic chamber. Blank tests using the inoculum alone were also conducted to measure the quantity of methane produced by the biomass alone. All tests were performed in triplicate.

Biogas volume produced during BMP tests was measured by means of the water displacement method in phases I, II, and mesophilic III. Methane volumes produced in the time interval between each measurement [t - (t-1)] were calculated using a model taking into consideration the gas concentration at time t and time t-1, together with the total volume of biogas produced at time t, the concentration of specific gas at times t and t-1, and the volume of head space of reactors (Van Ginkel et al., 2005). The following equation 2 (see section 1.1) was applied:

$$V_{C,t} = C_{C,t} * V_{G,t} + V_H * (C_{C,t} - C_{C,t-1}) \quad [2]$$

In phase thermophilic III, biogas generated from each reactor was collected using a 10-liter Tedlar® sampling bag connected to the upper gas port, and biogas volume was measured daily by means of a volumetric flow meter (Cossu et al., 2016).

Methane and carbon dioxide composition in the gas were determined using a gas chromatographer (Hewlett Packard 5820).

To have an empirical evaluation of the lag phase only visually hypnotized from the methane production curves obtained during the batch tests, data were interpolated on the basis of the Gompertz model (Van Ginkel et al., 2001). The Gompertz mathematical expression is described in the following equation 3:

$$P(t) = P_{max} e\left\{-e\left[\frac{R*e}{P_{max}}\right](\lambda - t) + 1\right\} \quad [3]$$

where P (t) is the cumulated methane production at time t; P_{max} is the maximum methane production, R is the maximum production rate and λ is the lag phase.

Data on methane productions are expressed at a temperature of 0 °C and pressure of 1 atm (Normal conditions).

Table 4.1.3: Substrate composition for anaerobic digestion batch tests under mesophilic conditions (experimental phase III).

Sample	%KW	%BW	%YW
A	10.0	90.0	0.0
B	9.4	84.6	6.0
C	8.0	72.0	20.0
D	7.0	63.0	30.0
E	5.0	45.0	50.0

Table 4.1.4: Substrate composition for anaerobic digestion batch tests under thermophilic conditions (experimental phase III).

Sample	%KW	%BW	%YW
F	10.0	90.0	0.0
G	9.4	84.6	6.0
H	8.0	72.0	20.0
I	7.0	63.0	30.0
L	5.0	45.0	50.0

4.1.3 RESULTS AND DISCUSSION

Biochemical methane production tests – phase I

As expected, the amount of methane recoverable from YW was virtually imperceptible both under mesophilic and thermophilic conditions. YW was characterised by high ammonia concentration (around 2000 mg/L), which inhibited the metabolism of anaerobic bacteria. Yenigün and Demirel (2013) reported that, although ammonia is an essential nutrient for bacterial growth, the presence of high concentrations (1700-5000 mg/L) may inhibit methanogenesis during the anaerobic digestion process, particularly when dealing with complex type of substrates such as manure or the organic fraction of municipal solid waste.

Under mesophilic conditions, KW and BW allowed recovery of 260 and 160 m^3CH_4/tVS, respectively. Using equation 3 it was possible to highlight that the lag phase for KW (8 days) was 1.5 times shorter than that for BW. The KW sample featured a COD and VS removal rate of 55.1% and 73.7%, respectively, whilst in BW they were 15.9% and 6.1%, respectively.

Under thermophilic conditions, methane productions were 310 and 240 m^3CH_4/tVS from KW and BW, respectively. Lag phase was the same (6 days) for KW and BW. In this case, COD and VS removal rates for KW were 56.5% and 69.9%, respectively, and for BW they were 83.2% and 65.6%, respectively.

In agreement with Clarke and Alibardi (2010) and Girotto et al. (2015), KW is clearly a more suitable substrate for anaerobic digestion although, as temperature increases, methanogenesis also performed well in the presence of BW alone. Thermophilic anaerobic digestion is capable of improving mass transport and increasing reaction speed (Krebs and Larsen, 1997). Furthermore, this process contributes towards ensuring improved substrate sanitation.

Biochemical methane production tests – phase II

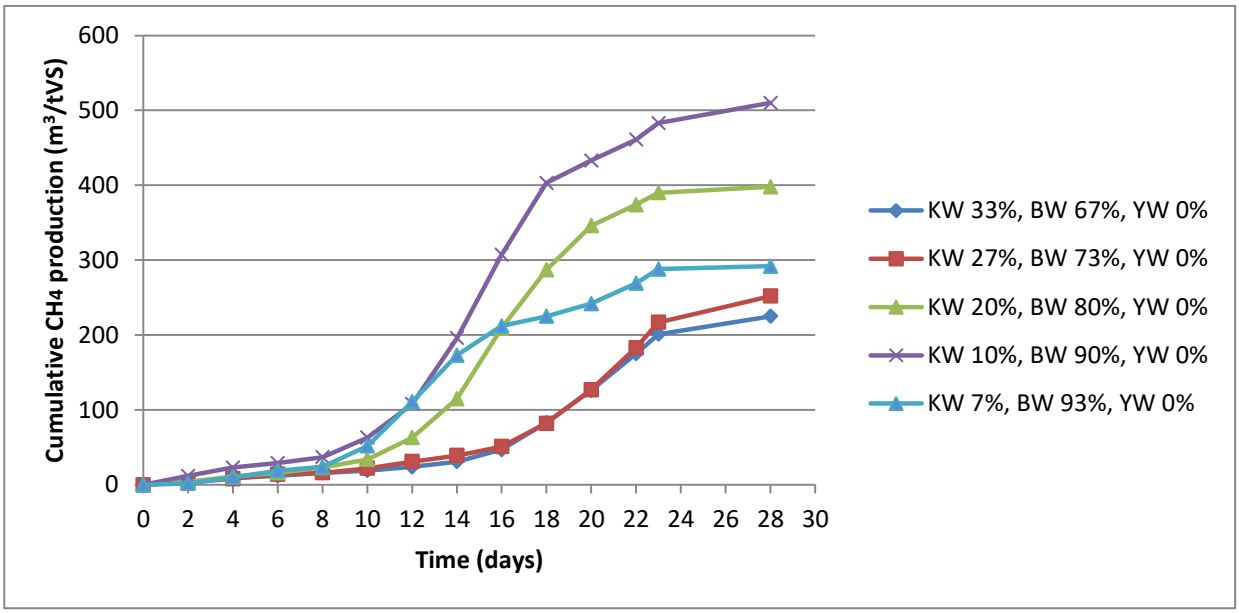
Anaerobic co-digestion resulted in higher methane yields than KW and BW alone, in agreement with Rajagopal et al. (2013). Tests were carried out by mixing different percentages of KW, namely 7%, 11%, 20%, 27%, and 33% initially with BW alone, and subsequently with a percentage of YW ranging from 6% to 4%.

Figure 4.1.2 illustrates the biogas production measured under mesophilic conditions.

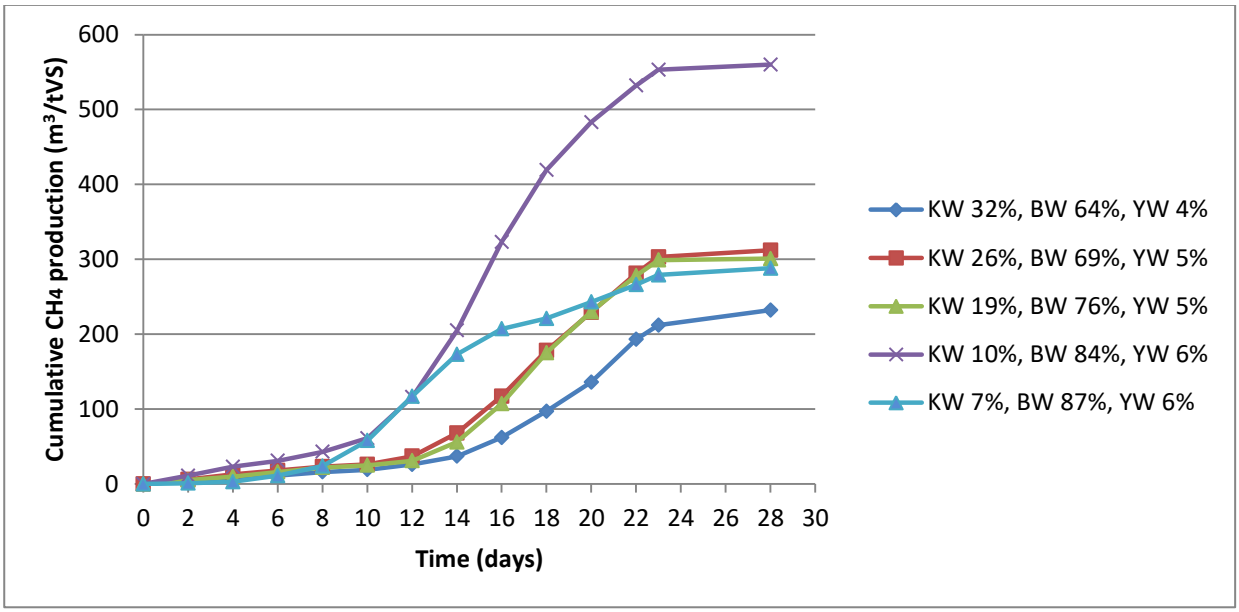
The process carried out with YW percentage varying between 0 and 6% displayed a complete absence of inhibition,

Mixtures with a percentage of KW ranging from 7 to 20% featured a much higher methane production than that observed with individual substrates. The best degree of mixing was obtained with approximately 10% KW, both with and without addition of low YW content. The addition of 6% YW elicited an increased biogas production and improved kinetics. Methane production increased from 510 m^3CH_4/tVS to 560 m^3CH_4/tVS, and a plateau was reached after 22 days instead of 28. This is probably due to the fact that bacteria benefit from the presence of nutrients both in BW and YW (e.g. N, P, K, Cu). When anaerobically digesting faeces with urine up to a ratio of 1:1, Creamer et al. (2008) reported that the substrate did not inhibit the anaerobic inoculum and provided a good biogas yield.

Both with and without YW addition, lag phases are directly proportional to the increasing concentration of KW (from 7% to 33%) changing between 8 and 13 days.



a



b

Figure 4.1.2: Cumulative methane production from different mixtures of kitchen waste (KW) and brown water (BW), under mesophilic conditions without (a) and with (b) urine (YW) addition up to 6% (experimental phase II).

Biochemical methane production tests – phase III

On the strength of the promising results obtained with the addition of YW in phase II, a new set of BMP tests were performed with the aim of investigating the impact of ammonia on methane production yielded by the different mixtures. As the anaerobic digestion process is particularly sensitive to ammonia content, a maximum concentration of 1700 mg/L (Yenigün and Demirel, 2013) and larger reactors were adopted to avoid a scale effect on the results of thermophilic phase III.

The results of the BMP tests performed under mesophilic and thermophilic conditions with a higher YW concentration up to 50% in the mixed substrates (see Tables 4.1.3 and 4.1.4) are shown in Figures 4.1.3 and 4.1.4, respectively.

Methane production for samples A and B (YW concentration is 0% and 6%, respectively) is virtually identical (Figure 4.1.3) with values around 210 m^3CH_4/tVS. On increasing YW concentration up to 30%, methane production likewise increases (samples C and D). A production peak is observed in D, corresponding to 280 m^3CH_4/tVS. By mixing KW and BW with 50% YW (sample E), methane production dropped to 160 m^3CH_4/tVS and terminated after 48 days. High ammonia concentration undoubtedly influenced the AD process, resulting in a lower methane production.

Under thermophilic conditions (Figure 4.1.4) the lowest methane production of 170 m^3CH_4/tVS was yielded by the sample containing 50% YW (sample L) and a long lag phase time of 20 days, whilst the highest methane production of 370 m^3CH_4/tVS was observed with sample G (6% YW). Methane yield from sample G exceeded that produced by sample F in the absence of YW addition (334 m^3CH_4/tVS). All samples, with the exception of L, reached a maximum methane production by the 25th day of AD.

Data relating to free ammonia concentrations calculated using equation 1 in the different samples on the basis of chemical composition of individual substrates (Table 4.1.2), revealed a decrease in methane production under mesophilic conditions up to values of around 100 mg/L of free ammonia; in this specific case study, these values corresponded to approximately 1000 mg/L of ammonia nitrogen and to a percentage of more than 30% YW. Under thermophilic conditions however, peak methane production was obtained by mixing KW and BW with 6% YW, corresponding to 59 mg/L of free ammonia, while it began to decrease at values exceeding 150 mg/L. Not taking into account the sample with a 50% YW content, the lag phase time during the thermophilic run (max. 3 days) was shorter than that monitored under mesophilic conditions (max. 7 days). This suggests that bacteria were able to adapt very well under thermophilic conditions, although the finding was not confirmed when comparing sample E and sample L. In sample L methane production commenced after 20 days compared to the previously observed 15 days (sample E). An excessively high ammonia concentration inhibits methanogenesis (Angelidaki and Ahring, 1994), with several Authors reporting how methane fermentation is more easily inhibited at thermophilic rather than mesophilic temperatures (Braun et al., 1981; Van Velsen and Lettinga, 1981; Angelidaki and Ahring, 1994).

Moreover, yellow waters likely contribute to bacterial growth due to the high micronutrient concentration. Indeed, peak methane production was achieved in sample G (6% YW) under

thermophilic conditions; conversely, under mesophilic conditions, the best yield was achieved by the sample containing 30% YW (sample D).

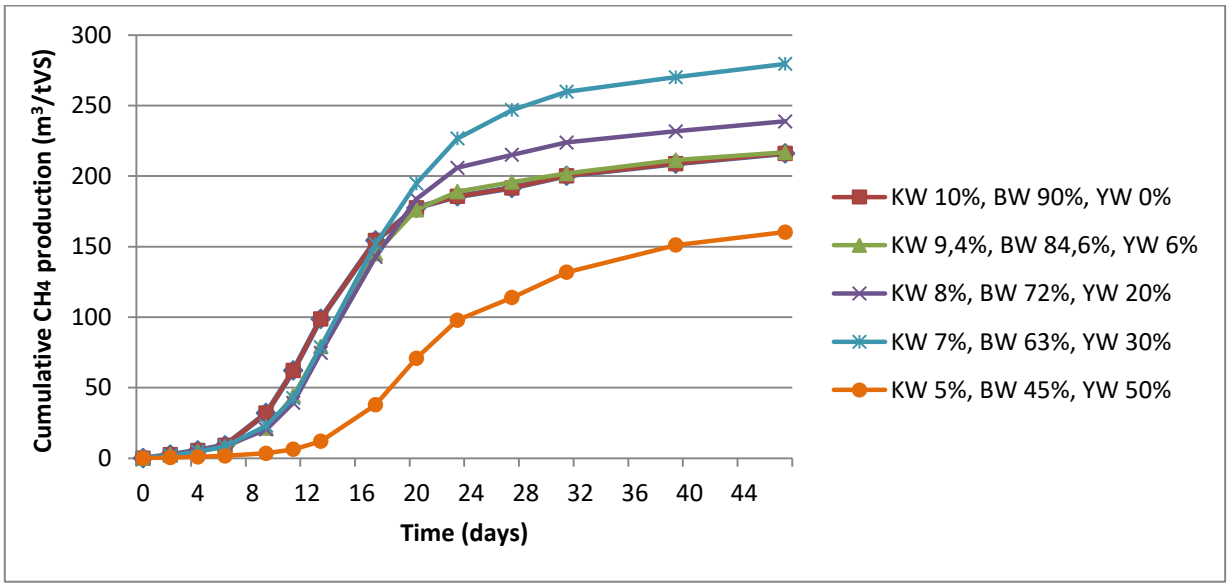


Figure 4.1.3: Cumulative methane production from different mixtures of kitchen waste (KW), brown water (BW), and urine (YW) under mesophilic conditions (experimental phase III).

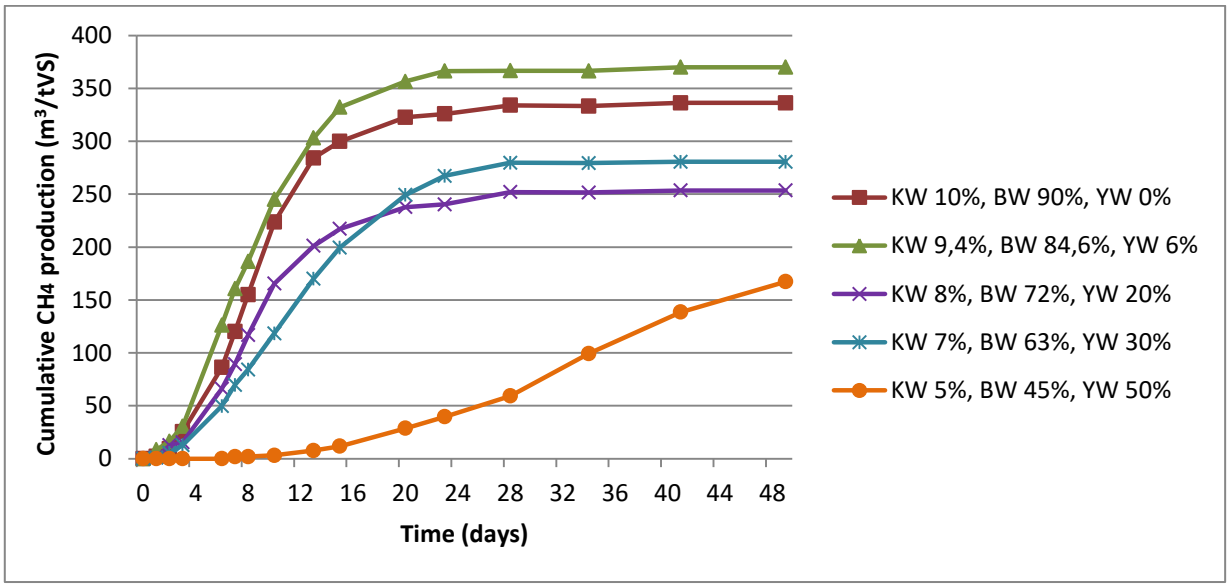


Figure 4.1.4: Cumulative methane production from different mixtures of kitchen waste (KW), brown water (BW), and urine (YW) under thermophilic conditions (experimental phase III).

4.1.4 CONCLUSIONS

The production of methane from the anaerobic co-digestion of kitchen waste and brown waters was tested both with and without the addition of yellow water under both mesophilic and thermophilic conditions.

BMP batch tests showed that when KW and BW were digested separately, they performed much better under thermophilic rather than mesophilic conditions. Moreover, in terms of methane yields, organic mixtures performed much better (max. 520 m^3CH_4/tVS) than the individual substrates alone (max. 220 m^3CH_4/tVS). Interesting results have been obtained on the influence of YW, and numerous studies have acknowledged the inhibitory effect of ammonia on bacterial activity. Nevertheless, in the experiments conducted, a small concentration of urine exerted a positive effect on the methanogenic phase, possibly due to the presence of micronutrients in yellow waters. In the presence of higher YW concentrations in the anaerobically digested mixtures, no inhibition was observed prior to reaching a 30% YW content under mesophilic conditions (30% YW corresponds to an optimum under mesophilic conditions, whilst under thermophilic conditions optimal YW content is 6%).

The anaerobic co-digestion of KW, BW, and a small amount of YW, may be further optimized to ensure that each decentralized scenario is rendered autonomous from the point of view of energy supply. Since the characteristics of kitchen waste vary significantly from one country to the next, optimal composition rate should be specifically tested prior to installation of an anaerobic digestion system.

4.2 THE BROAD SPECTRUM OF POSSIBILITIES FOR SPENT COFFEE GROUNDS VALORISATION

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Coffee is the world's second most traded commodity and the most renowned drink worldwide. The increasing production of coffee has been accompanied by a rise in consumption, and consequent increment in the amount of spent coffee grounds (SCGs) remaining as a solid residue from coffee brewing. In view of the high content of biodegradable compounds, if disposed, SCGs will certainly need to be biostabilized, although they should preferably be exploited in a biorefinery chain scheme. A wide range of alternative options is available for use in recycling SCGs as a valuable resource: food additives, pharmaceutical components, bio-sorbents, bio-fuels, and bio-products. The option of producing biogas from SCGs was tested and lab-scale bio-methane potential experiments were performed using different substrate to inoculum (S/I) ratios, namely 0.5, 1, and 2. A S/I ratio of 2 was found to be the optimal condition, resulting in a methane yield of 360 m^3CH_4/tVS.

4.2.1 INTRODUCTION

Coffee is the world's second most traded commodity after oil, and the most renowned drink worldwide. The International Coffee Organization (2016) has published the latest data relating to coffee production throughout the different nations of the world, with the top ten comprising: Guatemala 224˙871 tons, Mexico 257˙940 tons, Uganda 314˙489 tons, Honduras 380˙296 tons, India 385˙786 tons, Ethiopia 423˙287 tons, Indonesia 814˙629 tons, Colombia 892˙871 tons, Vietnam 1˙818˙811 tons, and Brazil 2˙859˙502 tons (International Coffee Organization, 2016). These production data all show an increase ranging from 10 to 12% in comparison with the yield obtained in 2015. However, in terms of exports, the ranking changes. According to the latest report (International Coffee Organization, 2016), Brazil remains in first place with 1˙708˙700 tons of coffee exported every year, followed by Vietnam (1˙147˙500 tons/y), Colombia (601˙860 tons/y), India (300˙360 tons/y), Indonesia (290˙820 tons/y), Honduras (284˙760 tons/y), Uganda (169˙020 tons/y), Ethiopia (150˙840 tons/y), Guatemala (145˙920 tons/y), Peru (136˙800 tons/y). Europe, USA, and Japan are the main coffee importing countries with 3˙140˙400, 1˙125˙960, and 319˙980 tons/y, respectively.

In line with the increasing production of coffee, consumption of the beverage, and consequently the amount of spent coffee grounds (SCGs) remaining as a solid residue from coffee brewing, are on an upwards trend. Murthy and Naidu (2012) reported that for every ton of green coffee beans, 650 kg of

residues remain as SCG. As assessed by Obruca et al. (2015) the composition of SCGs is made up of hemicellulose (30-40 ww%), lignin (25-33 ww%), oil (10-20 ww%), cellulose (8.6-13.3 ww%), proteins (6.7-13.6 ww%), and polyphenols (2.5 ww%). Approximately 5˙817˙500 tons of SCGs are generated worldwide every year as a municipal solid waste (Park et al., 2016). These residues are of no economic value (Al-Dhabi et al., 2017) and are usually discarded without further valorisation. In view of the high content of biodegradable compounds, if disposed, SCGs will certainly require biostabilization, although they should preferably be exploited in a biorefinery chain scheme. On identifying a potentially profitable use for energy and goods produced from any waste source, investments should focus increasingly on alternative biorefinery options rather than on waste disposal. In the context of a biorefinery concept applicable to food waste in general (Girotto et al., 2015), SCGs feature an incredibly wide range of potential applications. In some cases, due to the high fibre and polyphenol content of these residues, they are utilized in the food industry (Lopez-Barrera et al., 2016; Bravo et al., 2013) or the pharmaceutical sector (Lopez-Barrera et al., 2016; Fki et al., 2005).

Worldwide, the major drivers of a bioenergy approach are represented by an enhanced supply of renewable energy and mitigation of climate change. New sources of sustainable and green energy are needed to reduce the disproportionate use of common fossil fuels or substitute for these. Over the last decade, numerous studies have been performed with the aim of valorising SCGs as a raw substrate for use in the production of ethanol (Sampaio et al., 2013; Machado et al., 2000), bio-sorbents (Franca et al., 2009; Nakamura et al., 2003; Hirata et al., 2002; Rufford et al., 2008; Jung et al., 2016), biodiesel (Murthy and Naidu, 2012; Park et al., 2016; Burton et al., 2010; Kondamudi et al., 2008), pyrolysis oil (Couto et al., 2009; Bok et al., 2012; Li et al., 2014; Yang et al., 2016), and polyhydroxyalcanoates (Obruca et al., 2015; Al-Hamamre et al., 2012; Cruz et al., 2014; Pan et al., 2012) for bio-plastic production.

Studies focused on investigating energy recovery from SCG by means of anaerobic digestion (AD) were first set up in 1983 (Lane, 1983), although no in-depth assessments of optimal operating conditions were carried out.

The aim of this study is to provide an updated overview of the series of possibilities available to promote the exploitation of SCGs as a valuable resource for energy and product recovery. A comparison of the energy obtainable via different routes is provided. Moreover, the results of an original batch scale evaluation of the best AD conditions, in terms of substrate to inoculum (S/I) ratio, to enhance recovery of bio-methane from SCGs under mesophilic conditions are illustrated. The Authors tested different S/I ratios, namely 0.5, 1, and 2.

4.2.2 ALTERNATIVE BIOREFINERY OPTIONS FOR SCGS: STATE OF THE ART

Innovative solutions for the recycling of SCGs within a circular economy approach are summarized in Figure 4.2.1. The introduction of cutting-edge management solutions for these huge amounts of waste has contributed towards significantly reducing the amount of materials to be returned to the environment either as soil amendment or in a non-mobile form in artefacts (Back to Earth Alternatives, BEA).

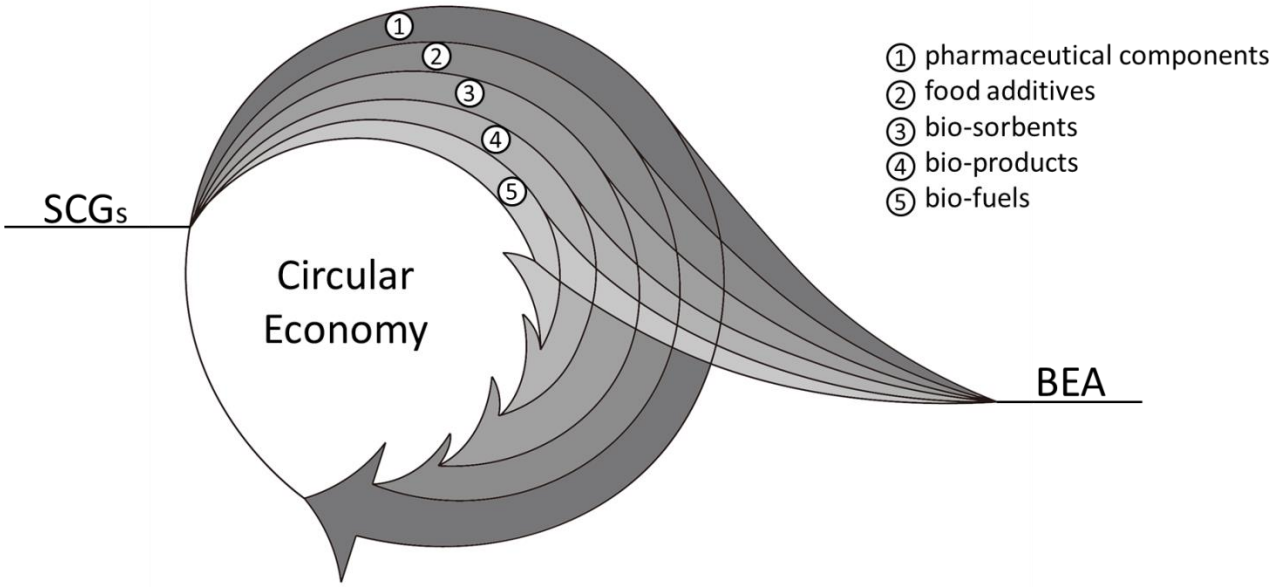

Figure 4.2.1: Alternative biorefinery options for SCGs within the circular economy.

Food and feed products

Spent coffee grounds constitute an excellent substrate for mushroom production, requiring no treatment prior to start-up of cultivation. Accordingly, several studies have cultivated a series of different types of edible fungi, including *L. edodes*, *Pleurotus spp*., and *Flammulina velutipes*, with a biological efficiency of up to 88.6 % (Murthy and Naidu, 2012; Leifa et al., 2001; Pushpa and Manonmani, 2008) signifying that each kg of dry SCGs used as a substrate resulted in the growth of nearly 0.9 kg of mushrooms.

Although featuring a high lignin content (Obruca et al., 2015; Cruz, 1983; Mussatto et al., 2011), SCGs have been investigated for potential use as animal feed. Claude (1979) and Givens and Barber (1986) demonstrated the suitability of SCGs as a source of nutrition for ruminants, pigs, chickens, and rabbits. However, the presence of polyphenols, caffeine and other substances in SCGs severely limits their application as animal feed (Obruca et al., 2015).

SCGs represent an excellent source of bioactive, particularly phenolic, compounds (Ramalakshmi et al., 2009), known to exert beneficial effects on human health due to their antioxidant properties (Lopez-barrera et al., 2016; Fki et al., 2005). Moreover, the high amount of total fibre contained in SCGs (80%) (Murthy and Naidu, 2012) has resulted in an increasing interest on the identification of alternative

options for the reuse of this residue in the food industry. Bravo et al. (2013) demonstrated the feasibility of exploiting SCGs as a food ingredient or additive with potential preservation and functional properties (Bravo et al., 2013). Subsequent to coffee brewing, the remaining grounds are suitable for use as a source of natural antioxidants, nutraceuticals, and preservatives in food formulations (Murthy and Naidu, 2012). López-Barrera et al. (2016) reported how dietary fibres contained in SCGs are fermented by colon microbiota producing short-chain fatty acids (SCFAs) capable of preventing inflammation. Further, due to their chemical composition, SCGs are a rich source of polysaccharides; indeed, several studies (Mussatto et al., 2011; Passos and Coimbra, 2013; Passos et al., 2014; Simões et al., 2014) have been undertaken to assess the extraction yield of galactomannans and arabinogalactans known for their immunostimulatory properties.

SCGs may even be applied as a starter substrate in the production of distilled beverages. Sampaio et al. (2013) produced liquor from SCGs, the organoleptic quality of which was considered acceptable for human consumption. This was achieved by three main steps, namely hydrothermal extraction, fermentation, and distillation. After being subjected to acid hydrolysis, SCGs hydrolysate may be used as a fermentation medium by *Saccharomyces cerevisiae* yeast and yield a 50.1 ww% ethanol production (Machado et al., 2000).

Bio-sorbents and energy storage

In addition to the exceptional properties associated with the use of SCGs as a mushroom-growing substrate, animal feed, and as food compounds, other effective re-use opportunities for SCGs have been investigated.

These studies have found that SCGs may be used as an inexpensive and easily available adsorbent for the removal of cationic dyes from aqueous solutions (Franca and Oliveira, 2009; Nakamura et al., 2003). Accordingly, SCGs can be applied efficiently in wastewater treatment units. Hirata et al. (2002) applied a microwave treatment to SCGs with the aim of obtaining carbonaceous materials to be used as adsorbates for the removal of basic dyes in wastewater (Hirata et al., 2002). Namane et al. (2005) treated SCGs with $ZnCl_2$ to produce activated carbon. The newest form of biomass-based granular activated carbon was successfully prepared by entrapping granular activated carbon (GAC) powder derived from spent coffee grounds into calcium-alginate beads (SCG-GAC) (Jung et al., 2016). Regeneration tests further confirmed that SCG-GAC has a promising reuse potential, showing a dye removal efficiency of more than 80% (expressed as percent ratio of removed dye concentrations to their initial concentrations) and an adsorption capacity up to 57 mg/g even after seven consecutive cycles (Jung et al., 2016).

Thomas et al. highlighted the potential of using SCGs in the production of electrode materials for cost effective energy storage systems. Supercapacitor electrodes prepared from coffee ground carbon displayed excellent stability with high charge–discharge rates (Rufford et al., 2008).

Bioplastic

The relatively high acid value (caused by the presence of free fatty acids) exhibited by SCG oil, although complicating transesterification during biodiesel production (Al-Hamamre et al., 2012), significantly stimulates the accumulation of polyhydroxyalcanoates (PHA) in the cytoplasm of microorganisms during batch scale experiments ((Obruca et al., 2015; Cruz et al., 2014). Oil extraction from SCG by means of n-hexane (Al-Hamamre et al., 2012) or supercritical carbon dioxide (Cruz et al., 2014) yields up to 12% on a dry weight basis. The conversion rate of SCGs into PHA ranges between 8 and 20% (ww). Cruz et al. (2014) succeeded in obtaining 97 kg of PHA from 1 ton of SCGs (Cruz et al., 2014), while Obruca et al. (2015) reached a yield of 14% (ww), both employing *Cuprividus Necator* as PHA cumulating bacteria. A limiting factor in the production of PHA from SCGs is represented by the presence of polyphenols (Pan et al., 2012), due to their inhibitory effect on the growth of some microorganisms.

Bio-fuels

SCGs have a high calorific power of approximately 24.9 MJ/kg (dw), thus representing an excellent substrate to be fed into industrial boilers (Silva et al., 1998). A few industries have attempted to exploit SCGs for the generation of heating and electricity (Yang et al., 2016); however, combustion of these wastes resulted in the generation of particulate matter and hazardous gases, particularly high nitrogen oxidants, thereby dramatically limiting the direct use of SCGs as solid fuels (Sprules, 1999;Limousy et al., 2013).

Recent interest has focused on the use of SCGs in the production of liquid biofuel (Yang et al., 2016) such as bioethanol, biodiesel, and pyrolysis oil. A comparison of the amount of energy obtainable from SCGs via different routes is shown in Figure 4.2.2.

The enzymatic rate of conversion of SCGs to fermentable sugars is around 85 dw% (Choi et al., 2012). Pressure applied in the pretreatment step is fundamental in producing swelling and degradation of the SCG cell wall, which improves subsequent enzymatic hydrolysis and fermentation by increasing the surface area of SCGs, and making it more accessible to hydrolytic enzymes. Under optimal popping pretreatment conditions of 1.47 MPa and 18.3 mgCellulase/gSCGs, the ethanol concentration and yields (based on sugar content) obtained by means of enzymatic hydrolysis subsequent to simultaneous saccharification and fermentation were 15.3 g/L and 87.2%, respectively (Choi et al., 2012).

Conversely, the oil extracted from SCGs may be transesterified for use in biodiesel production (Murthy and Naidu, 2012; Park et al., 2016; Burton et al., 2010; Kondamudi et al., 2008). The conversion of oil into biodiesel is nearly complete, with Burton et al. reaching a biodiesel production yield of 98.5% (Burton et al., 2010). Nonetheless, the oil content of SCGs is quite low, ranging between 10 and 20%

((Park et al., 2016; Burton et al., 2010; Kondamudi et al., 2008) depending on the coffee species (Arabica or Robusta), thus highlighting the scarce economic feasibility of extraction.

Accordingly, the practicability of using supercritical fluid extraction processes to obtain lipid fraction from SCGs has also been investigated (Couto et al., 2009; de Melo et al., 2014), together with a combination of ultrasonication (Abdullah and Koc, 2013) and conventional solvent extraction.

The conversion of SCGs into pyrolysis oil however, produced a higher oil yield (Bok et al., 2012; Li et al., 2014; Yang et al., 2016), ranging from 55% to 85% of wet mass depending on the moisture content of the feedstock. Reaction temperature and moisture content of the feedstock are the most important variables in fast pyrolysis of SCGs. Bok et al. (2012) obtained the highest yield of bio-crude oil (55%) at 550 °C (Bok et al., 2012), while Li et al. (2014) recorded maximum liquid yield of 66% at around 630 °C (Li et al., 2014). Unfortunately, SCGs feature a moisture content of between 50 and 60% mass fraction (Kondamudi et al., 2008), therefore a pre-drying process should be applied prior to feeding SCGs into a pyrolysis system (Yang et al., 2016), with consequent negative economic consequences. A low temperature conversion pyrolysis process was also applied (Romeiro et al., 2012) to a sample of SCGs at 380 °C, although a lower pyrolysis oil yield, approximately 50% mass fraction (Romeiro et al., 2012), was achieved compared to regular pyrolysis process.

For the above reasons, the emerging technology of hydrothermal liquefaction (HTL) has also been applied to SCGs with the aim of producing bio-oil. Optimal liquefaction conditions were assessed as 275 °C, retention time of 10 min and water/feedstock mass ratio of 20:1 (Yang et al., 2016). The highest crude bio-oil yield of 47.3% mass fraction was achieved with a higher heating value of 31.0 MJ/kg (much higher than that of SCGs, which was only 24.9 MJ/kg) and a consequent energy recovery percentage of 72.6% (Yang et al., 2016).

Another innovative approach is represented by biogas production (Neves et al., 2006). The biogas obtained could be used for numerous purposes, including the roasting of coffee grounds, thus closing the loop of the coffee production unit.

To date, very few studies have been conducted to investigate potential bio-methane production from SCGs.

Lane (1983) evaluated methane production using SCGs alone, reporting a biogas yield of 540 m^3/tVS (56–63% methane) (Lane, 1983).

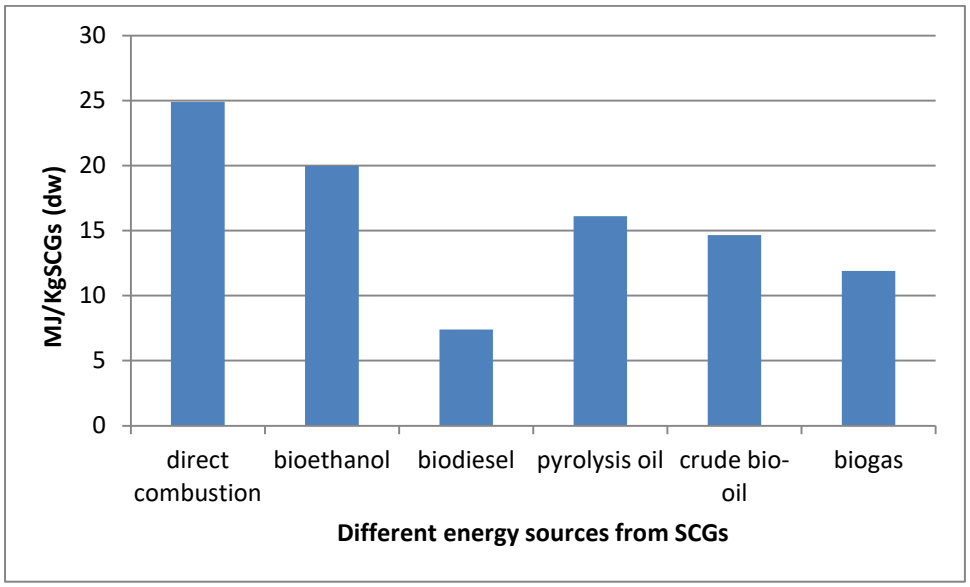

Figure 4.2.2: Energy obtainable from SCGs via different routes.

4.2.3 BIO-METHANE POTENTIAL TESTING ON SCGs

On the scenario of the diverse bio-refinery approaches currently available, the promising option of biogas production has not yet been investigated in depth. The Authors therefore decided to assess the potential for bio-methane production of SCGs using different substrate to inoculum (S/I) ratios to clarify optimal conditions for anaerobic digestion (AD) and the feasibility of applying the AD process to this specific kind of waste when processed alone.

Materials

SCGs were collected after the brewing of coffee using a moka coffee pot from the Environmental Engineering Laboratory of Padova University. SCGs were tested for TS and VS content (AOCS,1997), which were found to be 37 ww% and 36.5 ww%, respectively. VS/TS ratio was 0.99. Elemental analysis (C, H, N, and S) was determined using an elemental analyzer (Vario MACRO CNS, Hanau, Germany).

Table 4.2.1: Chemical characteristics and final analysis of SCGs used in this study.

Parameter	**Spent Coffee Grounds**
TS (ww%)	37
VS (ww%)	36.5
pH	6.3
C (dw %)	58.8
H (dw%)	8.9
O (dw %)	28.7
N (dw %)	3.4
S (dw %)	0.2
Fibre composition	
Cellulose (dw%)	24.3
Hemicellulose (dw%)	24.8
Lignin (dw%)	13.5

Oxygen content was calculated by difference. SCGs were also analysed in terms of hemicellulose, cellulose, and lignin content following the crude fibre procedure of APHA et al. (1999). Final analyses are illustrated in Table 4.2.1.

Granular sludge (5.2 gVS/L) from a full-scale Upflow Anaerobic Sludge Blanket (UASB) digester of a brewery factory located in Padova, Italy was used as inoculum.

Method

Lab scale tests were performed to evaluate the Biochemical Methane Potential (BMP) of SCG following anaerobic digestion. Tests were carried out in 1-Litre batch reactors under mesophilic conditions (35 ± 1 °C) (see Figure 4.2.3a). In each reactor substrate concentration was kept constant at 10 gVS/L while the amount of inoculum was changed according to the desired S/I ratio. After water addition, the total liquid volume in the reactors was 500 mL. Reactors were hermetically closed by means of a silicon plug enabling sampling of the gas and liquid produced during fermentation. The three different investigated ratios between the volatile solids of the substrate to be degraded and volatile solids of the inoculum biomass (S/I) were 0.5; 1; 2 gVS/gVS. After preparation, the reactors were flushed with N_2 gas for 3 minutes and incubated under static conditions in a thermostatic chamber. Blank tests using the inoculum alone were also prepared to measure the quantity of methane produced only by the biomass. All tests were performed in triplicate.

The biogas volume produced during BMP tests was measured by means of the water displacement method (see Figure 4.2.3b). The produced gas composition in terms of CH_4 and CO_2 was analysed using a portable gas analyzer (LFG 20-ADC, Gas Analysis Ltd). Methane volumes produced in the time interval between each measurement [t – (t-1)] during BMP tests, were calculated using a model, taking into consideration the gas concentration at time t and time t-1, together with 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 reactors (Van Ginkel et al., 2005). The following equation (see section 1.1) was applied:

$$V_{C,t} = C_{C,t} * V_{G,t} + V_H * (C_{C,t} - C_{C,t-1})$$

Data on methane production are expressed at a temperature of 0 °C and pressure of 1 atm (Normal conditions).

Figure 4.2.3: Lab tests experimental equipment. Mesophilic water bath containing the BMP bottles (a) and manual measurement of the biogas produces through water displacement (b).

Results and discussion

Biogas production reached a plateau after approximately 20 days.

No differences were detected between biogas and methane productions using the ratios S/I=0.5 and S/I=1 (Figure 4.2.4).

Compared to the methane yield reached at a S/I ratio of 0.5, a 23% increase was obtained using a substrate to inoculum ratio of 2. Maximum productions of biogas and methane obtained were 0.56 m^3/kgVS and 0.36 m^3/kgVS, respectively, considering the sole volatile solids of the substrate. These results are in agreement with the outputs published by Lane (1983). A 64% concentration of methane within biogas highlights the good quality of the latter. AD efficiency was also evaluated in terms of VS reduction. An increased biostability of the digestate was noticed when setting the S/I ratio at 2 in correspondence of which VS degradation (32.6 ± 1.0%) was 8% and 10% higher than the ones obtained in the other tests with S/I of 0.5 and 1, respectively, which is in agreement with the highest biogas production at this condition (S/I=2).

Therefore, when dealing with SCGs, a S/I of 2 improved the performances of the AD treatment both in terms of energy recovery and final by-product biostability. Digestate could be promptly turned into a safe soil amending material without long and energy consuming treatment. Being hemicellulose and lignin, components of SCGs, resistant to enzymatic hydrolysis (Kong et al., 1992), a substrate pre-treatment can be effective in enhancing the already high methane production yield.

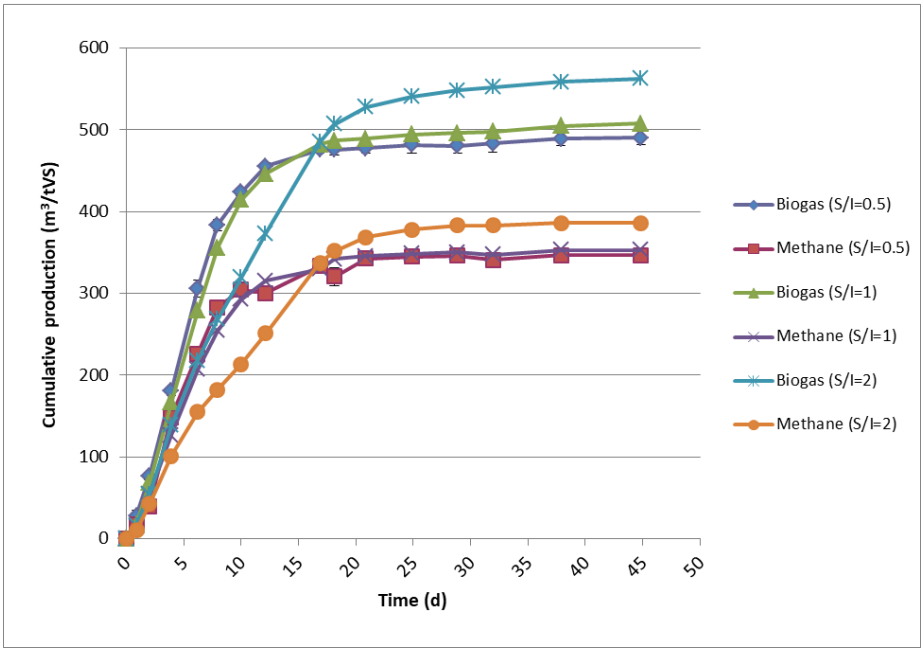

Figure 4.2.4: Comparison between the three cumulative biogas and methane productions with S/I ratios of 0.5, 1, and 2.

4.2.4 CONCLUSIONS

Despite the potential options available for the exploiting of SCGs as a valuable resource in the form of food additives, pharmaceutical components, bio-sorbents, bio-products, and bio-fuels, to date scarce emphasis has been placed on these alternatives.

On the scenario of the diverse biorefinery approach currently available, the Authors chose to assess the potential for bio-methane production of SCGs using different S/I ratios to clarify optimal conditions for anaerobic digestion, which remain to be fully investigated.

The highest bio-methane potential (360 m^3CH_4/tVS) was obtained with a S/I ratio of 2, a remarkable yield compared to those obtained for other digested substrates, and which may justify the source segregation of SCGs when produced. The construction of a small-scale anaerobic biodigestor may constitute an innovative means of raising awareness into food waste management issues and the need for new sources of energy. If small-scale AD reactors were installed on the site of large cafes or restaurants, the energy recovered could be utilized by customers to charge their mobiles or supply power for lighting, TVs, or radios. This would undoubtedly favourably impress and attract the attention of the public, potentially acting as an effective campaign to promote renewable energies within the biorefinery concept.

4.3 SPENT COFFEE GROUNDS ALKALINE PRE-TREATMENT AS BIOREFINERY OPTION TO ENHANCE THEIR ANAEROBIC DIGESTION YIELD

Authors: Francesca Girotto, Maria Cristina Lavagnolo, Alberto Pivato, Raffaello Cossu

Spent coffee grounds (SCGs) are potentially optimal substrates for methane production but the content of organic compounds refractory to anaerobic digestion reduces the yield of the process. Alkaline pre-treatment was applied to enhance the methane recovery from SCGs through anaerobic digestion. NaOH was applied with different loadings, namely 2%, 4%, 6%, 8% w/w for 24 h, to assess the efficiency of the process and the optimal amount of the basifying solution applied. The highest concentration of NaOH (8% w/w) led to the best anaerobic digestion performances (392 m^3CH_4/tVS) as a consequence of the slightly higher lignin degradation which was 24% higher than that of the untreated substrate, and of the higher DOC concentration.

4.3.1 INTRODUCTION

Agro-industrial sector of coffee production is growing in parallel with the increasing demand for the drink obtained at the end of the food production chain. Consequently, the amount of spent coffee grounds (SCGs) remaining as a solid residue from the brewing of the coffee powder, is increasing (Girotto et al., 2017). Park et al. (2016) reported that about 5˙817˙500 tons of SCGs are generated worldwide every year as a municipal solid waste. Developing practical solutions based on the concepts of the circular economy is the big challenge that in the last decade motivated many researches to find appropriate ways to exploit SCGs as a precious feedstock. Because of their composition consisting in 30-40 %w/w hemicellulose, 25-33 %w/w lignin, 10-20 %w/w oil, 8.6-13.3 %w/w cellulose, 6.7-13.6 %w/w proteins, and 2.5 %w/w polyphenols (Obruca et al., 2015) the biorefinery options are many. The demonstration of the huge versatility of this substrate and the combination of the several SCGs valorisation alternatives can be easily found in literature where data related to the use of SCGs as a raw substrate for the production of ethanol (Sampaio et al., 2013; Machado et al., 2000), bio-sorbents (Franca et al., 2009; Nakamura et al., 2009; Hirata et al., 2002; Thomas et al., 2008; Jung, 2016), biodiesel (Burton et al., 2010; Kondamudi et al., 2008; Park et al., 2016; Murthy and Naidu, 2012), pyrolysis oil (Couto et al., 2009; Bok et al., 2012; Li et al., 2014; Yang et al., 2016), and polyhydroxyalcanoates (Obruca et al., 2015; Al-Hamamre et al., 2012; Cruz et al., 2014; Pan et al., 2012) are plenty.

Lane (1989) made the first intent to prove the possibility of recovering energy from SCGs through anaerobic digestion (AD) and a deeper investigation by Girotto et al. (2017) confirmed the high methane yield of this substrate, 360 m^3/tVS. Being a promising substrate to be digested alone or together with other types of organic waste streams, the Authors decided to investigate the

possibility to increase SCGs bio-energy potential through pre-treatment. Hemicellulose and lignin, the main components of SCGs, are resistant to enzymatic hydrolysis due to their structure and composition. Other two not-readily biodegradable components are proteins (6.7-13.6 %w/w) and oil (10-20 %w/w) (Obruca et al., 2015). Alkaline pre-treatment, used successfully for organic substrates such as food waste (Menon et al., 2016), is one of the best performing pre-treatments compared to acidic, oxidative, steam explosion, and thermo-chemical ones (Milella et al., 2002) when applied to lignocellulosic biomass. Alkali addition causes swelling of lignocelluloses (Kong et al., 1992) and partial lignin solubilisation, moreover it can help the neutralization of acidic compounds produced during degradation (Pavlostathis and Gossett, 1985; Hendriks and Zeeman, 2009). Compared with acid or oxidative reagents, alkali pre-treatment appears to be the most effective method in breaking the ester bonds between lignin, hemicellulose, and cellulose (Taherzadeh and Karimi, 2008; Gaspar et al., 2007). Sodium hydroxide (NaOH) has been proved as a good pre-treatment to improve AD performances in the treatment of recalcitrant substrates such as corn stover (Zhu et al., 2010; Zheng et al., 2009) and olive pomace (Battista et al., 2016; Pellera et al., 2016) at mesophilic conditions. Comparing the effects of NaOH, KOH, and lime on rice straw, Yang et al. (2009) assessed that the strongest lignin breakdown and the highest methane yield was related to the use of NaOH.

The aim of this study was to assess the effects of alkaline treatment prior AD of SCGs and to measure the appropriate NaOH dosage, between 2% and 8% (w/w), to be mixed with SCGs in order to furtherly enhance methane production. Batch anaerobic digestion tests were performed using the untreated and the pre-treated investigated substrate and outputs in terms of substrate solubilisation, methane yield, and digestate quality were compared.

4.3.2 MATERIALS AND METHOD

Substrate

SCGs were collected from the Environmental Engineering Laboratory of Padova University. Brewed coffee powder was 100% Arabica. Their chemical composition is shown in Table 4.3.1.

Granular sludge (5.2 gVS/L) collected from a full-scale Upflow Anaerobic Sludge Blanket (UASB) digester of a brewery factory located in Padova, Italy, was used as inoculum.

NaOH pre-treatment

In order to perform alkaline pre-treatment, four samples containing 100 g of SCGs were mixed evenly with 100 mL NaOH solution with different molarities. The corresponding NaOH loadings over the substrate solids were 2%, 4%, 6%, and 8% (w/w), respectively. Samples were named as C, T, F, S, and E accordingly to the alkali addition percentage which was 0, 2, 4, 6, and 8%,

respectively. The NaOH soaked SCGs were kept at constant ambient temperature (20 ± 0.5 °C) in 250 mL glass bottles for 24 h. At the end of the pre-treatment, samples were taken for compositional analysis before anaerobic digestion tests.

Total solids (TS), volatile solids (VS), pH, total carbon (TC), and total nitrogen (TN) were analysed according to standard methods (APHA, 1999); hemicellulose, cellulose, and lignin content were evaluated following the crude fibre procedure of AOCS (1997). In order to better evaluate the action of NaOH addition in terms of cell walls breakdown, dissolved organic carbon (DOC) values were analysed by a TOC analyser (TOC-V CSN, Shimadzu) after filtration of liquid pre-treated samples at 0.2 μm.

Table 4.3.1: Characteristics of spent coffee grounds used in the experiment.

Parameter	**%**
Total solids (TS) (wb)	37
Volatile solids (VS) (wb)	36.7
Total carbon (TC) (db)	57.9
Total nitrogen (TN) (db)	2.9
Cellulose (db)	24.3
Hemicellulose (db)	24.8
Lignin (db)	13.5

Note – wb: wet basis; db: dry basis

Anaerobic digestion tests

Lab scale biomethane potential tests (BMP) were performed on SCGs with and without pre-treatment to compare their methane yields and the process efficiency in terms of digestate quality. Tests were carried out in 500 mL batch reactors under mesophilic conditions (35 ± 1 °C). Reactors were hermetically closed using silicon plugs. The liquid volume in each reactor, consisting of substrate plus inoculum, was 250 mL. Substrate to inoculum ratio (S/I) was fixed at 2 gVS/gVS (Girotto el al., 2017) and substrate concentration was 8 gVS/L inside each bottle. After preparation, the reactors were flushed with N_2 gas for 3 min and incubated under static conditions in a thermostatic chamber. Blank tests using the inoculum alone were also prepared to measure the quantity of methane produced only by the biomass.

The volume of biogas produced during the BMP tests was measured by means of the water displacement method (Alibardi et al., 2012). The produced gas composition in terms of CH_4 and CO_2 was analysed using a portable gas analyser (LFG 20-ADC, Gas Analysis Ltd). Methane volumes produced in the time interval between each measurement [t – (t-1)] during BMP tests, were calculated using a model taking into consideration the gas concentration at time t and time t-1,

together with 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 reactors (Van Ginkel et al., 2005). The following equation (see section 1.1) was applied:

$V_{C,t} = C_{C,t} * V_{G,t} + V_H * (C_{C,t} – C_{C,t-1})$

All tests were performed in triplicate. Data on methane productions are expressed at a temperature of 0 °C and pressure of 1 atm (Normal conditions).

Statistical analysis

In order to verify whether statistical differences could be observed as an effect of the NaOH pre-treatment with 95% of confidence level, one-way ANOVA F-test together with a Tukey pairwise comparison on the cumulative methane yields after 10, 20, and 40 days of BMP test were performed. Both analyses were run with the Statgraphics Centurion XVII software program (Wilkinson, 1992).

4.3.3 RESULTS AND DISCUSSION

Pre-treatment

Table 4.3.2 shows the change in the SCGs fibres components due to the 24 h pre-treatment together with the DOC content. An increased NaOH concentration resulted in a slight increased lignin removal. The highest hemicellulose degradation was instead obtained in sample S (6% NaOH). Cellulose degradation did not reach high values probably because of its structural characteristics, being surrounded, and therefore protected, by a lignin and hemicellulose wall. These results are in accordance to those reported by Zhu et al. (2010) who reached the highest lignin degradation (about 46%) when pre-treating corn stover with 7.5% NaOH. However, when using the same substrate, Zheng et al. (2009) reported a 2% NaOH loading as best for corn stover alkaline pre-treatment. Alkaline pre-treatment of olive mill solid waste also resulted to give the best results when performed with the lowest amount of NaOH tested (Pellera et al., 2016). Because of the small particle size of SCGs, the evidence of a substantial variation in fibre composition did not result to be so evident compared to other studies dealing with lignocellulosic biomass characterized by wider sizes like corn stover or olive pomace (Zhu et al., 2010; Pellera et al., 2016).

DOC, normally increasing during the first step of anaerobic digestion, the hydrolysis, can also be released from broken cell walls after a pre-treatment such as thermal or alkaline. It provides a substrate for microorganisms growth and it is correlated with the biogas potential of the feedstock (Nagler et al., 2016).

In this research it came out that the DOC highly increased in parallel with the increased NaOH loading. In particular sample T showed a 165% increase in DOC with respect to the sample without

NaOH addition, while sample E, characterized by the highest NaOH content, showed a 725% increase. This solubilisation trends linearly correlated (see Figure 4.3.1) to the NaOH loading is in agreement with what reported in other studies (Pellera et al., 2016; Li et al., 2012; Vlyssides and Karlis, 2004 ; Xie et al., 2011), where it was explained that increased concentrations of solubilized organic matter are due to the disruption of chemical bonds within the solid matrix. NaOH contributes to hydrolysis of complex organic matter leading to its transfer from the solid to the liquid phase (López Torres and Espinosa Lloréns, 2008; Pellera et al., 2016). Being fibres composition values after pre-treatment not so significantly far from those of the untreated SCGs, DOC is the most interesting parameter that effectively enables to recognize the strong action of NaOH, probably over proteins and oil fractions, in increasing the bioavailability of SCGs for the bacteria acting in the following step of anaerobic digestion.

Table 4.3.2: Effect of 24h alkaline pre-treatment on spent coffee grounds fibres composition.

Sample	NaOH (%db)	Cellulose (%db)	Hemicellulose (%db)	Lignin (%db)	DOC (mgC/L)
C	**0**	24.3 ± 1.6	24.8 ± 1.8	13.5 ± 0.9	343.5
T	**2**	24.2 ± 1.2	24.5 ± 0.9	13.6 ± 0.8	910.0
F	**4**	24.2 ± 1.2	24.1 ± 1.1	12.9 ± 1.1	1745.0
S	**6**	23.9 ± 1.3	23.2 ± 1.2	12.5 ± 1.2	2665.0
E	**8**	23.9 ± 0.9	23.6 ± 0.8	12.0 ± 1.1	2850.0

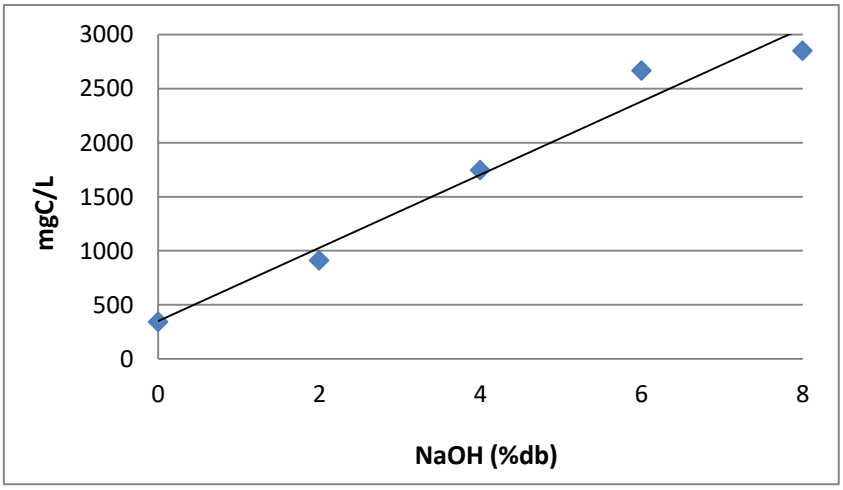

Figure 4.3.1: Linear correlation between DOC concentration and NaOH loading after 24h alkaline pre-treatment.

Anaerobic digestion

BMP tests were performed directly after the 24 h pre-treatment, without any substrate washing or pH adjustment. In fact, an interesting outcome of the experiment is related to the study of pH immediately after alkali addition and at the end of the pre-treatment (Table 4.3.3). SCGs resulted to

be a substrate with a initial slightly acid pH and a good buffer capacity. After the 24 h alkaline pre-treatment, pH decreased from a value much higher than 12 up to a minimum of 8.8 for sample T. After the inoculum (whose pH is 8) addition, there was no need for acid addition because the highest pH of 8.5 (sample E) was still within the acceptable range for anaerobic microorganisms. A pH range of 6.3-7.8 has been reported as optimal for the growth of methanogens (Pan et al., 2008). However, the process can tolerate a slightly wider range from 6 up to 8.5 (Saedi et al., 2008).

Table 4.3.3: Change of pH at the beginning (Alk_i) and at the end (Alk_f) of alkaline pre-treatment, and before (AD_i) and after (AD_f) anaerobic digestion tests (subsequently to inoculum addition).

Sample	% NaOH	pH			
		Alk_i	Alk_f	AD_i	AD_f
C	**0**	6.7 ± 0.0	6.7 ± 0.0	8 ± 0.1	8 ± 0.1
T	**2**	> 12	8.8 ± 0.1	8.1 ± 0.0	7.9 ± 0.0
F	**4**	> 12	10.3 ± 0.1	8.3 ± 0.1	8 ± 0.0
S	**6**	> 12	11.3 ± 0.2	8.3 ± 0.2	7.9 ± 0.1
E	**8**	> 12	12 ± 0.1	8.5 ± 0.1	8 ± 0.1

Anaerobic degradation resulted not to be inhibited for any of the pre-treated substrates. On the contrary, from Figure 4.3.2 it is possible to see a very general sharp increase in the methane production, especially for samples S and E characterized by the highest NaOH dosages. This might be due to the presence of a lot of promptly biodegradable organic matter in the substrate and, probably, also to the very broad contact surface area of SCGs which are therefore easily attacked by microorganisms. Comparing the untreated SCGs' methane yield (316 m^3CH_4/tVS) with the others, a maximum 24% increase was observed from sample E (8% NaOH). Accordingly to the increasing lignin degradation in parallel with the increasing amount of NaOH as seen in Table 4.3.2, the methane production yields grew from sample C to E. For 2% NaOH pre-treated SCGs, methane production was nearly equal to that of the untreated substrate. Alkaline pre-treatment lead to an increased organic carbon dissolution which favoured hydrolysis resulting in an easier and more efficient anaerobic digestion of SCGs. These results are in line with the improvements reported by Lopez-Torres and Llorens (2008) showing a 11.5% increase in the methane yield of the organic fraction of municipal solid waste by applying alkaline agents as a pre-treatment. Furthermore, Neves et al. (2006) reported improvements of 100% by applying NaOH as alkaline agent pretreating barley waste.

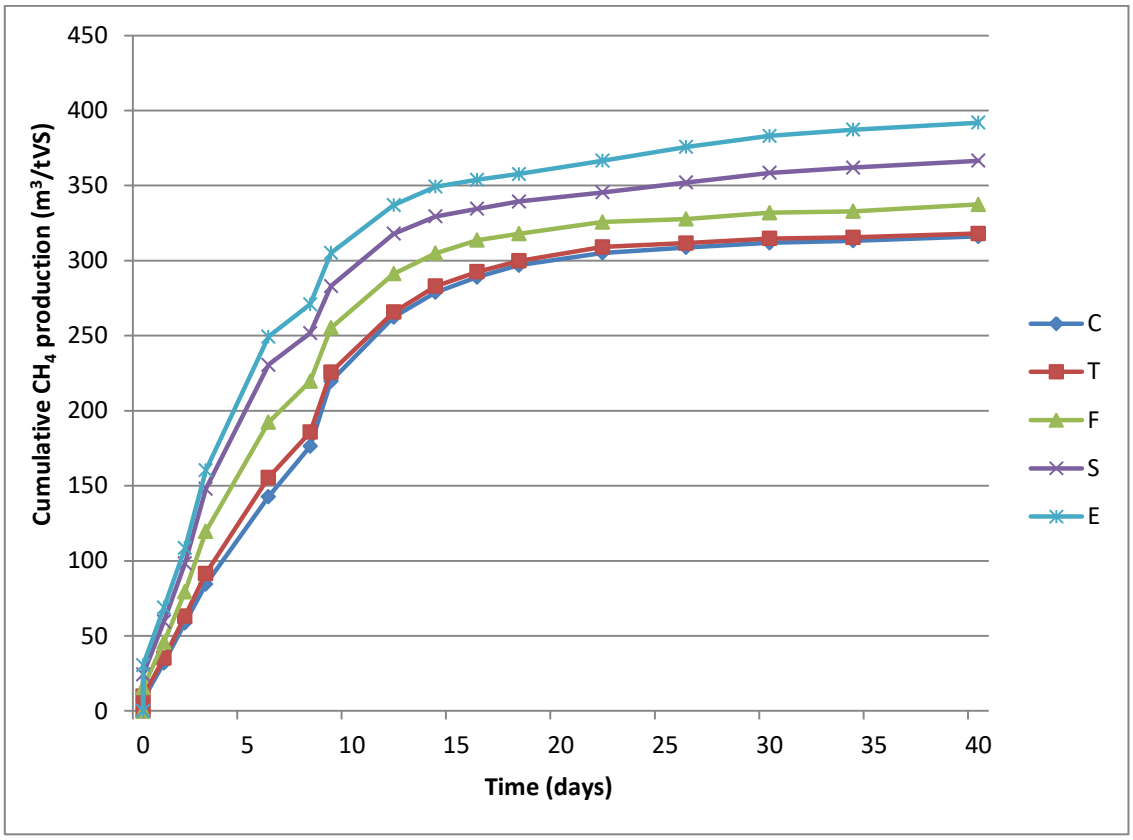

Figure 4.3.2: Cumulative methane production from SCGs samples pre-treated with different NaOH dosages: C (0% NaOH); T (2% NaOH); F (4% NaOH); S (6% NaOH); E (8% NaOH)

The efficiency of the whole SCGs treatment was also evaluated testing the TS and VS reduction after the digestion process of the substrate with and without alkali addition. As shown in Table 4.3.4 the TS and VS reduction increased in parallel with the increase in NaOH loading even if the highest VS reduction (36.2%) was obtained in sample S (6% NaOH).

Table 4.3.4: Biochemical methane potential and degradation of the substrate after 40 days anaerobic digestion.

Sample	% NaOH	m^3CH_4/tVS	Degradation (%)	
			TS*	VS*
C	**0**	316 ± 6.6	23.1 ± 0.5	32.6 ± 1.0
T	**2**	318 ± 1.8	25.5 ± 0.4	34.1 ± 0.9
F	**4**	337 ± 11.8	25.4 ± 0.4	34.5 ± 1.0
S	**6**	367 ± 12.4	26.7 ± 0.2	36.2 ± 0.8
E	**8**	392 ± 3.0	26.9 ± 0.3	35.9 ± 0.8

*****These values were obtained keeping into consideration the inoculum presence inside the reactors.*

Alkaline pre-treatment was not only successful in increasing the methane production yield during AD of SCGs but it also enabled to reach higher digestate stability.

Statistical analysis

The one-way ANOVA F-test showed significant differences ($P < 0.05$) among the treatments after 10, 20, and 40 days of anaerobic digestion batch tests. Unlike the results reported by Janke et al. (2017), the Tukey's pairwise comparisons after 10, 20, and 40 days of experiment (Figure 4.3.3) did not show a strong effect of NaOH pre-treatment on process acceleration, since the number of groups statistically different to each other was 3 at each step of the comparison. However, samples S and E were constantly showing a significantly higher methane production with respect to samples C and T. With 95% confidence level, it can be stated that NaOH pre-treatment led to an increased total methane potential during the anaerobic digestion of spent coffee grounds.

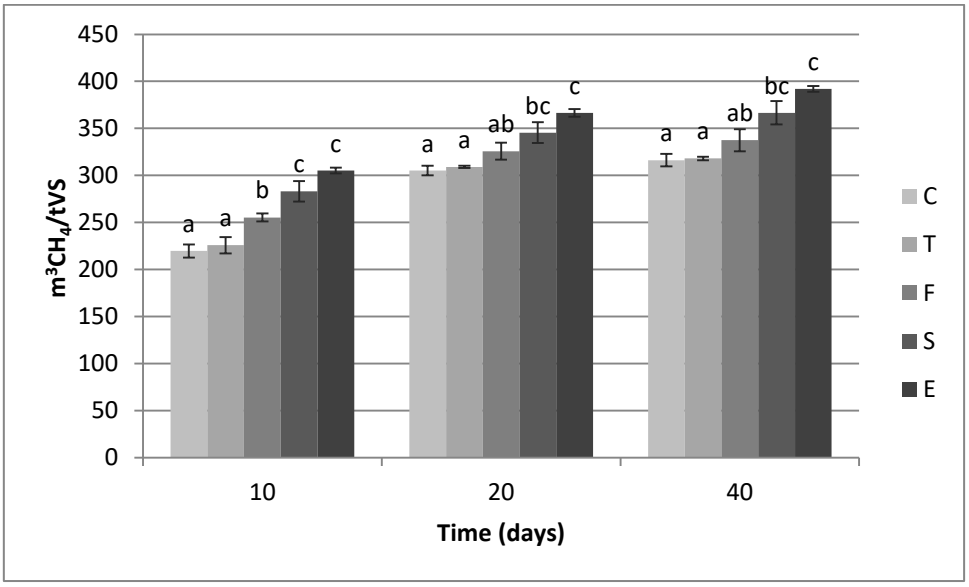

Figure 4.3.3: Tukey's pairwise comparison on the cumulative methane yields after 10, 20, and 40 days of BMP test. Different letters (a, b, and c) indicate significant differences ($P < 0.05$).
C (0% NaOH); T (2% NaOH); F (4% NaOH); S (6% NaOH); E (8% NaOH)

4.3.4 CONCLUSIONS

24 h NaOH pre-treatment was effective in improving the depolymerisation of spent coffee grounds and, consequently, the hydrolysis and the methane yield of the treated substrate. Anaerobic digestion of 8% NaOH pre-treated SCGs produced 24% more methane when compared to that of the untreated substrate.

SCGs resulted to be a substrate with a very good buffer capacity and no washing was needed after their pre-treatment. Lignin degradation increased in parallel with the increasing NaOH loading but without showing any particularly high peak of reduction. In particular, the main effect of NaOH was the very high increase in the DOC value. Bioavailability of organics contained in SCGs was favoured with consequent benefits for the overall anaerobic digestion process.

More tests could be performed increasing furtherly the NaOH concentration but taking into consideration the economic issues related to the pre-treatment, the Authors decided not to exceed a 8% NaOH loading (Chen et al., 2013). Moreover the change between the yield obtained from samples S (6%NaOH) and E (8% NaOH) was lower than 7%.

In view of a full scale application, a cost and benefit analysis would be required to see the final balance between the gain in methane potential production and the pre-treatment costs.

5. ANAEROBIC DIGESTION PROCESS IMPROVEMENT AND VALORISATION

This chapter includes the AD lab test investigation over FW in order to enhance the energy yield of the process analysing the effects of pre-aeration and of two-stage AD.

5.1 EFFECT OF AERATION APPLIED DURING DIFFERENT PHASES OF ANAEROBIC DIGESTION – A REVIEW

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Aerobic treatment has been investigated as a method to enhance putrescible substrate degradation and biogas production through anaerobic digestion (AD). A series of aeration methods has been studied in different phases of anaerobic digestion (before, during, or at a late stage of AD). Several research groups have applied aeration together with anaerobic digestion to improve hydrolysis and increase substrate conversion efficiencies. Aeration has been proven to reduce volatile fatty acids (VFA) accumulation during AD, reducing pH inhibition for methanogens, and thus increasing process yields. Aeration may represent an effective method to reduce substrates' toxicity (e.g. sulphur compounds), particularly when digestate, resulting from their anaerobic digestion, is destined for use on the land. However, a potential drawback is represented by decreased methane production observed as a consequence of excessive soluble COD consumption prior to the AD phase. Duration and intensity of aeration, substrate type, aeration method, temperature during aeration, and air application phase are deemed important factors capable of affecting the efficiency of this treatment. The present review aims to provide a comprehensive insight into research studies performed over the past decades to test the combination of aerobic treatment and anaerobic digestion of organic substrates.

5.1.1 INTRODUCTION

To address the number of problems which characterize the anaerobic digestion (AD) process and to simplify the management of the final residue consisting of undigested organic compounds and humic substances, methods including the optimization of AD parameters (pH and substrate C/N adjustment, solid retention time prolongation, inhibitors removal, substrate pre-treatment, co-digestion) may be applied.

With the aim of ensuring a balanced environment for anaerobic archaea growth and better performance, enhancing biogas production and, concurrently, reducing the amount of final residues, numerous studies have been performed to develop strategies focused both on improving process yields and testing effective pre-treatments to incentivize hydrolysis (first crucial and rate limiting

step for the degradation of organic substances) (Tsapekos et al., 2016).

As reported by Ariunbaatar et al. (2014), mechanical, thermal, chemical and biological pre-treatment methods may be applied. To focus on the latter, biological pre-treatments may be of an aerobic or anaerobic (two-stage AD) nature, or envisage the addition of specific enzymes such as peptidase, carbohydrase and lipase to the AD system (Ariubaatar et al., 2014; Dionisi and Silva, 2016; Neumann et al., 2016).

Aerobic treatment appears to represent a promising option, in spite of the fact that oxygen has long been considered an inhibitor of methanogenesis. On the one hand, aerobic treatment may threaten the subsequent AD process, resulting in the consumption of part of the substrate and inhibiting anaerobic archaea. However, as long as the process is not too protracted, it may elicit the positive effect of merely removing the excess readily-biodegradable organic compounds, which commonly result in acidification due to accumulation of VFA inside the bioreactors and consequent drop in pH. The latter consideration becomes particularly useful when high organic loads are applied. Several studies indicated that implementation of an aeration step prior to AD may reduce VFA accumulation in anaerobic digesters by means of oxidization, subsequently resulting in enhanced methane yields (Chu et al., 1994; Zhou et al., 2007). On the other hand, however, aerobic treatment may also enhance hydrolysis of AD, stimulating enzyme activity and hydrolysing bacteria. A large group of facultative microorganisms underlying the hydrolysis of organic waste grow both in the presence of oxygen and under anaerobic conditions. Aerobic pre-treatment is an efficient method of increasing microbial growth of hydrolytic bacteria, and therefore enhancing production of extracellular enzymes, that catalyzes the hydrolysis of organic substrates (Lim and Wang, 2013).

Aerobic treatment has also been found to exert positive effects on methanogenic activity (Lagervirkvist et al, 2015; Ramos and Fdz-Polanco, 2013). Aeration may enhance AD performance in a number of ways according to the different modes of aeration employed. Indeed, aeration may improve hydrolysis, reduce the peak of VFA yielded by readily-biodegradable organics, thus contributing towards controlling pH throughout the AD process. The present paper undertakes a systematic review of the available data relating to the modalities and effects of aeration discussed in scientific literature for the purpose of AD process improvement.

Different ways to combine aeration and anaerobic digestion

Aeration can be applied either as a pre-treatment or during the anaerobic digestion process; it may however also be performed solely in the latter stages of AD to enable a further production of methane and to improve the stability of digestate prior its successive treatment for reuse or disposal (see Figure 5.1.1).

A series of different substrates, such as organic fraction of municipal solid waste (OFMSW), food

waste from agricultural and industrial activities, sewage sludge, corn straw, grass silage, have been tested, with the scale of experimentation varying from batch to semi-continuous. Aeration modalities (natural aeration, air or oxygen injection, electrolytic aeration) varied according to the test scale and the phase of application.

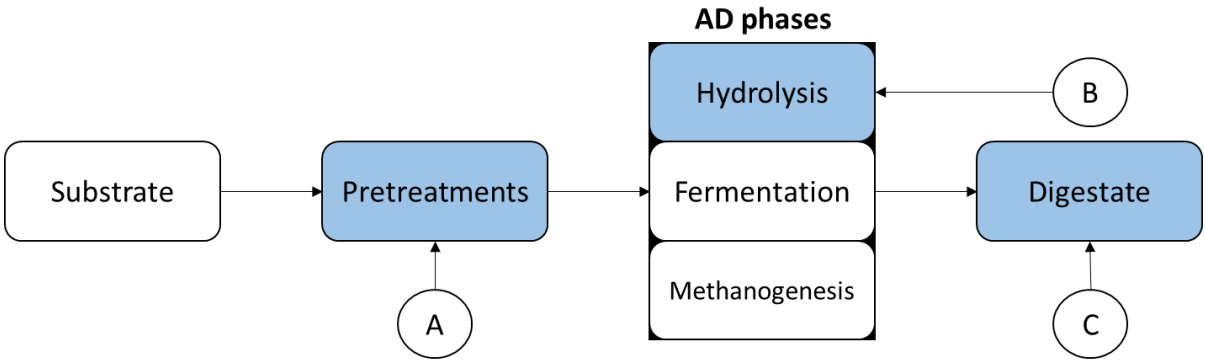

Figure 5.1.1: Possible alternatives in the use of aeration within Anaerobic Digestion (both one and two-stage) operational schemes.

(A - pre-aeration; B - micro-aeration during AD process; C - aeration of digestate)

5.1.2 AEROBIC TREATMENT PRIOR TO AD PROCESS

Several studies have focused on the use of aeration as a pre-treatment method to improve the biodegradation and treatability of organic substrate (Charles et al., 2009; Fu et al., 2015a; Nguyen et al., 2007; Wu et al., 2015; Zhu et al., 2009; Peces et al., 2015). The investigated substrates included corn straw, sewage sludge, OFMSW, vegetable wastes, horse dung, sisal pulp and olive mill wastewater. The majority of the experimentations highlighted the improvement of the first AD step, hydrolysis, as a consequence of pre-aeration (Charles et al., 2009; Fu et al., 2015a; Zhu et al., 2009; Ahn et al., 2014).

Tested aeration modalities were performed using air stones (Ahn et al., 2014), air pumps (Nguyen et al., 2007; Zhu et al., 2009; Kusch et al., 2008), compressed air (Charles et al., 2009; Jang et al., 2014), and air or oxygen injection (Fu et al., 2015a; Fu et al., 2015b; Gonzalez-Gonzalez and Cuadro, 2015). A less intense but simpler aeration method was also achieved by leaving the tested BMP bottles open with or without shaking to maintain semi-aerobic conditions (Peces et al., 2015; Mshandete et al., 2005). Optimum aeration time varied from 9 to 96 hours according to the different substrates and processes used (Peces et al., 2015; Jang et al., 2014; Mshandete et al., 2005). With regard to batch test conditions, optimum oxygen loading ranged from 5 mLO_2/gVS to 10 mLO_2/gVS when oxygen injection was applied (Fu et al., 2015a; Fu et al., 2015b). When CSTR reactors were run with semi-continuous oxygen or air introduction, oxygen supply rates varied from 37.5 $mLO_2/L_R/d$ to 16 $LO_2/L_R/d$ (Fu et al., 2015a; Fu et al., 2015b).

A summary of studies conducted using aerobic pre-treatment prior to AD process is provided in

Table 5.1.1.

Fdez.-Güelfo et al. (2011a) compared aerobic pre-treatment using a series of different inoculums: mature compost, fungus and activated sludge; the results obtained revealed how that the use of compost yielded the best performance. Fdez.-Güelfo et al. (2011a), Fdez.-Güelfo et al. (2011b), Fdez.-Güelfo et al. (2011c) reported that mixing OFMSW with mature compost for 24h resulted in a higher specific microbial growth rate (160–205% as compared to untreated OFMSW) and organic matter degradation, accompanied by a faster methane production. Optimum mature compost inoculation percentage was 2.5% (v/v), displaying maximum hydrolytic activity with an increased COD of 51% and a 35.5% improvement in specific methane production (Fdez.-Güelfo et al., 2011a; Fdez.-Güelfo et al., 2011b; Fdez.-Güelfo et al., 2011c). Giordano et al. (2014) applied aeration pre-treatment to prepare an inoculum for a two-stage hydrogen and methane production process. When food waste was used, a lower H_2 yield and a much higher CH_4 yield were obtained by the aerobic pre-treated inoculum compared to heat-shock, which suggested that aeration pre-treatment could preserve the hydrolytic activity and form a more structured microbial community (Giordano et al., 2014; De Gioannis et al., 2013; Favaro et al.; 2013).

Table 5.1.1: Effect of aerobic treatment prior to AD process.

Substrate	**Aeration method and amount**	**AD process and test scale**	**Optimum Results**	**Effects of pre-aeration**	**Reference**
Corn straw	Oxygen injection under thermophilic conditions (0, 5, 10, 20, 30, 40 mLO_2/g VS)	Batch mesophilic BMP test	16.24% methane yield improvement (5 mLO_2/g VS)	Micro-aerobic condition caused higher hydrolysis	(Fu et al., 2015a)
Digestate from primary fermentation of corn stover	Oxygen injection under thermophilic conditions (5, 10, 20 mLO_2/g VS)	Batch mesophilic BMP tests	28.45% methane yield improvement (10 mLO_2/g VS)	Not mentioned	(Fu et al., 2015b)
Food waste and brown water	Oxygen injection (37.5 mLO_2/L_R /d; 4 days)	Batch mesophilic BMP tests	10% acetate increase in the hydrolysate and 23.0% methane yield improved (37.5 mLO_2/L_R/d, 4 days)	The enhanced solubilisation of organics and control of VFA accumulation improved methane yield	(Lim and Wang, 2013)

Horse dung	Air injection at the bottom of the anaerobic digestion reactor (0.053 $Lair/L_R/min$; 48 h)	Mesophilic CSTR	18% methane yield decreased and no process kinetics enhanced	Excessive soluble COD consumption during the long aeration (48hours) led to a decreased methane production	(Kusch et al., 2008)
OFMSW	Air injection (air was introduced into the sealed reactor until internal pressure was raised to a predetermined level)	DiCOM® reactor	Pre-aeration and wet thermophilic anaerobic digestion was able to stabilize OFMSW within a period of only 12 days	Increase of both cellulase and protease exoenzyme activities was observed	(Charles et al., 2009)
OFMSW	Air injection at the bottom of the anaerobic digestion reactor (0.0067 Lair/kg/min in; 2h run/4h stop)	Mesophilic CSTR	Biogas production doubled in AD reactors with micro-aeration	Better hydrolysis and acidification were observed	(Nguyen et al., 2007)
OFMSW	Partial composting as pre-aeration (20 °C 0.21 Lair/kg-OFMSW/min)	Batch mesophilic BMP tests	20% of VS was degraded during partial composting and 40% methane yield was lost	Start-up of the dry anaerobic batch digestion of OFMSW was accelerated	(Tenbrummeler and Koster, 1990)
Olive mill wastewater	Air injection for 5 days (5 $Lair/L_R/min$)	Mesophilic CSTR	Methane production doubled from 0.16 $m^3/kgCOD$ without pre-treatment to 0.39 m^3/kg COD	Polyphenol concentration was reduced	(Gonzalez-Gonzalez and Cuadros, 2015)
Primary sludge and waste activated sludge	Natural aeration (bottles were left open to maintain semi-aerobic conditions) at 20 °C (12, 24, 48, 72, 96 hours)	Batch mesophilic BMP test	VFA recovery (43 $gCOD_{VFA}\ kg^{-1}$ VS) and 14% methane potential improved (20 °C, 72 hours)	Pre-aeration positive effect was linked to growth of fungi which have a strong biodegradation activity	(Peces et al., 2015)

Sewage sludge	Air injection (0.05 Lair/L_R/min; 0.5,1, 6, 24, 48, 96 h)	Batch mesophilic BMP tests	40% siloxane concentration decreased; 108% NH_3-N decreased; 25% methane yield improved (24 hours)	SCODcr from sewage sludge microbial cell disruption increased	(Ahn et al., 2014)
Sewage sludge	Air injection under thermophilic conditions (air rate 2.5 Lair/L_R/min; 1day)	Batch mesophilic BMP tests	42% higher methane production rate and 15% higher TCOD removal (air rate 2.5 L/L_R/min, 1days)	A greater diversity of bacteria and archaea populations was observed during mesophilic anaerobic digestion	(Jang et al., 2014)
Sewage sludge	Aerobic thermophilic pre-treatment by adding 1.2% VS of aerobic thermophilic sludge to methanogenic anaerobic sludge	Batch mesophilic BMP tests	Biogas production enhanced 2.2-fold	Aerobic thermophilic bacteria enhanced the production of biogas from anaerobically digested sewage sludge	(Miah et al., 2005)
Sisal pulp	Natural aeration (digesters were left open to maintain semi-aerobic conditions and shaken at 135 rpm at 37 °C (0, 3, 6, 9, 12, 24, 48, 72 h)	Batch mesophilic BMP tests	The highest activity of hydrolytic enzymes was obtained at 9h of pre-treatment.	Higher activities of some extracellular hydrolytic enzymes	(Mshandete et al., 2005)
Vegetable and flower waste	Air injection (0.5 Lair/L_R/min, 5min injection every 1, 4, 12, 24 h)	Batch mesophilic BMP tests	Hydrolysis efficiency with aeration treatment was 30.9%-56.4% while hydrolysis efficiency was 57.7% under anaerobic conditions	Acidogenesis was promoted and accumulation of lactic acid was prevented	(Zhun et al., 2009)

Aeration intensity and aeration methods

Aeration intensity or oxygen level should be given careful consideration during the pre-aeration stage due to the possibility of over-aeration reducing the methane potential of the digested substrate, and of too little oxygen potentially failing to produce any effect. A varied range of optimum aeration times, aeration intensity and aeration frequency have been adopted in lab-scale experiments

due to the variety of substrates and AD processes. As shown in Table 5.1.1, when different aeration methods were used, the testing aeration time range varied considerably between 0 and 96 hours. Ahn et al. (2014) used air stones in the pre-aeration of sewage sludge. The results obtained showed an optimum aeration time of 24 hours, resulting in a 25% methane yield improvement (Ahn et al., 2014). However, Peces et al. (2015) also used primary sludge and waste activated sludge as substrates and left the bottles open to maintain semi-aerobic conditions. Their results demonstrated a 14% methane potential improvement following 72 hours pre-fermentation (Peces et al., 2015). From this comparison, the aeration time gap between the two studies could be attributed to the intensity of aeration. By simply leaving the substrate in contact with air it would be much more difficult for the oxygen to access the deeper parts of the medium.

Mshandate et al. (2005) observed how 26% more methane was obtained after 9 h of aerobic pre-treatment (activated sludge mixed population as a source of inoculum in batch cultures) than with untreated sisal pulp waste. However, there was a loss of potential methane production with a longer pre-treatment of sisal pulp waste (Mshandate et al., 2005). In this study moreover, aeration was performed simply by keeping bottles open and shaken, thus applying a more intensified aeration than that performed by Peces et al. (2015). The optimum aeration time was 9 hours. Optimum aeration time may be linked not only to aeration intensity but also to substrate types. Other studies opted to apply intermittent aeration by injecting air (oxygen) or using controlled air pumps. Fu et al. (2015a) injected oxygen into the digester to pre-treat inoculated corn straw. The result showed a methane yield improvement of 16.24% under 5 mLO_2/gVS (Fu et al., 2015a). However, when these Authors used the same aeration method to treat digestate from primary digestion of corn straw (Fu et al., 2015b) which was still containing a high amount of VS, the optimum oxygen level for obtaining the highest methane yield was 10 mLO_2/g VS; this suggests that more easily degradable substrate (raw corn straw) requires less oxygen compared to a more refractory substrate (digestate from corn straw).

Botheju et al. (2010) reported how, under batch feed condition, oxygenation (0–16 %) in anaerobic digestion produced no effect on volumetric biogas generation but lead to enhanced methane yield under the lowest oxygenation level of 1.3%. Increased oxygenation reduced CH_4/CO_2 ratio due to the significant increase in CO_2 production due to substrate oxidation (Botheju et al., 2010a).

Botheju and Bakke (2011) have suggested that “electrolytic aeration” may represent an innovative concept for use in undertaking aeration pre-treatment. An electric current is used to introduce an aeration effect by means of water electrolysis, and electrolytically-generated hydrogen, together with oxygen, may also represent a substrate for AD. This concept has been tested in a study by Tartakovsky et al. (2011), which demonstrated enhanced methane production (10–25%) from

synthetic wastewater in laboratory-scale anaerobic reactors equipped with electrodes for water electrolysis. The electrodes were installed in the reactor sludge bed and a voltage of 2.8–3.5 V was applied. Reactor stability was also improved in comparison to a conventional anaerobic reactor. Further, Tartakovsky et al. (2014) reported a significant improvement of process performance and biogas quality (26% methane production improvement) by creating aerobic conditions in an electrolysis-enhanced anaerobic digestion process. In this study, cow manure and switchgrass were used as co-digestion substrates. Methane production from hydrolytic hydrogen and enhancement of hydrolysis of organic matter was used as a possible mechanism (Tartakosky et al., 2014). Chen et al. (2016a) applied direct voltage to create bio-stimulation, with the results obtained showing how methane production increased by 76.2% with an enhanced VS removal rate 26.6% with the applied voltage of 0.6 V. They also suggested that the positive effect might have been elicited by micro-aerobic conditions and from the available hydrogen resulting from water electrolysis. It is moreover worthy of mention that electricity consumption of 0.6 V can easily be compensated by the incremental energy producible from the higher recovery of methane (Chen et al., 2016a). Although the economic efficiency of AD reactors with electrolysis remains to be thoroughly evaluated, this electrical stimulation technology may constitute an innovative feasible method, and further studies should be carried out.

Effect of aerobic treatment on hydrolysis

Aerobic treatment is capable of enhancing hydrolysis of AD, particularly when using lignocellulosic biomass and other refractory organic matters as a substrate.

The characteristics of organic substrates should be carefully evaluated prior to undertaking studies to investigate the impact of aeration pre-treatment. Physical and chemical characteristics of the substrate after aeration are largely dependent on the specific characteristics of the initial substrate. Pre-aeration of readily-biodegradable substrates, such as OFMSW, may reduce easily-degradable organics underlying the potential accumulation of VFAs (Charles et al., 2009) during the anaerobic process. In the case of not readily-biodegradable substrates, such as corn straw, pre-aeration improves degradability of the substrate (Fu et al., 2015a). A summary of pre-aeration effects focusing on hydrolysis performances for different substrates types is shown in Table 5.1.2.

Hydrolysis is catalysed by extracellular enzymes (such as protease, amylase, etc.) excreted by the facultative microorganisms. By investigating wet thermophilic anaerobic digestion of OFMSW through introduction of pressurized air, Charles et al. (2009) reported improved hydrolysis during pre-aeration as a consequence of stronger cellulase and protease exo-enzyme activities. Zhu et al. (2009) found that during hydrolysis of vegetable and flower wastes, cumulative protease and carboxymethyl cellulase activities increased with higher pre-aeration frequencies. Nonetheless, no

increase of cumulative soluble organic matter is required as facultative bacteria consume VFA by means of aerobic respiration.

Table 5.1.2: Dynamics of physical and chemical characteristics of organic wastes when applying aeration.

Substrate	Aeration method and amount	Indicators change		Reference
		Without aeration	With aeration	
OFMSW	Air injection (air was introduced into the sealed reactor until internal pressure was raised to a predetermined level)	VFA=7.2g/gTS	VFA=1.8 g/gTS Aeration time: 3 days	(Charles et al., 2009)
Primary sludge	Natural aeration (bottles were left open)	Acetate=0.92 gCOD/L	Acetate=1.76gCOD/L 20 °C semi-aerobic	(Peces et al., 2015)
Sewage sludge from a WWTP	Air injection (0.15 L/min; 0.5, 1, 6 24, 48, 96 h)	SCODCr=2.65g/L	SCODCr=3.24g/L Aeration time: 2 days	(Ahn et al,. 2014)
Vegetable and flower wastes	Air injection (0.7 L/min, 5min injection every 1, 4, 12, 24h)	-	TOC=3397-4665 mg/L in the hydrolytic effluent Aeration time: 3 days	(Zhu et al., 2009)

Pre-aeration with inoculum addition, resulting in biological hydrolysis, may prove to be more energy-saving and considerably more efficient than pre-aeration without inoculum. Lim and Wang (2013) showed how inoculum addition influenced pre-aeration, as they observed 21% and 10% improvement in methane yields when pre-aerating inoculum-added substrates and pre-aerating substrates without inoculum, respectively. Accordingly, pre-aeration of substrate with inoculum is recommended in the absence of sufficient indigenous hydrolytic and acidogenic bacteria in the AD feedstock. Moreover, pre-aeration before AD may have indirect effects on hydrolysis, potentially enhancing biomass of facultative acidogenes and extracellular enzymes (Botheju and Bakke, 2011).

Pre-aeration under thermophilic conditions

Performing pre-aeration at high temperatures may lead to a stronger synergistic effect. Jang et al. (2014) evaluated the influence of thermophilic aerobic digestion as a sludge pre-treatment method on mesophilic anaerobic digestion. At the optimal conditions of 1-day thermophilic aerobic digestion and 20 days' mesophilic anaerobic digestion, the two-stage process yielded an approximately 42% higher methane production rate and 15% higher TCOD removal. This aerobic biological pre-treatment led to a higher diversity of the bacterial community in the anaerobic digester, resulting in enhanced hydrolysis and methane production (Jang et al., 2014).

Miah et al. (2005) performed aerobic thermophilic pre-treatment prior to AD for enhanced methane

production. They incubated sewage sludge at 65 °C for several months; by adding 1.2% VS of this aerobic thermophilic sludge to methanogenic anaerobic sludge they enhanced biogas production 2.2-fold (Miah et al., 2005).

Charles et al. (2009) suggested that combined pre-aeration and wet thermophilic anaerobic digestion may be capable of stabilizing OFMSW over a 12-day period (Charles et al., 2009). Fu et al. (2015a) investigated the effect of a combination of micro-aerobic and thermophilic (55 °C) pre-treatment on the anaerobic digestion of corn straw, with positive results (16.24% methane yield improvement). However, it cannot be concluded with any certainty that the hydrolysis process was accelerated under thermophilic conditions, as the study lacked control experiments at lower temperatures. However, high temperatures are also prone at times to producing negative effects: Peces et al. (2015) applied semi-aerobic fermentation to pre-treat primary sludge; under aerobic conditions, pre-fermentation at 37, 55, and 70 °C resulted in higher soluble COD, but elicited a 20% reduction in sludge methane potential at 55 and 70 °C (Peces et al., 2015). Fdez-Güelfo et al. (2011a) combined thermochemical pre-treatments (air and sodium hydroxide) with biological pre-treatments (using mature compost, fungus *Aspergillus awamori* and activated sludge). Under aggressive temperature and pressure conditions, synthetic air employed as an oxidant agent may degrade a significant fraction of organic matter in OFMSW. However, amongst all assays the best result was obtained by thermochemical pre-treatment without air (Fdez- Güelfo et al., 2011a). It may therefore be feasible to combine pre-aeration with other biological pre-treatments, such as the addition of compost, fungi or enzymes.

Degradation of toxic organics

Recent studies have revealed a reduction in the concentration of polyphenols, inhibitors of methanogenic archaea, and a doubling of methane yield following aerobic pre-treatment (Gonzalez-Gonzalez and Cuadro, 2015; Mshandete et al., 2005; Fdez-Güelfo et al., 2011a). Gonzalez-Gonzales and Cuadros (2015) reported that 5 days of pre-aeration were enough to avoid polyphenols inhibition during the AD of olive mill wastewater. The mechanism of polyphenols reduction can be attributed to the oxidative action of laccases which require oxygen as a second substrate (Mshandete et al., 2005 Fdez-guelfo et al., 2011a). Moreover, Wu et al. (2015) reported how limited aeration was successful in aiding the removal of benzene, toluene, ethylbenzene and xylenes (BTEX), thus lessening the toxicity and enhancing degradability of petrochemical wastewaters. BTEX can be degraded in aerobic conditions since dioxygenases and monooxygenases enzymatic systems acts on metabolic pathways for their degradation (Zhang et al., 2013; Khan et al., 2001). Further, Gavazza et al. (2015) applied electrolysis to produce oxygen in order to achieve the aerobic degradation of aromatic amines in textile wastewaters. Simple aromatic amines can be mineralized under

methanogenic conditions but sulfonated aromatic amines, on the other hand, are resistant and require specialized aerobic microbes for their mineralization (Pandey et al., 2007). Common substrates from solid wastes, such as food waste, corn straw and OFMSW did not usually raise the issue of the presence of toxic organics; however, when using sewage and industrial sludge means of reducing toxic or harmful substances should be considered. Ahn et al. (2014) employed air stones for aeration to pre-treat sewage sludge prior to introduction into the AD process. The results obtained showed a 40% decrease in siloxane after 96 hours' aeration because of its release into the atmosphere after the breakdown of extracellular polymeric substances (released by the disruption of microbial cells of sewage sludge) in which siloxane was adsorbed (Ahn et al., 2014). To date however, very few papers have been published that relate to the elimination of toxic organics during AD of organic wastes. Lastly, with regard to land use of digestate, the presence of toxic organics should be carefully evaluated before the application of pre-treatment during the AD process (Vallini et al., 1989).

Assisting the start-up of AD reactor

Pre-aeration has also been used to generate the heat required to meet AD energy requirements both under mesophilic and thermophilic conditions (Charle et al., 2009; Kusch et al., 2008). Lagerkvist et al. (2015) reported the efficiency of aeration in establishing methanogenic conditions in a full-scale AD reactor treating food waste. At start-up no methanogenic sludge was available. The reactor was therefore fed with 1200 kg of total solids (TS) for 8 days and filled with thickened and slightly acidic sewage sludge. It was subsequently heated to 55 °C. Compressed air was subsequently blown into the digester and, within a month, a fully functional methanogenic culture was established. Charles et al. (2009) found that pre-aeration of 48 h generated enough biological heat to increase the temperature of bulk OFMSW to 60 °C, sufficient for self-heating prior to start-up of thermophilic anaerobic digestion, without the need for an external heat source. Pre-aeration also reduced the excess of readily-degradable organic compounds in OFMSW, i.e. a common cause of acidification during start-up of the batch system (Charles et al., 2009). Again, it is fundamental that over-aeration, which would consume the available substrate, should be carefully avoided, thus leaving the substrate available to methanogens for the production of biogas. Indeed, the results obtained showed how increased duration of aerobic pre-treatment limited the amount of methane gas formed rather than stimulating it. The highest cumulative CH_4 production was yielded by non-pre-aerated samples. Tenrummeler and Koster (1990) applied an aerobic partial-composting step to solve the start-up unbalance of a dry anaerobic batch digestion of OFMSW, which resulted in the rapid degradation of easily-degradable organic compounds. The aerobic pre-treatment step lowered initial acid-formation rate and accelerated start-up of the dry anaerobic batch digestion of OFMSW.

Nonetheless, partial composting caused a loss of 40% of potential methane yield (Tenrummeler and Koster, 1990). As for the dry anaerobic digestion, pre-aeration or micro-aeration during the start-up of AD may reduce VFA accumulation whilst, at the same time, exerting no negative effect on methanogens due to the rapid oxygen consumption ability of facultative fermentative organisms, and the shielding effect of microbial aggregates (Botheju and Bakke, 2011).

Possible drawbacks of pre-aeration

Despite the several positive effects reported above, other researchers have referred to a clear drawback when performing aeration as pre-treatment prior to the AD process. According to the results obtained by Tenbrummeler and Koster (1990), a partial pre-composting treatment of OFMSW resulted in a 19.5% VS loss. Aerobic pre-treatment resulted in a 10% lower methane production, likely due to the lengthy aeration period and excessive consumption of soluble COD prior to the anaerobic digestion phase (Kusch et al., 2008).

Another important issue to consider is the additional operational cost due to pre-aeration. Literature provides only one study by Rafieenia et al. (2016) mentioning that the cost and benefit analysis does not support the economic feasibility of the pre-treatment as the energy consumed for pre-aeration is much higher than the recovered one. Studies are still needed in order to investigate through a complete Life Cycle Assessment the affordability of pre-aeration.

5.1.3 AERATION THROUGHOUT THE ENTIRE AD PROCESS

When applying aeration throughout the entire AD process in batch, CSTR, or UASB anaerobic bioreactors the investigated substrates used included sewage sludge, brown water, food waste, corn straw, cassava wastewater, grass silage, and evaporator condensate from a sulphite pulp mill (Zou et al., 2007; Lagerkvist et al., 2015; Ramos et al., 2013; Peces et al., 2015; Fdez-Güelfo et al., 2011b; Botheju et al., 2010; Ramos et al., 2014a; Lim et al., 2014; Fu et al., 2016; Xu et al., 2014; Botheju et al., 2010b; Khongsumran et al., 2014; Diaz et al., 2011). The approaches implemented to aerobically stimulate anaerobic digestion performances included micro-aeration or limited oxygen injection throughout the entire AD process (Ramos et al., 2013; Khongsumran et al., 2014; Diaz et al., 2011; Jagadabhi et al., 2010). Micro-aeration is defined as the introduction of small amounts of oxygen into an anaerobic biochemical process to enable both anaerobic and aerobic biological activities to occur within a single bioreactor (Cesaro and Belgiorno, 2014). When using micro-aeration or oxygen injection, the main purpose is to sustain the stability of AD reactors and to enhance the methane yield. Micro-aeration may be of use in reducing sulphides content and, therefore, minimizing the toxic effect of aqueous sulphides on acetogenic, methanogenic and micro-organisms (Krayzelova et al., 2015; Nghiem et al., 2014; Fdz-Polanco et al., 2009). Several studies

have shown that limited oxygen may exert positive effects on hydrolysis (Lim et al., 2014; Khongsumran et al., 2014; Jagadabhi et al., 2010).

A summary of studies that have used limited aeration during anaerobic digestion is reported in Table 5.1.3.

Table 5.1.3: Effect of limited aeration during the AD process.

Substrate	**AD process and test scale**	**Aeration method and amount**	**Results**	**Effects of pre-aeration**	**Reference**
Brown water and food waste	Mesophilic two-phase CSTR	Oxygen injection to the liquid part of the reactor (5mLO_2/L_R/d and 7 mLO_2/L_R/d)	43% total VFA concentration increase with 5 mLO_2/L_R/d	Micro-aeration led to diverse bacterial communities. *Firmicutes* enabled to metabolize a greater variety of substrates in the acidogenic reactor	(Lim et al., 2014)
Cassava wastewater	CSTR without temperature control	Oxygen injection into the CSTR (1.5 to 6.0 mLO_2/L_R/d)	Maximum hydrolysis efficiency: 62.57% for cellulose applying 3.0 mLO_2/L_R/d	Anaerobic hydrolysis was enhanced; Facultative bacteria increased resulting in higher population and more secreted enzyme	(Khongsumran et al., 2014)
Cellulose	Batch mesophilic BMP tests	Oxygen injection (5 mLO_2/L_R/d)	No hydrolysis improvement, but shorter lag-phase time	Transformation of the cellulose to simple organic compounds in the early stages of AD was accelerated	(Diaz et al., 2011a)
Corn straw	Batch thermophilic BMP tests	Air injection (0, 12.5, 25, 50, and 100 mL/L_R/d)	16.5% methane yield improvement (12.5 mL/L_R/d)	Hydrolysis-associated microorganisms, *Firmicutes*, class *Clostridia* and order *Clostridiales* raised; Oxytolerant *Methanosarcina* and doubled	(Fu et al., 2016)
Evaporator condensate from a sulphite pulp mill	Mesophilic UASB	Direct limited aeration in the UASB (aeration flow rate: 6 mL/L_R /min)	COD removal rate increase from 40% to 80%	Limited aeration caused no oxygen inhibition to the anaerobic microorganisms but instigated sulphide oxidization and H_2S removal	(Zhou et al., 2007)

Grass-silage	Mesophilic leach-bed reactor	Air injection flow rate: 1.33L/L_R/min (for three minutes) on day 11 and 5.33L/L_R/min (for six minutes) on day 22	4-fold increase in cumulative VFA production (for air flow of 1.33L/L_R/min on day 11)	The conversion of the produced leachate SCOD into VFA improved	(Jagadabhi et al., 2010)
OFMSW	Mesophilic CSTR	Air injection at the bottom of the anaerobic digestion reactor (1 L/min; 2h run/4h stop)	Reduction of DOC with an aeration time of 3 days	DOC reduced from 140gC/kgTS (without aeration) to127 gC/kgTS (with aeration)	(Nguyen et al., 2007)
Sewage sludge	Mesophilic CSTR	Pure oxygen supply into the sludge recirculation 0.0033 LO_2/L_R/min	Better capability to deal successfully with overloads	The growth of hydrogenotrophic methanogens was promoted. This could favour acetic formation and help to maintain a stable pH	(Ramos and Fdz-Polanco, 2013)
Sewage sludge	Mesophilic CSTR	Oxygen injection every 10 min into different O_2 dosing points with O_2 flow rate (0.005-0.034 LO_2/L_R/d)	Oxygen did not have a significant impact on digestion	O_2 transfer rate positively affected long-term microbial diversity	(Ramos et al., 2014a)
Sewage sludge and food waste	Thermophilic CSTR	Compressed air injection (41.7 mLair /kgTS/min)	Aerobic treatment may amend occasional acidification problems	The aerobic degradation of organic acids led to increased pH, thus promoting growth of methanogens.	(Lagerkvist et al., 2015)
Synthetic food waste	Mesophilic two-phase UASB	Air pump at the bottom of the LBRs (0, 129, 258, and 387 Lair/kg TS/d)	5% COD faster leaching and 18% methane production enhancement with 258 Lair/kgTS/d	Micro-aeration could enhance acid fermentation and promote production of VFAs	(Xu et al., 2014)
Synthetic substrate (Starch 3.9, peptone 3.01 and yeast extract 3.58 g/L)	Mesophilic semi-CSTR	Daily air injection (0, 0.43, 0.86, 1.3 mLO_2/L_R/d)	30-55% methane yield increase	Initial VFAs accumulation was minimized	(Botheju et al., 2010a)
Synthetic substrate (Starch	Mesophilic semi-CSTR	Daily air injection after	Methane generation	Oxygen suppressed the formation of	(Botheju et al., 2010b)

3.9, peptone 3.01 and yeast extract 3.58 g/L)		daily feeding (increasing oxygen loads of 0, 2.52, 5.04 and 10.07% daily feed COD)	decreased linearly with the oxygen loading	VFAs due to aerobic respiration	
Synthetic wastewater	Mesophilic UASB	Oxygen supply through electrolysis (2.8-3.5V)	10-25% methane production enhancement and reactor stability improvement	Hydrolysis and hydrogen sulphide oxidation were enhanced	(Tartakovsky et al., 2011)

Aeration intensity and aeration methods

Due to the limited solubility of oxygen, intermittent aeration was performed in all reviewed studies. When micro-aeration was performed during the acidogenic phase in a two-stage anaerobic digestion, 258 Lair/kgTS/d (54 LO_2/kgTS/d) was recommended for acidogenic leach bed reactor (LBR) treating food waste (Xu et al., 2014). Jagadabhi et al. (2010) suggested that micro-aeration at low flow rates (10 LO_2/kgVS/min) during the first 11 days of AD may improve VFA production without any significant increase in COD solubilisation. Similarly, in their two-phase CSTR, Lim et al. (2014) added oxygen at a rate of 3 mL/min daily for one to two hours after feeding. The results showed that low micro-aeration conditions (5 mLO_2/L_R/d) performed better than higher conditions (7 mLO_2/L_R/d) (Lim et al., 2014).

Studies performed to investigate single phase CSTR revealed that aeration intensity was lower than that needed for a two-phase CSTR (Botheju et al., 2010a; Botheju et al., 2010b; Khongsumran et al., 2014). Khongsumran et al. (2014) supplied oxygen to a CSTR for 5 min every 2 hours obtaining the highest cellulose hydrolysis efficiency by applying 3.0 mLO_2/L_R/d. Botheju et al. (2010a) exposed four CSTR reactors to different oxygenation levels performing daily air injection after daily substrate feeding. Only the lowest oxygenation level (3.25 mLO_2/L_R/d) produced a positive effect on methane production (Botheju et al., 2010a). In a similar research study Botheju et al. (2010b) observed a linear decrease in methane generation in the presence of an increased oxygen loading. In line with the above-mentioned studies, it may be feasible to conclude that the oxygen level should be around 3 mLO_2/L_R/d in single CSTR systems (Botheju et al., 2010b).

Batch reactors were also used to test the feasibility of micro-aeration during the AD process. Fu et al. (2016) achieved a 16.5% improvement in methane yield during the thermophilic AD of corn straw by injecting air into their bottles with a syringe (2.63 mLO_2/L_R/d). Diaz et al. (2011a) when testing cellulose batch AD, shortened the lag-phase time with daily oxygen supply (5 mLO_2/L_R/d). For refractory substrates such as corn straw or cellulose, aerobic and anaerobic treatment

combination may not be sufficient.

Effect of aerobic treatment on hydrolysis

Three modes of aerobic treatment (during the acidogenic phase in a two-stage anaerobic digestion process, in CSTRs, in batch anaerobic bio-digesters) were applied to evaluate the effects on hydrolysis during anaerobic digestion tests. Lim et al. (2014) applied micro-aeration during the hydrolysis phase (acidogenic phase) in a two-stage anaerobic process performed to co-digest brown water and food waste. A 43% increase in total VFA concentration was achieved and the positive effect attributed to the growth and expansion of diverse bacterial communities promoted by micro-aeration (Lim et al., 2014). Xu et al. (2014) used synthetic food waste as a substrate and applied micro-aeration in a leach bed reactor (LBR). The results obtained showed that 2 hours aeration enhanced carbohydrate and protein hydrolysis by 38% and 64%, respectively (Xu et al., 2014). Jagadabhi et al. (2010) also introduced air into an LBR and detected a quadruplicated VFA production, thus improving conversion of the produced leachate soluble COD (SCOD) into VFA. On the contrary, Lagerkvist et al. (2015) introduced compressed air (41.7 mLair/min/kgTS) into their digester to achieve aerobic degradation of organic acids and to amend occasional acidification problems in a full scale AD plant. It was noted that aeration intensity should be chosen carefully, in accordance with the specific aim to be achieved, potentially improvement of hydrolysis or relieving of acid accumulation. Other studies provided aeration or injected air during the anaerobic process inside a CSTR. Khongsumran et al. (2014) supplied oxygen into a CSTR treating cassava wastewater; maximum hydrolysis efficiency was achieved, namely 62.57%, under 3.0 $mLO_2/L_R/d$ due to the enhanced growth of facultative bacteria. Nonetheless, other studies reported no obvious hydrolysis improvement. Ramos and Fdz-Polanco (2013) supplied pure oxygen during sludge recirculation and found that oxygen increased the ability to deal with overloads and enhanced digestion performances only under stressful conditions. Diaz et al. (2011) applied micro-aeration at an oxygen supply of 5 $mLO_2/L_R/d$ in a batch mesophilic AD reactor treating cellulose. No improvement was detected in hydrolysis, although a shorter lag-phase time, which resulted from accelerating the transformation of the cellulose to simple organic compounds in the early stages of AD, was observed.

Effect of aerobic treatment on oxidation of hydrogen sulphides

Limited oxygen injection into the anaerobic reactor was found to greatly reduce hydrogen sulphide accumulation (Diaz et al., 2011a; Nghiem et al., 2014; Ramos et al., 2014a). Oxygen is capable of oxidizing sulphides generated in anaerobic digesters in which S compounds are reduced to HS- and H_2S by the biological activity of anaerobic sulphate-reducing archaea (SRB). Sulphide content minimization enhances the entire AD process, see Table 5.1.4, by abating the toxic effect of

aqueous sulphides on methanogenic and acetogenic organisms (Diazet al., 2011) Other positive effects on AD performances reported in the literature are lag phase shortening, methane yield enhancement and biogas quality improvement (Nguyen et al., 2007; Diaz et al., 2011a). Micro-aeration may exert positive effects on the activity of anaerobic microorganisms. Other acknowledged explanations for the positive effects elicited under micro-aerobic conditions include a higher solubilisation of organic matter, intensified hydrolysis, and a more stable acidification (Nguyen et al., 2007). Furthermore, micro-oxygenation may prevent hydraulic overloads by promoting the growth of both acetoclastic and hydrogenotrophic methanogenic archaea (Ramos and Fdz-Polanco, 2013).

Table 5.1.4: Effects of limited aeration during the AD process on the H_2S reduction.

Substrate	**AD process and test scale**	**Aeration method and amount**	**Results**	**Reference**
Sewage sludge	Mesophilic CSTR (200 L)	Oxygen supply 0-0,00485 $LO_2/L_R/d$ for low H_2S concentration; 0-0.0705 LO_2/d for high H_2S concentration	97% H_2S removal efficiency at low concentration (0.33% in the biogas) and 99% at high concentration (3.38% H_2S in the biogas)	(Ramos et al., 2012)
Sewage sludge	Mesophilic CSTR (265 L)	Oxygen supply from a cylinder by a mass flow controller 0.16-0.46 LO_2/Lfed	99% H_2S removal efficiency applying 0.46 LO_2/Lfed	(Ramos et al., 2012)
Sludge from WWTP	Mesophilic CSTR (200 L)	Oxygen injection 0.013-0.024 $LO_2/L_R/d$	more than 99% H_2S removal	(Fdz-Polanco et al.,2009)
Synthetic brewery wastewater	UASB (2.7 L)	Air flow 0.37 $L/L_R/d$	73% H_2S removal efficiency	(Krayzelova et al., 2014)
Thickened surplus activated sludge	Mesophilic CSTR (11 L)	Air pump 1.1 Lair/d (0.1 $L/L_R/d$)	92.3% H_2S reduction	(Jenicek et al.,

Possible drawbacks

In this context of combined aerobic and anaerobic conditions during the AD process, a series of negative results have also been reported. Botheju et al. (2010b) concluded that total volumetric biogas generation was not significantly influenced by aeration achieved by means of daily oxygen injection in a 5.5 L bioreactor. A reduced methane yield was replaced by an increased CO_2 yield, and an increasingly negative effect on methane generation was observed on a par with the increasing aeration level (Botheju et al., 2010b).

5.1.4 AERATION IN THE LATE AD STAGE

As the end of the AD process approaches, increasingly lower quantities of organic matter can still

be consumed to further produce methane. Nonetheless, digestate can be treated to further increase the substrate methane potential and improve the stability of digestate. It is worth to mention that digestate will, anyhow, need further post-treatment prior agronomic use or disposal. It was recently reported that by applying aeration during the late stages of AD, methane yield could be furtherr improved (Fu et al., 2015a). In a study conducted by Fu et al. (2015a), digestate from primary fermentation of corn stover was treated under 55 °C with oxygen loads of 5, 10 and 20 mL/gVS (VS of residual substrate). The results obtained showed a 28.45% improvement in methane yield during secondary thermophilic micro-aerobic treatment with an optimal oxygen load of 10 mL/gVS. The Authors attributed this positive effect to the high hydrolysis rate under micro-aerobic thermophilic conditions. Indeed, the VS removal efficiency was 29.43% higher than that registered in the reactor without aeration.

Tomei et al. (2016) studied the hygienization performance for sewage sludge under a sequential anaerobic-aerobic process. The study showed that implementation of an aerobic phase after anaerobic digestion may reduce *E.coli* population, a conventional microbial indicator originating from human and animal intestines. An increase in temperature during the aerobic stage was found to have no effect on lowering *E.coli* population.

It should however be underlined that to date little is known about the biological stability of digestate subjected to a micro-aerobic AD process. Previous studies have been strictly divided into anaerobic and aerobic processes with aeration being applied as post-treatment (composting) to achieve the stability of digestate (Abdullahi et al., 2008; Kaparaju and Rintala, 2006; Cavinato et al., 2013). However, it is possible to improve both methane yield and digestate bio-stability by combining anaerobic/aerobic treatment in the same AD process (Peces et al., 2015; Fu et al., 2015a; Xu et al., 2014). Further research should be conducted on the aerobic treatment in the late AD stage, with particular focus on easier digestate stability.

Effect of aeration on microbial diversity

Information relating to the presence of microbial communities in AD reactors operating under strictly anaerobic and limited aeration conditions is crucial to provide a better insight into the metabolic reactions involved in the process. The availability of this information promotes an easier and better control of the AD process parameters, and contributes significantly towards improving process performance. However, very few reports focus on the presence of microbial communities following limited aeration. Micro-aeration may potentially shorten the lag phase, increase the specific growth rate and improve tolerability of microorganisms to substrate inhibition (Ghaly and El-Taweel, 1994). Moreover, micro-aeration leads to a more diverse microbial variety, a crucial issue for deep substrate decomposition. An interesting study revealed that the presence of

Acetobacterperoxydans, an aerobic bacterium, led to high VFA production inside the acidogenic reactor (Lim and Wang, 2013). Tang et al. (2004) investigated the effect of micro-aeration on thermophilic anaerobic digestion of municipal solid waste (MSW), with no significant difference in CH_4 production being observed between micro-aerobic and anaerobic conditions. Although no significant difference was observed in microbial diversity under micro-aeration conditions, however, the number of Methanosarcina decreased while Methanoculleus population increased. This was mainly due to the difference in oxygen tolerability of the two different methanogenic archaeal species. Although micro-aeration did not suppress the activity of sulphate-reducing bacteria (SRB), H_2S production under micro-aeration was much lower. The Authors concluded that limited oxygen chemically improved sulphide oxidation in partially aerated reactors. In contrast to Tang et al. (2004), Ramos et al. (2014b) did not observe any significant impact on archaeal populations following micro-aeration.

Another study conducted to investigate AD treatment in a sulphite pulp mill showed how limited aeration is capable of reducing sulphide inhibition of methanogens, highlighting how inhibition decreased gradually in the presence of aeration due to an increase in sulphide oxidation. Predominant microorganisms present before aeration were rod-shape methanogens, while after limited aeration cocci-shaped methanogens were dominant (Zhou et al., 2007).

A study carried out by Lim et al. (2014) under conditions of micro-aeration revealed a greater diversity in the microbial population compared to that observed under strictly anaerobic conditions. This diversity led to improved hydrolysis rates and subsequently better process performance.

5.1.5 CONCLUSIONS

Aerobic treatment exerts both positive and negative effects when combined with AD. The overall influence reported in the literature does not allow any definitive conclusions to be drawn. The effect produced is mainly associated with duration of specific aeration, intensity, and methods used (air or oxygen only, injected or pumped in through intermittent or continuous mode), on the air application phase (before, during, or at a late stage of AD), on the type of AD test (batch reactors or CSTR), and on the type of digested substrate (e.g. corn straw, sewage sludge, food waste). Numerous reports have referred to an enhanced methane production during AD following the application of aeration. Aerobic treatment is capable of removing hydrogen sulphides that accumulate during the AD of sulphur-rich substrates; micro-aeration improves hydrolysis of recalcitrant organic matters such as corn straw; moreover, the problem of acidification when starting AD operations in batch reactors can be prevented by pre-aeration of organic matter such as OFMSW.

Aerobic treatment may be capable not only of improving the performance of AD, but also of

ensuring an easier post-treatment of digestate. However, some Authors have obtained lower methane yields after aerobic pre-treatment of the digested substrates or a poorer biogas quality after aeration during AD.

The conducting of microbial diversity studies would likely be beneficial to investigate the effect produced by a series of substrates and aeration conditions on different methanogenic microorganisms. Ecotoxicological tests considering the potential pollutants contained in the digestate are suggested as a further tool to assess environmental benefits of using aeration in combination with anaerobic digestion processes (Pivato et al., 2016a; Pivato et al., 2016b; Pivato et al., 2014).

Use of a combination of aerobic treatment with other pre-treatment methods (e.g. thermal chemical pre-treatment, inoculum recirculation) should be taken into consideration in an attempt to achieve a synergistic effect. Several novel aeration methods, such as electrolytic aeration, should also be further investigated.

Economic feasibility studies should likewise be undertaken to examine additional aeration costs (Rafieenia et al., 2016); this parameter, together with the demonstration of improved AD performance, should represent the key tool in the decision-making process.

5.2 EFFECT OF AEROBIC PRE-TREATMENT ON HYDROGEN AND METHANE PRODUCTION IN A TWO-STAGE ANAEROBIC DIGESTION PROCESS USING FOOD WASTE WITH DIFFERENT COMPOSITIONS

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Aerobic pre-treatment was applied prior to two-stage anaerobic digestion process. Three different food wastes samples, namely carbohydrate rich, protein rich and lipid rich, were prepared as substrates. Effect of aerobic pre-treatment on hydrogen and methane production was studied. Pre-aeration of substrates showed no positive impact on hydrogen production in the first stage. All three categories of pre-aerated food wastes produced less hydrogen compared to samples without pre-aeration. In the second stage, methane production increased for aerated protein rich and carbohydrate rich samples. In addition, the lag phase for carbohydrate rich substrate was shorter for aerated samples. Aerated protein rich substrate yielded the best results among substrates for methane production, with a cumulative production of approximately 351 m^3/tVS. With regard to non-aerated substrates, lipid rich was the best substrate for CH_4 production (263 m^3/tVS). Pre-aerated P substrate was the best in terms of total energy generation which amounted to 9.64 KJ/gVS. This study revealed aerobic pre-treatment to be a promising option for use in achieving enhanced substrate conversion efficiencies and CH_4 production in a two-stage AD process, particularly when the substrate contains high amounts of proteins.

5.2.1 INTRODUCTION

The use of renewable energy sources is a critical issue worldwide due to the serious negative environmental consequences caused by the use of fossil fuels, in addition to the proximate depletion of the latter in the near future. Anaerobic digestion (AD) is one of the most widely investigated methods used in the production of energy from different kinds of organic waste. During this process, strictly anaerobic bacteria and archaea are utilized to produce biofuels such as hydrogen and methane when growing on organic substrates. Hydrogen has been indicated as one of the most promising fuels for the future (Ozkan et al., 2010; De Gioannis et al., 2013). However, subsequent to anaerobic hydrogen production substrate conversion remains incomplete, with the majority remaining as a residue after the process. A promising system is represented by a two-stage AD process combining H_2 and CH_4 productions. During the first stage, organic compounds are hydrolysed and utilized by hydrogen producing bacteria to produce H_2 and volatile fatty acids (VFAs), whilst in the second stage, VFAs are used as substrates for CH_4 production by methanogens. Two-stage AD provides a positive energy yield (40-90% available energy), thus

underlining the highly important process sustainability (Ruggeri et al., 2010). Several studies have demonstrated the ability of two-stage AD to improve CH_4 yields during the second stage, likely due to better hydrolysis (Liu et al., 2006; Pakarinen et al., 2011). Moreover, compared to one-stage AD, process control would be simpler and stability would be improved (Lim et al., 2013; Ariunbaatar et al., 2015).

During hydrolysis, the rate limiting step of anaerobic digestion, organic compounds including proteins, carbohydrates and lipids are broken down by hydrolytic bacteria into amino-acids, sugars and long chain fatty acids, respectively. Substrate pre-treatment methods are aimed at promoting and improving hydrolysis of high molecular weight compounds to readily-biodegradable constituents, and subsequently increasing the AD process product yields.

Hydrolysis occurs under both aerobic and anaerobic conditions; however, hydrolysis rates are significantly higher under aerobic conditions, likely due to the higher production of enzymes (Botheju et al., 2009). In addition, pre-aeration reduces accumulation of VFAs, resulting in a drop of pH during the process, thus improving the start-up stability of anaerobic digestion. Limited pre-aeration prior to anaerobic digestion has been shown to improve hydrolysis and biogas production (Charlset et al., 2009; Zhu et al., 2009; Ahn et al., 2014; Cossu et al., 2016; Peces et al., 2016).

Composition of organic wastes varies according to the source from which the wastes are collected. Slaughterhouse wastes may be rich in proteins and lipids, while food wastes and organic fraction of municipal solid wastes (OFMSW) are rich in carbohydrates. An in-depth understanding of effective pre-treatment methods for each kind of waste is fundamental in improving biogas production.

To the best of the Authors' knowledge, no scientific reports have been published to date on the effects of aerobic pre-treatment on food waste with different compositions for either H_2 and/or CH_4 production in a two-stage AD process. Moreover, the effect of carbohydrate, lipid and protein content of food waste on pre-aeration efficiencies has not been addressed before. Therefore, the present work aims to study the aerobic pre-treatment effect of carbohydrate rich (C), protein rich (P), and lipid rich (L) food waste prior to two-stage anaerobic digestion on both H_2 and CH_4 production.

5.2.2 MATERIALS AND METHODS

Organic Waste Samples

Synthetic food waste samples were prepared in order to simulate industrial or municipal food waste with different compositions as indicated in a previous study (Alibardi and Cossu, 2016).

Three different substrates were prepared and classified as C, P, and L substrates. The composition of samples is shown in Table 5.2.1. The percentages are based on wet weight.

Food waste samples were shredded after preparation and characterized (Table 5.2.2) in order to have more detailed information for each substrate category.

Table 5.2.1: Composition of synthetic food wastes (%W/W).

Ingredients	**C**	**L**	**P**
Tuna (%)	6.7	7.5	31.1
Butter (%)	5.5	22.3	5.5
Apple (%)	27.8	27	7.85
Banana (%)	27.8	27	7.85
Chicken breast (%)	6.7	7.5	31.1
Bread (%)	5.4	1.5	3.2
Pasta (%)	5.4	1.5	3.2
Minestrone soup (%)	14.7	5.5	10.2

Table 5.2.2: Average characteristics of food wastes with different compositions.

Parameters	**C**	**L**	**P**
TS(%)	28.56	30.72	43.2
VS(%TS)	95.4	96.1	97.3
TOC(%TS)	58.7	65.9	66.3
TKN(%TS)	3.34	3.05	7.98
Lipid (%TS)	16.1	41	17.3
Protein(%TS)	19.8	18.1	47.3
Glucose(%TS)	4.2	1.54	3.11
Fructose (%TS)	12.36	5.29	2.75
Sucrose (%TS)	15.56	7.42	2.78

Aerobic pre-treatment of the substrates

In order to compare the two-stage AD process with and without pre-aeration on the prepared substrates featuring different compositions, half the waste samples from each category were air injected using an aquarium pump (EIN WELTWEIT-Elite799) connected to a porous stone for better air diffusion. The air flow rate was fixed at 5 L/h using a flow meter (BROOKS SHO-RATE 1355). After 24 h, aeration was stopped. The inoculum was then added to each bottle with and without pre-treatment.

Two-stage AD – Hydrogen production

Laboratory scale tests were performed to evaluate Biochemical Hydrogen Potential (BHP) of the examined substrates. Batch tests were carried out using 1-litre glass bottles which were subsequently sealed with silicon plug. Substrate concentration and substrate to inoculum ratio (S/I) were 5gVS/L and 0.3 gVS/gVS, respectively. Granular sludge was used as inoculum for BHP and was collected from a full-scale Upflow Anaerobic Sludge Blanket (UASB) digester of a brewery factory located in Padova, Italy.

Heat treatment was carried out on granular sludge in a rotary water-bath incubator at a fixed temperature of 80 °C for 15 minutes in order to suppress methanogenic bacteria (Alibardi and Cossu, 2016). pH was set at 6.0 using phosphate buffer before the start of tests. Three main H_2-producing enzymes are used by anaerobic microorganisms: [Fe/Fe]/hydrogenases, [Ni/Fe]/hydrogenases and nitrogenases. These H_2-producing enzymes are generally all highly oxygen-sensitive and presence of oxygen may reduce their activities (Mathews and Wang, 2009). Accordingly, H_2 production should be carried out under strictly anaerobic conditions. Therefore, following aerobic pre-treatment, the bottles were flushed with N_2 gas for 3 minutes to ensure anaerobic conditions and incubated at a temperature of 35±1ºC. All tests were performed in duplicate.

Two-stage AD – Methane production

After completing the H_2 production phase, the bottles were opened and pH, dissolved organic carbon (DOC) and VFAs were measured. Non-pre-treated granular sludge (at the same amount as the first stage) was then added to each bottle and all were sealed again, flushed with N_2 gas for 3 min, and incubated at the same initial mesophilic conditions of 35±1ºC.

Analytical Methods

Total solids (TS), volatile solids (VS), and TKN were analysed according to standard methods (APHA, 1999). Total organic carbon (TOC) values were calculated on the basis of the difference between total carbon and inorganic carbon present in the samples. Concentrations of carbohydrates, proteins, lipids and free sugars were obtained according to official methods (AOAC, 2003). The volume of biogas produced during the anaerobic digestion process was measured by means of the water displacement method. The produced gas composition in terms of H_2 first, and then CH_4, was analysed using a micro-GC (Varian 490-GC) equipped with an MS5A column to measure H_2 and CH_4, and a PPU column for CO_2 and two Thermal Conductivity Detectors. Argon was used as the carrier gas at a pressure of 60 kPa. Temperatures of column and injector were set to 80°C.

VFAs concentrations were measured using a gas chromatograph (Varian 3900) equipped with a CP-WAX 58 WCOT fused silica column and a Flame Ionization Detector. Nitrogen was used as carrier gas with a flow of 4 mL/min in column. The oven temperature was set at 80°C for the first minute and then increased at a rate of 10°C/min to 180°C for two minutes. Column and injector temperatures were set to 250°C.

Hydrogen and methane production calculations

Hydrogen, methane and carbon dioxide volumes produced during the first and second stages of AD were calculated according to the following equation (see section 1.1) (VanGinkel et al., 2005):

$$V_{c,t} = C_{c,t} * V_{b,t} + V_H * (C_{c,t} - C_{c,t-1})$$

5.2.3 RESULTS AND DISCUSSION

Effect of aeration pre-treatment on the first stage of AD

Hydrogen production

The results obtained for hydrogen production potential from three different food waste samples are shown in Figure 5.2.1. Data obtained through GC analysis revealed a lack of methane in the emitted gas, due to efficiency of the thermal pre-treatment of inoculum. In the first stage of AD, substrate C without aeration produced considerably more hydrogen (55.31 m^3H_2/tVS) compared to L (27.93 m^3H_2/tVS) and P (7.96 m^3H_2/tVS) substrates. This finding is in agreement with Alibardi and Cossu (2015), who concluded that carbohydrate rich food waste is capable of producing much higher quantities of H_2 compared to lipid or protein rich substrates. This could be attributed to faster hydrolysis rate of carbohydrates (almost 20 times faster) compared to lipids and proteins (Lay et al., 2003). Since the duration of H2 production is short (around 3 days) it is not enough for the hydrolysis of proteins and lipids. In addition, conversion of long chain fatty acids from hydrolysis of lipids to H_2 is feasible only at very low hydrogen partial pressure (Hallenbeck, 2009). Degradation of some aminoacids from hydrolysis of protein is H_2 consuming. According to Hallenbeck (2009), readily-biodegradable carbohydrates are the preferred substrates by anaerobic microorganisms during dark fermentative H_2 production. Similarly, Chu et al., (2012) showed that H_2 yield is strongly dependent on the carbohydrate content of organic wastes.

For samples subjected to aerobic pre-treatment, substrate C achieved the highest H_2 yield (44.4 m^3H_2/tVS), followed by substrate L (21 m^3H_2/tVS) and P (5.27 m^3H_2/tVS). Aerobic pre-treatment lowered average H_2 production for C (19%), L (24%), and P (33%) substrates. Although aerobic pre-treatment has been indicated as a strategy to increase hydrolysis and CH_4 production from sludge (Ahn et al., 2014; Peces et al., 2016), it proved ineffective in achieving an increase in H_2 production from food waste. The latter could convincingly be explained by a low solid retention time (SRT) for H_2 (3 days) compared to CH_4 production (15 days or more). In addition, during aeration, part of the available readily-biodegradable carbon (mainly free sugars) is converted to CO_2 or consumed for cell growth instead of in product formation (Botheju and Bakke, 2011). In the present study, the carbon loss after aeration was proved by 37%, 6%, and 12% decreased TOC content for C, P, and L substrates, respectively. Although this drawback may also be present in pre-aeration studies on CH_4 production, it may be compensated by a longer SRT, which enhances carbon hydrolysis with lower degradability, subsequently leading to higher product yields.

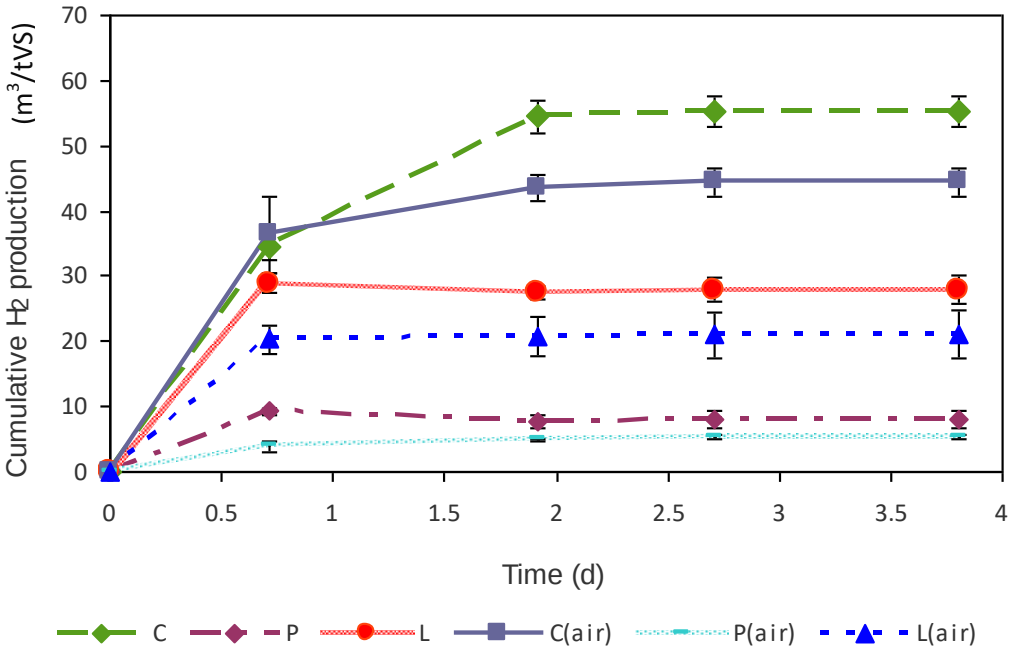


Figure 5.2.1: Hydrogen production potential for the three different substrates, namely C, P, and L, with and without aerobic pre-treatment. C, P and L are carbohydrate, protein and lipid rich substrates without aerobic pre-treatment, respectively. C (air), P (air) and L (air) are carbohydrate, protein and lipid rich substrates with aerobic pre-treatment, respectively.

VFA composition after the hydrogen-producing phase

The results of characterization of liquid samples at the end of the hydrogen-producing phase are shown in Tables 5.2.3 and 4. The major VFA components for all food waste samples were acetic acid (AC) and butyric acid (BU), while propionic acid (PA) was detected only in P-rich samples. Aerobic pre-treatment slightly increased PA production for substrate P. Presence of PA at the end of the first stage is not favourable since unlike AC and BU it is produced by a metabolic pathway which consumes substrate without producing H2 (Hawkes et al., 2007). The average concentrations of VFAs at the end of the first stage of AD are reported in Table 5.2.3. When compared to non-aerated samples, AC content in pre-aerated P samples was slightly lower (4%) while in pre-aerated samples and L AC decrease was much more significant, 33% and 25%, respectively. Similarly, aeration lowered BU production for sample C by 43%. On the contrary, in samples P and L, BU concentration increased by 34% and 10%, respectively. PA concentration in pre-aerated P samples was slightly higher (5%) than in non-pre-treated P ones.

Samples P, both with and without aeration, displayed the lowest BU/AC ratios amongst all substrate types. For non-aerated samples, correlation between BU/AC ratio and H_2 production is in agreement with previous studies which suggested that BU/AC ratio is directly proportional to H_2 yield (Kim et al., 2006). Conversely, other studies reported the absence of a correlation between

BU/AC ratio and H_2 yield (Guo et al., 2013; Ghimire et al., 2015). Indeed, Table 5..2.3 highlights how for aerated P and L samples the BU/AC ratios increased in comparison to non-aerated samples, although lacking any positive effect on H_2 production.

pH, DOC concentrations and cumulative CO_2 production values obtained following the first stage for samples with and without aerobic pre-treatment are shown in Table 5.2.4. Pre-aeration was not found to have significantly affected cumulative CO_2 production at the end of the first stage. However, DOC values were lower for all pre-aerated samples in comparison to samples without pre-aeration. This could be mainly due to lower amount of easily degradable carbon in pre-aerated samples as a result of partial loss of carbon during aeration.

Table 5.2.3: Average volatile fatty acid (VFA) production for the three different substrates, namely C, P, and L, with and without pre-treatment.

Specific VFA(mg/L)	**C**	**C (air)**	**P**	**P (air)**	**L**	**L (air)**
Acetic acid	593±66	392±28	490±63	473±51	510±43	381±29
Butyric acid	413±50	236±23	139±35	187±32	220±38	243±43
Propionic acid	0	0	88.9±11	94.2±21	0	0
BU/AC ratio (mmol/mmol)	0.47	0.41	0.19	0.26	0.29	0.43
Total VFAs (mg/L)	1006	628	717.9	754.2	730	624

Table 5.2.4: Average CO_2 and DOC concentration and pH at the end of first and second stages of AD process for the three different substrates, namely C-rich, P-rich, and L-rich with and without aerobic pre-treatment.

	Parameter	**C**	**C (air)**	**P**	**P (air)**	**L**	**L (air)**
First stage	CO_2 (m^3/tVS)	82.42±2.11	72.5±1.41	42.81±2.02	39.41±2.11	65.64±3.87	65.41±2.5
	DOC (mg/L)	1003.5±23.11	626.5±31.33	734.5±2.08	690.5±8.12	790.25±28.14	678.5±16.2
	pH	4.56±0.01	4.88±0.02	5.51±0.1	5.31±0.03	4.56±0.1	4.56±0.1
Second stage	CO_2 (m^3/tVS)	169.76±3.2	159.18±2.65	150.16±3.37	214.62±4.8	151.94±2.12	151.95±3.19
	DOC (mg/L)	<15	<15	<30	<25	<20	<30
	pH	7.49±0.03	7.39±0. 1	7.69±0.02	7.56±0.02	7.3±0.07	7.14±0.1

pH values were measured at the end of the first stage for all samples. Substrate P (both with and without pre-aeration) showed higher values compared to other substrates. Generally, substrates with high nitrogen content (such as protein rich wastes) prevent excessive acidification due to their buffering capacity (Boni et al., 2013).

In terms of pH, during the first stage, aeration led to a slightly higher pH in substrate C as a consequence of the lower VFA concentration (see Table 5.2.3). Instead, aeration led to a lower pH in substrate P compared to non-aerated samples. For substrate L, with and without aerobic pre-treatment, pH values at the end of the first stage of the AD process were substantially similar.

Effect of pre-aeration on the second stage of AD

Methane production

Cumulative methane productions for the three investigated food waste substrates, with and without aeration, are shown in Figure 5.2.2. Long lag phase of almost one week (except for non pre-aerated sample C where the lag phase lasted about 3 weeks) was observed for all substrate types. The most probable reason was a low pH following completion of the first AD stage. However, for substrate P without aeration the lag phase lasted only 3 days.

For substrate C, aerobic pre-treatment ensured a better acclimatization of bacteria and increased cumulative CH_4 production by 6% was observed at the end of second stage of AD. Cumulative CH_4 production for the aerated substrate C was 600% higher than the non-aerated substrate C at day 14. Significant CH_4 production in non pre-aerated samples C started after about three weeks. Similarly, Charles et al., (2009) observed an accelerated CH_4 production after aerating OFMSW, a carbohydrate rich substrate.

For substrate P with aeration, cumulative CH_4 production was lower compared to non-aerated samples until day 50. After this time, CH_4 production remained virtually constant for non-aerated samples, whilst it increased significantly (45.6%) for samples with pre-aeration. Cumulative CH_4 production for P-rich with and without aerobic pre-treatment was 351.69 and 241.52 m^3CH_4/tVS, respectively.

Cumulative CH_4 productions for substrate L were around 263 and 240 m^3CH_4/tVS for non-aerated and pre-aerated samples, respectively. L was the only substrate that produced less CH_4 with pre-aeration. However, when taking into consideration non-aerated substrates, L was the best substrate type for CH_4 production in two-stage AD. Similarly, Johansen and Bakke (2006) reported that micro aeration led to higher hydrolysis of carbohydrates and proteins of primary sludge, while lipids hydrolysis failed to increase without the addition of inoculum.

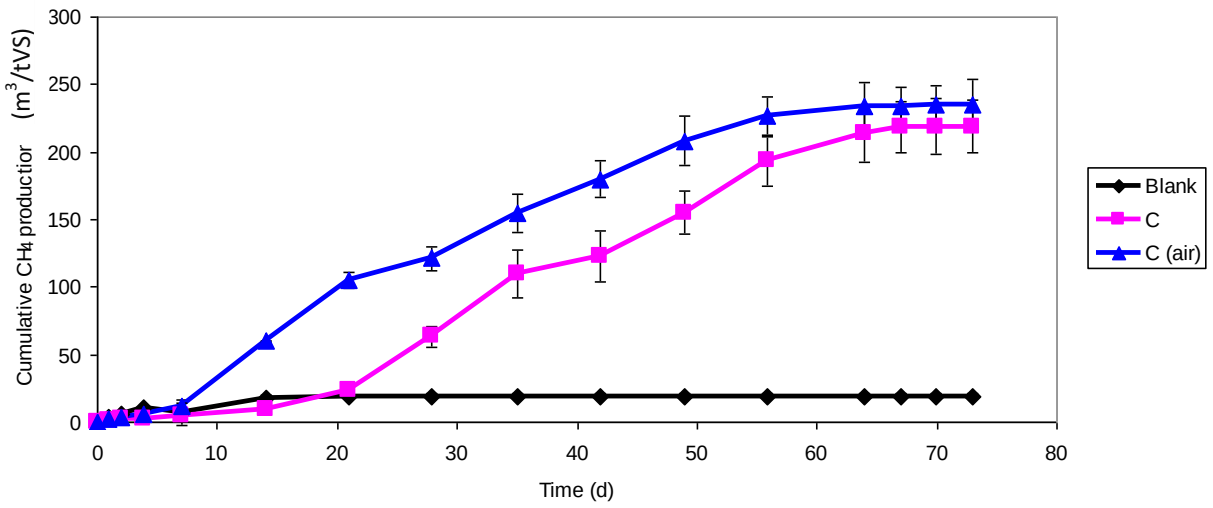
Alibardi and Cossu (2015) reported that proteins and lipids produce higher quantities of CH_4 than carbohydrates. These Authors separated different fractions of municipal solid waste to produce CH_4 in single stage AD from each single fraction. They observed that the highest CH_4 production was achieved using the fraction containing meat, cheese and fish, and the lowest was produced using a fraction containing bread and pasta alone. This finding is in agreement with the present study in which substrates P and L produced more CH_4 compared to substrate C.

Several researchers have observed higher CH_4 production in single stage AD process following aerobic pre-treatment. Lim and Wang (2013) showed that aerobic pre-treatment of a mixture of brown water and food waste improved AD treatment performance with a 10% increase in CH_4 production. Pre-aeration of sewage sludge and primary sludge increased cumulative CH_4 production by 25% (Ahn et al., 2014), and 14% (Peces et al., 2016), respectively. According to Botheju et al. (2009) pre-aeration may increase substrate conversion efficiency due to enhanced hydrolysis.

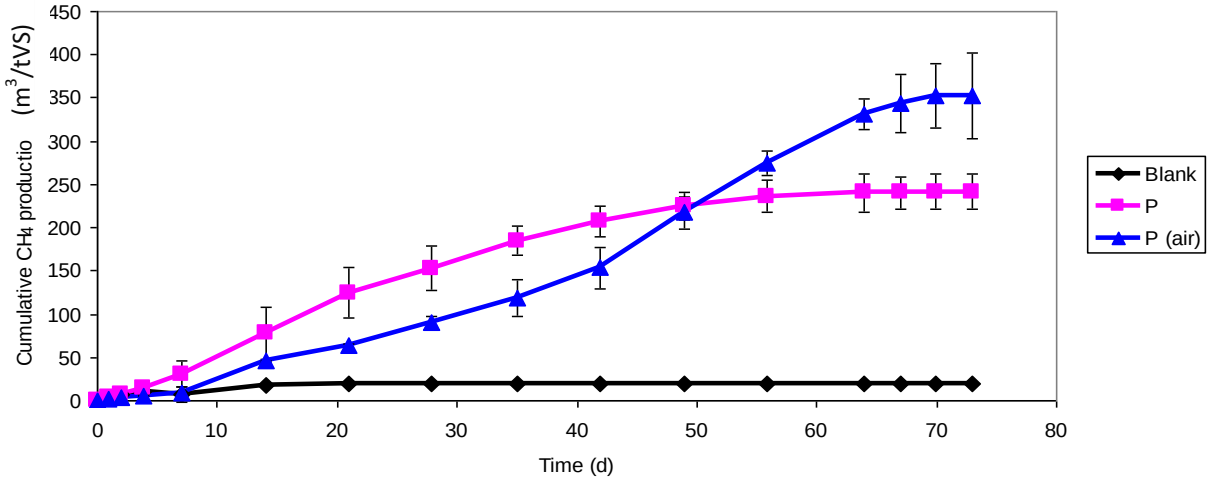
Jang et al., (2014) applied aerobic thermophilic pre-treatment prior to mesophilic AD for sludge digestion. They obtained higher CH_4 production and higher carbon conversion efficiencies with aeration. The study was reported as using sludge with a higher protein compared to carbohydrate content.

In anaerobic digesters VFA accumulation, and consequent drop in pH, is a major issue during CH_4 production. Limited aeration may remove excess degradable carbon, thus promoting the onset of methanogenesis. Several studies have reported lower VFA accumulation during anaerobic digestion following aerobic pre-treatment (Chu et al., 1994; Zhou et al., 2007). The present study likewise showed positive effects of pre-aeration on the reduction of VFA accumulation during the second stage of AD (Figure 5.2.3). A high VFA concentration was observed for substrate C without aerobic pre-treatment; lower VFA concentrations were observed in pre-aerated C samples, as well as higher CH_4 production (Figure 5.2.2a).

Table 5.2.4 illustrates pH, CO_2 and DOC values after the second stage of AD. At the end of the second stage, pH values for all samples ranged from 7.1 to 7.6. Similar to the first stage, pH was slightly lower in pre-aerated samples, possibly due to higher carbon conversion to VFAs. The most significant difference between cumulative CO_2 production for pre-aerated and non-aerated samples was observed for P-rich substrate (214.62 and 150.16 m^3/tVS, respectively). This sample also showed the highest CH_4 production.



a



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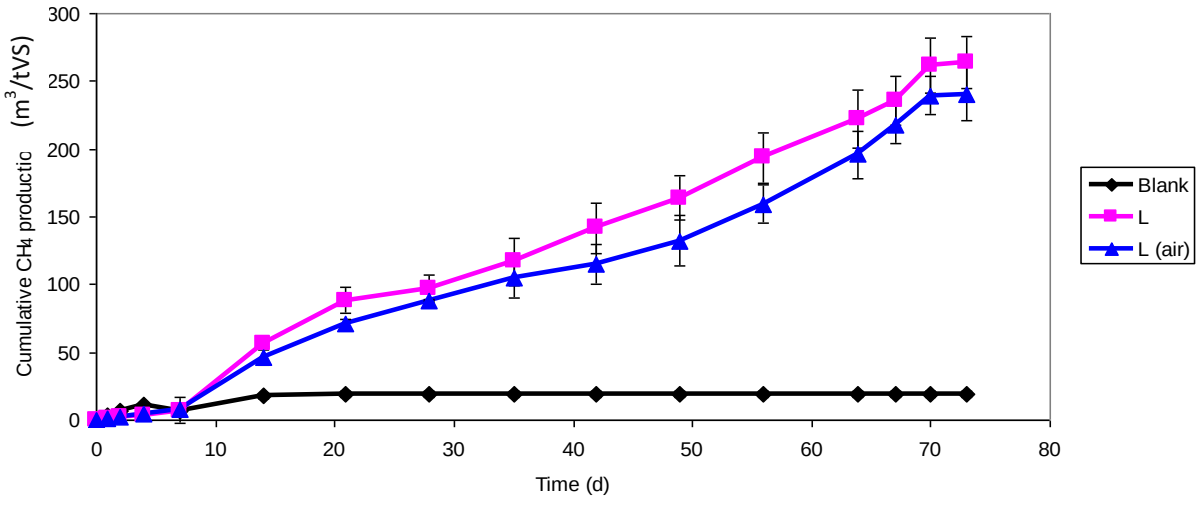

c

Figure 5.2.2: Methane production potential of the three different substrates, namely C (a), P, (b) and L (c), with and without aerobic pre-treatment, after hydrogen production phase. C (air), P (air) and L (air) are carbohydrate, protein and lipid rich substrates with aerobic pre-treatment, respectively. C, P, L are carbohydrate, protein and lipid rich substrates without aerobic pre-treatment. B is blank.

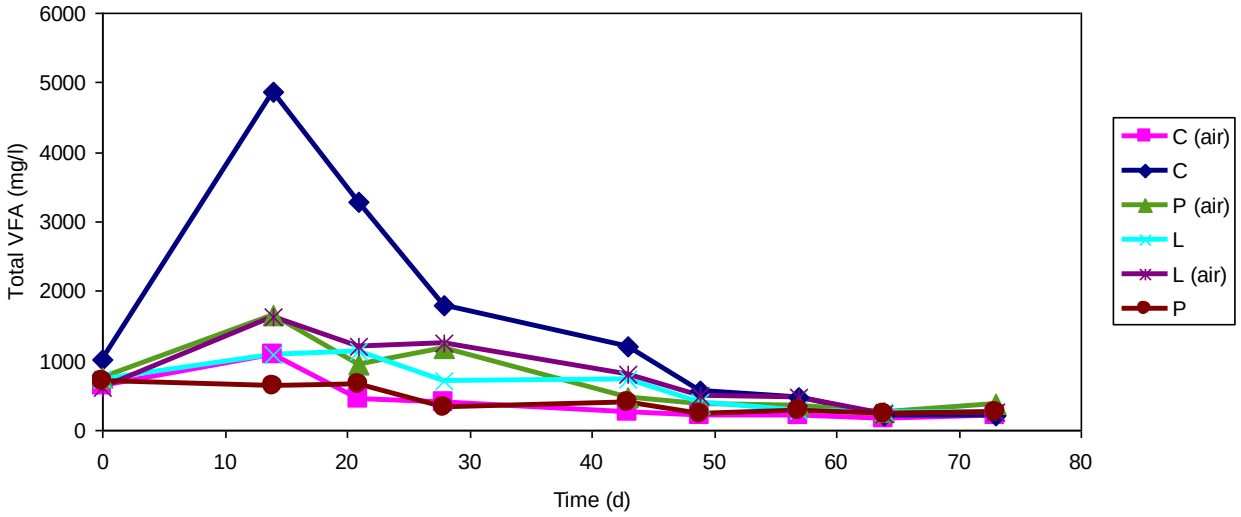

Figure 5.2.3: VFA accumulation during the second stage of the AD process.

Total Energy yields from H_2 and CH_4 in the two-stage AD

H_2 and CH_4 productions obtained with each substrate revealed that more H_2 were accompanied by a lower production of CH_4 and vice versa. In order to define the efficiency of the two-stage process in terms of energy generation, total energies from CH_4 and H_2 have been calculated and presented in Table 5.2.5. Pre-aerated P substrate was the best in terms of total energy generation which amounted to 9.64 KJ/gVS, 45.8% higher than non pre-aerated P substrate. The increase of total energy yield was lower for pre-aerated C samples (6.3% higher than without aeration). Conversely, L samples performed better without aeration.

Table 5.2.5: Total energy generation in the process in terms of H_2 and CH_4.

Substrate	Energy from H_2 (kJ/gVS)	Energy from CH_4 (kJ/gVS)	Total Energy (kJ/gVS)
C	0.23	5.98	6.21
C (air)	0.2	6.4	6.6
P	0.038	6.58	6.61
P (air)	0.017	9.63	9.64
L	0.14	7.22	7.36
L (air)	0.14	6.58	6.72

5.2.4 CONCLUSIONS

The efficiencies of a two-stage AD treatment using organic wastes with different compositions in both the presence and absence of aeration as a treatment were compared by evaluating the H_2 and CH_4 production. This study suggested that pre-aeration of organic waste did not constitute an effective treatment for the purpose of improving H_2 production potential during the first stage of the

AD process. However, during the subsequent stage of AD, carbon conversion to CH_4 was higher for pre-aerated P and C samples than in non-pre-aerated ones, resulting in an increase of total energy potential. Conversely, pre-aeration of substrate L was not effective, causing a decrease of energy potential compared to non pre-aerated sample.

Further studies should however be undertaken using shorter and therefore less expensive, pre-aeration times in order to assess whether this may result in a positive effect on both H_2 and CH_4 productions. From the very beginning of the food waste treatment up to digestate management and disposal, the totality of the two-stage AD processes, with and without aeration, should be investigated and compared in terms of a complete Life Cycle Assessment, considering also the chemical and ecotoxicological quality of the produced digestate (Pivato et al., 2016a).

5.3 TWO-STAGE ANAEROBIC DIGESTION OF THE ORGANIC FRACTION OF MUNICIPAL SOLID WASTE – EFFECTS OF PROCESS CONDITIONS DURING BATCH TESTS

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Two-stage anaerobic digestion (AD) batch tests were performed using the organic fraction of municipal solid waste as substrate. Effects of different combination of initial pH (5.5, 7, and 9) and substrate to inoculum (S/I) ratio (from 0.5 to 6 gVS/gVS) were investigated for hydrogen and methane productions during the first and the second stage of AD, respectively.

Results showed that both initial pH and S/I ratio had an impact on hydrogen yield, hydrogen production rate and duration of lag phase. The highest hydrogen yield of 29.8 m^3H_2/tVS was obtained at initial pH of 5.5 and S/I ratio of 6. However, the highest hydrogen production rate (65 m^3H_2/tVS/d) was recorded at pH of 9 and S/I ratio of 6. Increasing the initial pH from 5.5 to 9, led to shorter lag phases for all S/I ratios. Premising that the S/I ratios were all adjusted at the value of 0.5 at the beginning of the second digestion phase, methane production was not significantly influenced by the different S/I ratios set at the beginning of the first digestion phase. When compared to single-phase AD, two-stage AD tests resulted in enhanced methane production rates from 37.3 to 68.5 m^3CH_4/tVS/d, reducing by half both the lag phase and the time required to reach maximum methane production.

5.3.1 INTRODUCTION

Two-stage anaerobic digestion process has recently been suggested as an option to maximize the amount of energy recoverable from biodegradable organic waste in terms of hydrogen (H_2) and methane (CH_4) (Girotto et al., 2016; Rafieenia et al., 2017; Ruggeri et al., 2010). H_2 is a clean energy carrier and has a high-energy density. H_2 has, in fact, the highest calorific value among other fuels and its combustion does not lead to carbon emissions. H_2 can be produced from cheap organic wastes and wastewaters in a process called dark fermentation (Ma et al., 2017; Rafieenia et al., 2017). Biomethane can play a central role in the development of the circular economy principle. It is a source of energy that can be used for power and heat production but also as a gaseous vehicle fuel, it can replace natural gas and be fed into national gas grids or be used as a feedstock for producing chemicals and materials (Weiland, 2010).

Pre-treatments are often applied to enhance biogas productivity of substrates (Rafieenia et al., 2016; Zhang et al., 2016) and fermentation step for H_2 production itself could be seen as a pre-treatment to increase overall biodegradability. During the fermentation stage of AD, organic substances are

hydrolysed and converted to H_2 and volatile fatty acids (VFAs) by hydrogen producing bacteria. Optimisation of the H_2 production phase can lead to an improved hydrolysis and therefore higher energetic exploitation of waste materials.

The advantages traditionally indicated for two-stage digestion systems, if compared to single stage AD, are shorter substrate retention time, enhanced solids degradation efficiencies (Brummeler et al., 1992; Ghosh et al., 1985; Nguyen et al., 2014), enhanced hydrolysis with a subsequently higher CH_4 production (Liu et al., 2006; Massanet-Nicolau et al., 2015; Pakarinen et al., 2011) and potentially higher organic loading rates (Ariunbaatar et al., 2015). Despite these advantages, the higher complexity of two stage digestion plants, if compared to single digestion, limited the diffusion of this option to less than 10% of current digestion capacity (De Baere and Mattheeuws, 2010).

The possibility of simultaneous H_2 and CH_4 productions from the same feedstock, rather renovates the interest of this kind of plant configuration and this option is currently receiving growing interest with several investigations at lab and pilot scale level (Lee et al., 2010; Park et al., 2010; Zuo et al., 2013). Besides reaching higher energy yields, two-stage AD promotes a stronger bio-stabilisation of the treated organic waste (Lim et al., 2013; Wu et al., 2016) and could also lead to the production of metabolites to be used as renewable and biodegradable substitutes for petrochemical products (Girotto et al., 2017, 2015). Notwithstanding, there is still an on-going lack of optimal operational parameters and procedures to promote the successful succession of the two phases without compromising operational condition for the methanogenic stage. In particular, there is a considerable lack of comprehensive studies relating to the effects produced by initial operational parameters of fermentation on the second phase of the process.

Data on H_2 and CH_4 production yields reported in scientific studies on two-stage AD process are illustrated in Table 5.3.1. Results indicate that generally there is a good energy recovery potential from the treatment of organic waste suggesting that the identification of optimal operational conditions and energy potential can stimulate the application of two-stage AD and diffuse the production of renewable H_2 and CH_4 from organic residues.

The aim of this study was to investigate the effect of substrate to inoculum ratio (S/I) and initial pH on hydrogen and methane productions in a two-stage anaerobic digestion process using organic fraction of municipal solid waste (OFMSW) as substrate. In particular, three initial pH values, namely 5.5, 7, and 9, and six S/I ratios, namely 0.5, 1, 2, 4, and 6 gVS/gVS, were analysed. In addition, CH_4 production yields from two-stage AD were compared with those obtained from a single stage AD process treating the same substrate.

Table 5.3.1: Comparison of hydrogen and methane production yields in a two-stage AD process using different organic substrates.

Substrate	**Hydrogen potential production (m^3H_2/tVS)**	**Methane potential production (m^3CH_4/tVS)**	**Reference**
Dairy processing waste	40.15	34.2	(Zhong et al., 2015)
Kitchen waste	36	135	(Li et al., 2015)
OFMSW	43	500	(Liu et al., 2006)
OFMSW	90	560	(Wang and Zhao, 2009)
Potato residues	31	387	(Zhu et al., 2008)
Steam-peeling potato waste	134*	183*	(Giordano et al., 2011)
Common wheat waste	47*	202*	(Giordano et al., 2011)
Vinegar residue	53.2	192	(Wang et al., 2015)

* mL/gCOD

5.3.2 MATERIALS AND METHODS

Substrate and inoculum

OFMSW samples were collected from the waste receiving area of an anaerobic digestion plant treating organic waste located in Padova, Italy. The OFMSW delivered at the plant is source segregated at household level and the collection area involves a population of about 130,000 inhabitants. Samples were properly sorted and stored before use (Alibardi and Cossu, 2015). Samples were chopped with a food grinder and diluted with water at a ratio 1:2 (kg/L) prior to use as substrate lab scale tests. Granular sludge collected from a full-scale Upflow Anaerobic Sludge Blanket (UASB) digester of a brewery located in Padova, was used as inoculum. OFMWS and sludge samples were characterized for the following parameters: Total Solids (TS), Volatile Solids (VS), Total Carbon (TC), Total Kjeldahl Nitrogen (TKN), Chemical Oxygen Demand (COD) (Table 5.3.2**)**.

Table 5.3.2: Average substrate and inoculum characteristics.

Parameter	**OFMSW**	**Granular sludge**
TS (%)	75	15
VS (%TS)	90	53
TC (%TS)	50,2	29,6
TKN (gN/kgTS)	8.7	43
COD (gO_2/kgTS)	300	693

Two-stage digestion batch tests

Experimental design was planned in order to study the combination effects of each investigated initial pH and S/I ratio on two-stage AD process (Table 5.3.3). The following conditions S/I and pH

were tested during the first stage of digestion for H_2 production. S/I ratios were 0.5, 1, 2, 4, and 6 gVS/gVS. Initial pH values were 5.5, 7, and 9.

Two-stage digestion batch tests were carried out using 1L glass bottles sealed with a silicon plug and a working volume of 500 mL. Different S/I ratios were achieved changing the amount of inoculum in each test while substrate concentration was kept constant at 5gVS/L. MES ($C_6H_{13}NO_4S$) was used to obtain an initial pH of 5.5, while (sodium carbonate, Na_2CO_3) was used to reach an initial pH value of 9. No buffer was used for the initial tests at neutral pH (7.0).

For first AD stage (fermentation tests), the inoculum was thermally pre-treated for 4 hours at 100 °C to inhibit methanogenic archaea (Alibardi et al., 2012). To promote CH_4 production during the second AD stage (methane production), S/I ratio was fixed at 0.5 gVS/gVS, while initial pH was set at 8.5 by dosing Na_2CO_3.

A single-stage AD test (methane production only), characterized by a S/I ratio equal to 0.5 and a pH of 8.5, was run in parallel in order to compare methane production yields with hydrogen and methane yields obtained through the two-step process.

The bottles were flushed with N_2 gas for 3 minutes to ensure anaerobic conditions and incubated at a temperature of 35±1ºC. All tests were performed in duplicate.

Table 5.3.3: Initial operational conditions of two-stage and single stage batch tests.

Run	**First stage, fermentation**		**Second stage, methanization**	
	S/I (gVS/gVS)	**Initial pH**	**S/I (gVS/gVS)**	**Initial pH**
A	0.5	5.5	0.5	8.5
B	1.0	5.5	0.5	8.5
C	2.0	5.5	0.5	8.5
D	4.0	5.5	0.5	8.5
E	6.0	5.5	0.5	8.5
F	0.5	7.0	0.5	8.5
G	1.0	7.0	0.5	8.5
H	2.0	7.0	0.5	8.5
I	4.0	7.0	0.5	8.5
L	6.0	7.0	0.5	8.5
M	0.5	9.0	0.5	8.5
N	1.0	9.0	0.5	8.5
O	2.0	9.0	0.5	8.5
P	4.0	9.0	0.5	8.5
Q	6.0	9.0	0.5	8.5
	Single stage, methane production			
	S/I (gVS/gVS)		**Initial pH**	
R	0.5		8.5	

Analytical Methods

TS, VS, COD, TKN and alkalinity were analysed according to standard methods (APHA/AWWA/WEF, 2012). TC and DC were analysed by a TOC analyser (TOC-V CSN, Shimadzu). The volume of biogas produced during two-phase digestion tests was measured by means of the water displacement method (Lavagnolo et al., 2017). H_2, CO_2, and CH_4 concentrations in biogas were measured by a gas chromatograph (HP5890) equipped with thermal conductivity detector (TCD), HP-MOLSIV and HP-PLOT U columns, and nitrogen as carrier gas.

H_2 and CH_4 volumes produced in the time interval between each measurement [t – (t-1)] were calculated using a model taking into consideration the gas concentration at time t and time t-1, together with the total volume of biogas produced at time t, the concentration of specific gas at times t and t-1, and the volume of head space of reactors (Van Ginkel et al., 2005). The following equation (see section 1.1) was applied:

$$V_{C,t} = C_{C,t} * V_{G,t} + V_H * (C_{C,t} - C_{C,t-1}) \quad (1)$$

To compare the results obtained from the batch tests, data were interpolated on the basis of the Gompertz model (Chen et al., 2002). The Gompertz mathematical expression is described in Equation (2):

$$P(t) = P_{max} e\left\{-e\left[\frac{R*e}{P_{max}}\right](\lambda - t) + 1\right\} \quad (2)$$

where P (t) is the cumulated H_2 or CH_4 production at time t; P_{max} is the maximum H_2 or CH_4 production; R is the maximum production rate; and λ is the lag phase. The results related to production rate (R) and duration of the lag phase (λ) were applied to compare the different investigated operative conditions.

Data on H_2 and CH_4 productions are expressed at a temperature of 0 °C and pressure of 1 atm (Normal conditions).

5.3.3 RESULTS AND DISCUSSION

Effect of S/I ratio and initial pH during the first AD stage – fermentation

Hydrogen production yields obtained during the first stage (fermentation) are shown in Table 5.3.4. Hydrogen yields were slightly lower than the values reported in the literature for similar substrates (Table 5.3.1). This could be due either to a decreased hydrolytic activity after the long thermal pre-treatment on the sludge or to the specific substrate composition used in this study. A decreased hydrolytic activity could be a side effect of the heating process at 100 °C for 4 hours. Shah *et al.* (Shah et al., 2016) assessed the viability of isolates from granular sludge after a pre-treatment similar to the one applied in this study (2h and 4h at 100 °C) and observed that isolates were still

active after the heat shock exhibited a broad range of hydrolytic activities. It is, therefore, presumable that the slightly lower H_2 yields compared to similar studies could be due to the specific OFMSW composition. It was observed that yields of H_2 production from OFMSW collected in different seasons varied during the year due to changes in the OFMSW composition (Alibardi and Cossu, 2015). Various studies also confirmed that carbohydrate content of organic wastes directly affects H_2 production suggesting that a lack of fractions rich in sugars or starch could reduce the H_2 productions via biological fermentation (Kobayashi et al., 2012; Okamoto et al., 2000; Rafieenia et al., 2017).

Table 5.3.4: Hydrogen production yields (average values), final pH at the end of the first stage of AD batch tests and results of the data modelling with Gompertz equation (2).

Run	First stage, fermentation		Modelling results		
	Hydrogen production (m^3H_2/tVS)	Final pH	R (m^3H_2/tVS/d)	λ (h)	P_{max} (m^3H_2/tVS)
A	18.0	6.0	55.5	14.2	18.0
B	13.0	6.0	20.6	11.2	13.0
C	14.2	6.0	25.3	11.5	14.2
D	16.4	6.0	19.4	10.2	16.4
E	29.8	5.5	40.3	14.9	29.8
F	7.0	6.0	12.9	9.0	7.0
G	15.9	5.0	50.0	9.6	15.9
H	10.2	5.0	18.0	5.3	10.2
I	28.2	5.0	54.2	7.2	28.8
L	17.5	5.0	64.8	7.2	17.5
M	7.9	6.5	27.3	8.5	7.9
N	14.9	5.5	55.3	8.1	14.9
O	24.0	5.0	60.0	4.8	24.0
P	24.3	5.0	64.8	6.0	24.3
Q	23.6	5.0	65.0	5.9	23.6

In general, two days were enough to complete hydrogen production but a total of four days was waited to ascertain the plateau and no methane production was observed during the fermentation tests, indicating that the sludge pre-treatment was effective in inhibiting methanogens. Moreover, none of the conditions tested during first phase, in terms of S/I ratios and pH, favoured the reactivation of methanogens even after the fermentation stopped (data not shown).

Results of the data modelling with Gompertz equation (2) are reported in Table 5.3.4 and plots of H_2 yields vs. S/I ratios and of lag phase duration (λ) vs. initial pH are shown in Figure 5.3.1a and Figure 5.3.1b, respectively. The highest H_2 yield of 29.8 m^3H_2/tVS was recorded from test E, characterised by a S/I ratio of 6 and an initial pH of 5.5. This test was also characterised by a lag

phase of 14.9 h and a maximum production rate of 40.3 m^3H_2/tVS/d. The lowest H_2 yield was recorded from test F, characterised by a S/I ratio of 0.5 and an initial pH of 7.0 (Table 5.3.1). The H_2 production rate for this test was also the lowest (12.9 m^3H_2/tVS/d). Whilst the low yield and production rate, the lag phase for this test was shorter than that for test E.

In general, high H_2 yields were observed from tests with S/I ratios of 4 and 6 gVS/gVS (Figure 5.3.1a) and short lag phases were observed for tests with an initial pH of 7 or 9 (Figure 5.3.1b). Production rates (R) seemed not to be influenced neither by S/I ratio nor by initial pH, even though faster production rates are generally associated with higher hydrogen production yields.

In the present study, different S/I ratios were obtained by changing the sludge concentration in the reactors while substrate concentration was kept constant. Despite the presumable larger presence of hydrogen producing bacteria at lower S/I ratios, this condition did not lead to higher H_2 yields. A larger variability in bacterial populations present in the mixed microflora with low S/I ratios could have introduced also non-hydrogen forming bacteria competing for the same substrates or hydrogen consuming bacteria and this could have had a measurable impact on hydrogen yields. The higher S/I ratios, on the contrary, were obtained by lower biomass concentrations and this condition could have reduced the possibility of non-hydrogen forming bacteria or hydrogen consuming bacteria to have an effect on hydrogen yields. Alibardi *et al.* (2012) indicated that long heat pre-treatments strongly influence microbial viability, with reductions of order of magnitudes of active bacteria levels. Despite this effect, high bacterial concentrations could allow niches of non-hydrogen forming or hydrogen consuming bacteria to grow sufficiently to produce an effect on hydrogen yield. On the contrary, when biomass concentrations are kept low, these niches are not able to influence overall hydrogen productions that are only defined by the activity of fast growing hydrogen producing bacteria. S/I ratio has therefore a direct effect on microbial activities of different populations present in the mixed microflora and to maximise H_2 production, small concentrations are sufficient to obtain efficient hydrogen conversions (Alibardi et al., 2012). Pan *et al.* (2008) investigated how S/I ratio affects H_2 production from food waste under mesophilic and thermophilic conditions but with no pre-treatment to enhance hydrogen production. Differently from the approach in the present research study, a constant biomass concentration was used by Pan *et al.* (Pan et al., 2008) while substrate concentration was changed. Optimal S/I ratios of 6 and 7 were identified under mesophilic and thermophilic conditions, respectively. Low S/I ratios (< 3) led to high methane productions and at high S/I ratios (> 7) low H_2 yields were observed. These results confirm how an optimal balance between biomass and substrate concentrations needs to be identified to enhance hydrogen production and avoid the activity of other bacterial species not contributing or negatively affective hydrogen fermentation.

Initial pH and S/I ratio also influenced hydrogen production rate (R) and lag phase duration (λ) (Table 5.3.4). When initial pH was increased from 5.5 to 9, a shorter lag phase was observed for all S/I ratios (Figure 5.3.1b). The longest lag phase (14.9 h - Test E) was observed with S/I ratio and initial pH of 6 and 5.5, respectively. The shortest lag phase (4.8 h - Test O) corresponded to S/I ratio and initial pH of 2 and 7, respectively. These results suggest that a neutral to basic pH could speed up the activity of hydrogen forming bacteria after the heat pre-treatment while an initial acid condition imposes a longer acclimating phase before hydrogen production starts. These results are in accordance with Chen *et al.*, (2002) who reported longer lag phases when mixed microflora inoculum was cultivated at pH of 5 (compared to pH 6 and 7) after an enrichment phase at both acid or basic conditions. Similarly, Ferchichi *et al.* (2005) reported a significantly long lag phase of 43.26 h with an initial pH of 5 and a short lag phase of 3.06 h when the pH was 8, using cheese whey as substrate. The initial low pH conditions can result in the protonation of non-dissociated weak acids, which may pass freely through the cell's membrane into its cytoplasm causing its consequent acidification (Foster, 1992). This internal condition could result in loss of activity by the glycolytic enzymes and structural damage of the cell membrane that can lead to prolonged re-activation phases after external stresses to the inoculum and, consequently, longer lag phases (Ferchichi et al., 2005).

Operational pH is also one of the key factors in dark fermentative H_2 production. It may affect hydrogenase activity and metabolic pathways towards different by-products generation (Chaganti et al., 2011). In all tests, pH decreased to values between 5.5 and 6 at the end of the fermentation (Table 5.3.4). These results indicate that, despite the different pH set at the beginning of the tests, the fermentation products established an acid environment even at high initial pH conditions (pH 9). Optimal initial pH of 5.5-6.0 has been reported by many studies for mesophilic dark fermentation (Chaganti et al., 2013; Ferchichi et al., 2005; Ghimire et al., 2015; Rafieenia et al., 2017; Wu et al., 2010). Low pH (4.5-6) leads to a higher concentration of acetic and butyric acids which are soluble metabolites whose production pathways are accompanied by H_2 production (Luo et al., 2011). Moreover, the activities of H_2 consuming microorganisms like methanogens, homoacetogens, and propionic acid bacteria decrease at low pH conditions (Calli et al., 2008; Chaganti et al., 2011). This study also demonstrated that high initial pH speeded up the inoculum reactivation with short lag phases. It is, therefore, presumable that an optimal combination of initial pH and operational pH during the fermentation process, could enhance the overall hydrogen production by combining short lag phases with high hydrogen yields. Further studies are anyway required to confirm or rebut this hypothesis.

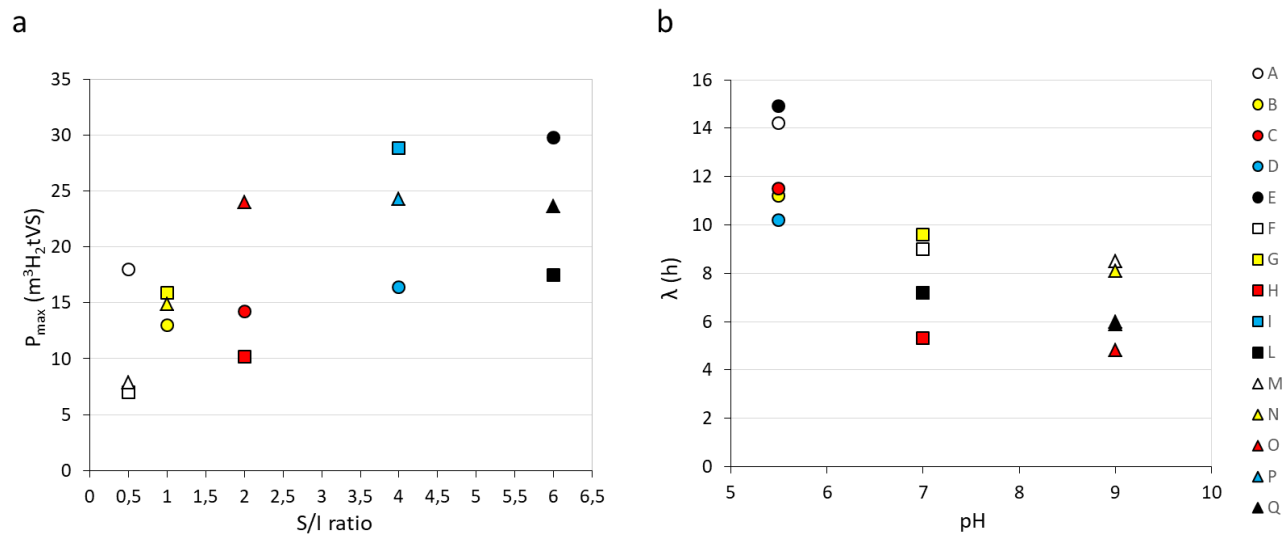

Figure 5.3.1: Distribution of maximum hydrogen (H_2) production (P_{max}) over S/I ratio (a) and lag phase (λ) over initial pH (b).

Effect of S/I ratio and initial pH during the second AD stage – methane production

Methane production yields during the second AD stage are reported in Table 5.3.5. The highest methane production of 620 m^3CH_4/tVS was obtained from test F, while the lowest was measured from test D (463 m^3CH_4/tVS). The average methane production of 544 m^3CH_4/tVS was obtained from all the tests at various S/I ratios and initial pH conditions. The maximum methane production from test R, carried out in a single digestion phase for methane production, resulted 633 m^3CH_4/tVS. The lower methane yields obtained from the double digestion process could be explained by the fact that hydrogen was produced in the first digestion phase. A portion of the total electrons released by the biodegradation process was already passed to H_2 and therefore a reduction of the total methane production from the second phase could be expected.

The outputs from the second digestion phase (Tests A to Q) and the single digestion process (Test R) displayed a similar pattern although the lag phase for single phase digestion was longer and time required to reach maximum methane production was almost doubled (Table 5.3.6). Indeed, for two-stage AD, hydrolysis and acidogenesis occur during the first stage resulting in enhanced VFAs production which can be converted to CH_4 rapidly during the second stage (Liu et al., 2006; Pakarinen et al., 2011; Voelklein et al., 2016). The optimal conditions for hydrolytic bacteria and methanogenic archaea may be different and splitting the process into two phases, provides the opportunity for the specific optimization of each phase. Differently, single-stage AD, for which hydrolysis is the rate limiting process, combines hydrolysis, acidogenesis and methanogenesis with a consequently longer lag phase than that of the second stage of a two-stage AD.

Average methane productions (Figure 5.3.2) decreased in line with an increase of the S/I ratio (applied in the first stage) up to 4. However, an opposite trend was observed when passing from a S/I ratio of 4 to 6, displaying a pattern similar to that observed for H_2 production during the first AD stage (Figure 5.3.1).

Table 5.3.5: Methane production yields, final pH at the end of the second stage of AD batch tests and results of the data modelling with Gompertz equation (2).

Run	Second stage, methane production		Modelling results		
	Methane production (m^3CH_4/tVS)	**Final pH**	**R ($m^3CH_4/tVS/d$)**	**λ (d)**	**P_{max} (m^3CH_4/tVS)**
A	527	8	101.4	7.9	517
B	550	8	76.4	5.4	549
C	499	7.5	68.0	5.4	493
D	489	7.5	52.8	5.0	463
E	582	7.5	62.5	5.1	562
F	619	8	67.4	4.9	620
G	590	8	62.1	4.8	600
H	532	8	56.6	4.7	541
I	474	7.5	50.1	4.2	482
L	523	7.5	53.1	4.2	534
M	606	8	94.9	6.8	614
N	566	8	73.2	5.9	573
O	554	8	75.2	5.8	553
P	532	7.5	67.8	5.9	529
Q	529	7.5	66.6	5.6	529
	Single stage, methane production		**Modelling results**		
	Methane production (m^3CH_4/tVS)	**Final pH**	**R ($m^3CH_4/tVS/d$)**	**λ (d)**	**P_{max} (m^3CH_4/tVS)**
R	626.1	7.0	37.3	12.0	633

Table 5.3.6: Comparison of Gompertz equation modelling results from single-stage and two-stage AD processes (average values between all tests). R - methane production rate, λ - lag phase, and t_{max} - time needed for maximum methane production.

	P_{max} (m^3CH_4/tVS)	**R ($m^3CH_4/tVS/d$)**	**λ (d)**	**t_{max} (d)**
Single-stage AD	633	37.3	12.0	40
Two-stage AD	544	68.5	5.4	20

Comparing trends in Figure 5.3.1a and Figure 5.3.2, it can be highlighted that lower hydrogen productions are associated with higher methane yields for all S/I ratios. In particular, test F, characterised by a S/I ratio of 0.5 and pH of 7, produced the lowest amount of hydrogen (7 m^3H_2/tVS) and the highest amount of methane (619 m^3CH_4/tVS). In accordance with Schievano *et al.* (Schievano et al., 2012), single-stage Biochemical Methane Potential (BMP) outputs featured higher methane yields than those achieved from a two-stage AD process, although with a longer lag phase and lower maximum production rate. The slightly lower CH_4 yields obtained for two-stage AD could be due to the fact that only acetoclastic methanogens are capable of functioning without the help of hydrogenotrophic methanogenic archaea due to the previous recovery of H_2. In fact, in a single-stage AD, CH_4 could be obtained both from VFAs conversion by acetoclastic methanogens and by H_2 and CO_2 conversion by hydrogenotrophic methanogenic archaea. In contrast to our results, Voelklein *et al.* (2016) reported a 23% increase in methane production from a two-stage AD of restaurant food waste rather than a single-stage process. Likewise, Liu *et al.* (2006) recovered 21% more methane from two-stage AD tests performed on mixed organic waste.

The first AD stage, aimed at hydrogen production, may also be viewed as an effective pre-treatment for the subsequent production of methane, providing a VFA-rich substrate ready to be digested by methanogenic archaea. The average CH_4 yield from two-stage AD (544 m^3CH_4/tVS) was lower than the one from single-stage AD (633 m^3CH_4/tVS) (Table 5.3.5 and Table 5.3.6). However, if similar conditions were considered (in terms of S/I = 0.5 with a neutral or basic initial pH), approximately equal yields were obtained from single-stage and two-stage AD (626 and 619 m^3CH_4/tVS, respectively). Methane production rate (R) doubled (Table 5.3.6), whilst both lag phase and time required to reach the maximum methane production were reduced by half when passing from single-stage to two-stage AD process. These findings are in accordance with Leite *et al.* (2016) who achieved a 15% increase of produced energy from single-stage to two-stage AD system. In fact, when splitting the AD process into two-stages, the first stage may be regarded as a pre-treatment to increase the methane production rate and to shorten the lag phase, as confirmed by the results reported in the present paper. The faster production rate, accompanied by a shorter lag phase,

proves a significant overall benefit of two-stage over single-stage AD. By comparing the potential energy output of the two processes, it is possible to highlight how a double phase digestion process could be energetically more favourable if compared to a single-phase digestion when the time for digestion (i.e. digester volume or solid retention time) is fixed. If a period of 2 and 20 days for hydrogen and methane productions, respectively, were taken into account for a two-stage digestion, and 22 days in the case of the single-stage process, the potential energy output for produced fuel gases is reported in Figure 5.3.3. These choices were made on the basis of the average time required to reach maximum hydrogen and methane productions in the two-stage process.

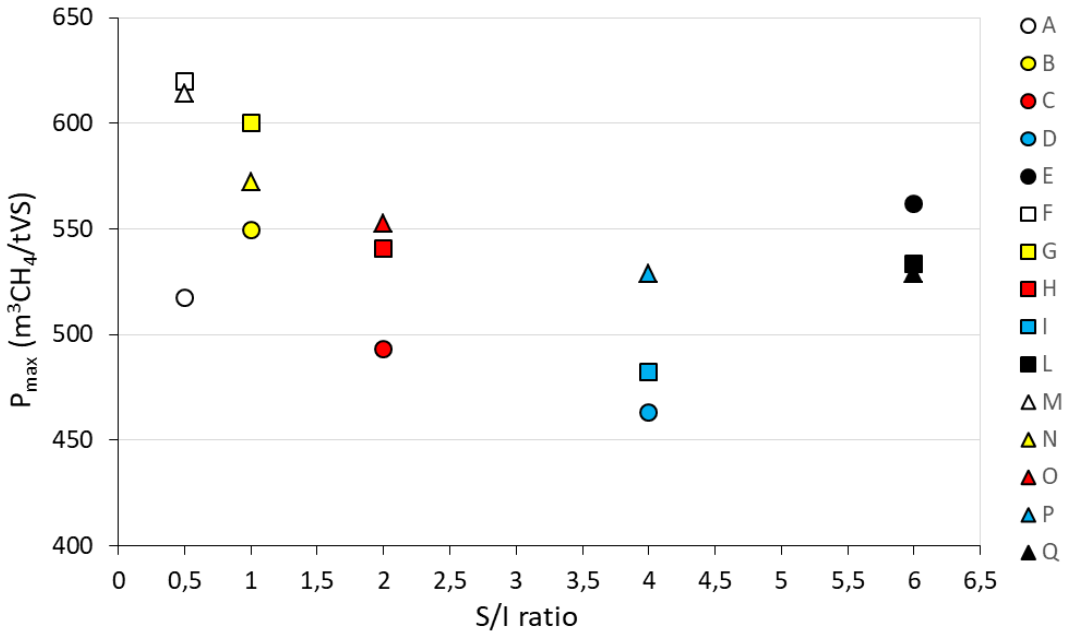

Figure 5.3.2: Distribution of maximum methane (CH_4) production (P_{max}) obtained from the second phase over S/I ratios tested during the first phase.

According to Figure 5.3.3, all two-stage tests were energetically more favourable than single-stage tests. These results confirm that the implementation of a two-stage digestion processes for sequential H_2 and CH_4 production from OFMSW could enhance methanogenic phase performances and increase the overall potential energy production thank to faster digestion processes.

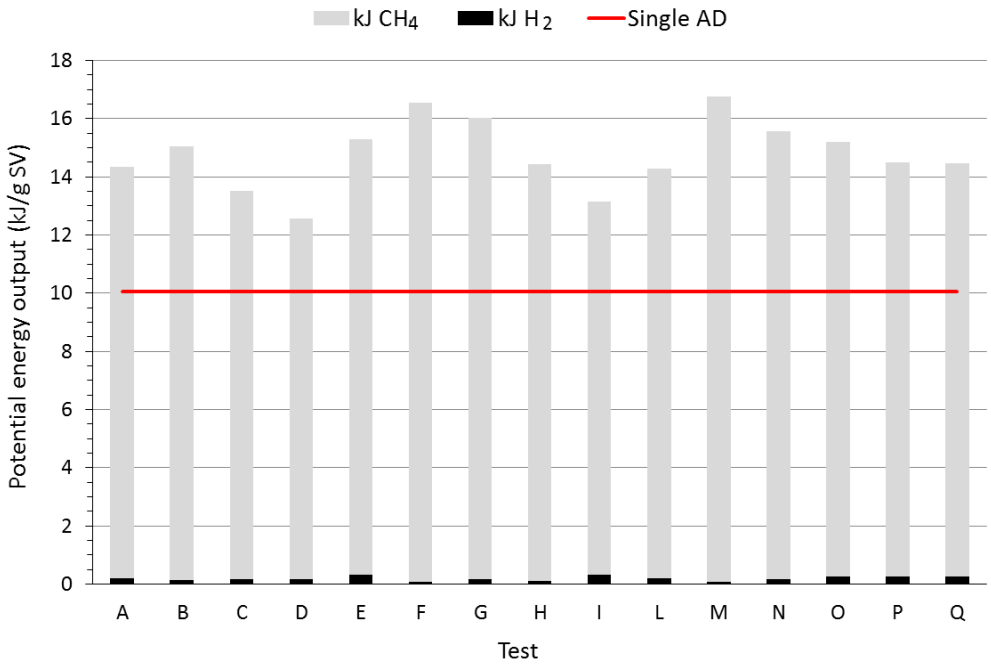

Figure 5.3.3: Potential energy output from single-stage and two-stage tests after 22 days of digestion (2 days first stage, 20 days second stage). H_2 energy density = 120 MJ/kg – CH_4 energy density = 50 MJ/kg.

5.3.4 CONCLUSIONS

The present study investigated the effects of two parameters, initial pH and substrate to inoculumratio, on hydrogen and methane productions obtained from the organic fraction of municipal solid waste in a two-stage AD process. Data analysis revealed how a variation in initial pH value influenced substrate degradation kinetics and total hydrogen production. Kinetics were favoured by initial alkaline conditions (pH=9) linked to faster production rates and shorter lag phase. High S/I ratios were found to facilitate hydrogen production, with the most favourable condition being identified at a S/I ratio of 6. Peak methane production (619 m^3CH_4/tVS) recorded during the second AD stage of BMP test characterized by a S/I ratio of 0.5 and an initial pH of 7, was close to the value of 633 m^3CH_4/tVS obtained during the single-stage process. There was no evident relationship between initial pH values during fermentation and methane production, probably due to pH adjustment performed on completion of fermentation tests, while an increase in S/I ratio from 0.5 to 4 at the beginning of the first stage resulted in a subsequent slight decrease in methane production even though it is worth reminding that the S/I ratios were adjusted at the value of 0.5 in all samples at the beginning of the second stage. The fermentation phase, in addition to promoting hydrogen recovery, represents an efficient means of pre-treatment aimed at enhancing subsequent methane production. In comparison with the single-stage AD process, a two-stage

process elicits faster methane production, a shorter lag phase, and a better energetic exploitation of OFMWS, as demonstrated by the achieved energy output.

5.4 ACIDOGENIC FERMENTATION OF THE ORGANIC FRACTION OF MUNICIPAL SOLID WASTE AND CHEESE WHEY FOR BIO-PLASTIC PRECURSORS RECOVERY - EFFECTS OF PROCESS CONDITIONS DURING BATCH TESTS

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The problem of fossil fuels dependency is being addressed through sustainable bio-fuels and bio-products production worldwide. At the base of this bio-based economy there is the efficient use of biomass as non-virgin feedstock. Through acidogenic fermentation, organic waste can be valorised in order to obtain several precursors to be used for bio-plastic production. Some investigations have been done but there is still a lack of knowledge that must be filled before moving to effective full scale plants.

Acidogenic fermentation batch tests were performed using food waste (FW) and cheese whey (CW) as substrates. Effects of nine different combinations of substrate to inoculum ratio (2, 4, and 6) and initial pH (5, 7, and 9) were investigated for metabolites (acetate, butyrate, propionate, valerate, lactate, and ethanol) productions.

Results showed that the most abundant metabolites derived from FW fermentation were butyrate and acetate, mainly influenced by the S/I ratio (acetate and butyrate maximum productions of 21.4 and 34.5 g/L, respectively, at S/I=6). Instead, when dealing with CW, lactate was the dominant metabolite significantly correlated with pH (lactate maximum production of 15.7 g/L at pH=9).

5.1.1 INTRODUCTION

During the last decades many studies about anaerobic digestion (AD) treatment of biomass have been published with a view on underlying the effectiveness of treating those residues with the double benefit of reducing the amount of waste to be disposed and valorising those biomass as a bio-energy source.

AD process includes four sequential stages: hydrolysis, acidogenisis, acetogenesis, and methanogenesis. Up to now, full-scale applications have been mainly developed to maximise the yield of the last stage in order to obtain the valuable energetic gas to be used as a fuel, namely methane. However, during the initial steps of AD, before methanogenesis, many valuable products with a wide spectrum of possible uses can be gathered.

In a bio-based economy, biomass is the sustainable non-virgin feedstock to be converted into energy or into raw materials for products (Márquez Luzardo and Venselaar, 2012). According to the biocascading pyramid illustrated in Figure 5.4.1, there are several routes through which it is possible to increase the resource use efficiency of biomass (Annevelink et al., 2017; Márquez Luzardo and Venselaar, 2012).

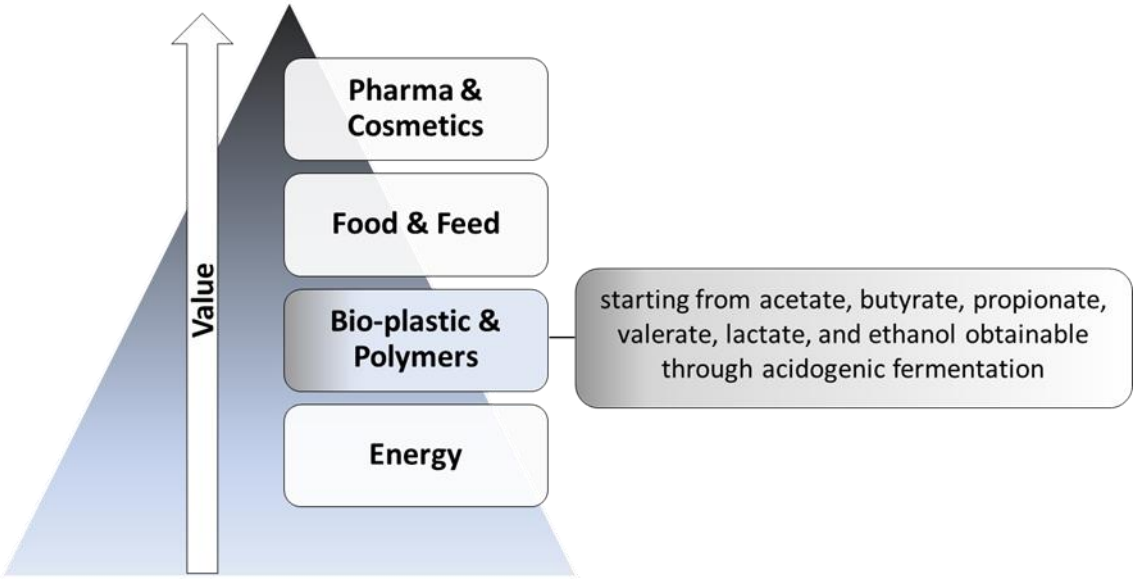


Figure 5.4.1: Biocascading pyramid referred to biomass-based by-products (modified from Annevelink et al., 2017; Márquez Luzardo and Venselaar, 2012). The highlighted block is the main focus of the present study.

Therefore, in the view of a bio-refinery process, valorisation of putrescible organics via acidogenic fermentation opens a wide variety of routes starting from the different end-products namely hydrogen and biological metabolites. Many types of microorganisms and biochemical pathways are involved in the acidogenesis and, consequently, a large number of valuable products are usually formed (Arroja et al., n.d.).

Focusing the attention on the latter, volatile fatty acids (VFAs), ethanol and lactate are not only the optimal feed for methane production through archaea (Stoyanova et al., 2017) but can be valorised as sustainable precursors for bio-plastic production (Tang et al., 2017; Wu et al., 2016a, 2015). In fact, these platform molecules can be versatile tool in many product formation routes with a higher added value than methane (Tamis and Joosse, 2015). This idea is at the base of the so called carboxylate platform concept (Agler et al., 2011; Holtzapple and Granda, 2009), which is aimed at supporting the conversion of organic feedstocks to short-chain carboxylates as intermediate feedstock chemicals, using hydrolysis and fermentation with undefined mixed cultures in engineered systems under anaerobic conditions (Agler et al., 2011; Angenent et al., 2004; Kleerebezem and Loosdrecht, 2007; Tamis and Joosse, 2015). Outputs can vary (Tamis and Joosse, 2015) from bio-polymers (Kleerebezem and Loosdrecht, 2007; Reis et al., 2003), to medium chain length fatty acids (Grootscholten et al., 2014; Spirito et al., 2014) which might be used as antimicrobials (Woolford, 1975), up to corrosion inhibitors (Kuznetsov and Ibatullin, 2002) and other bio-based chemical production processes (Levy et al., 1981). In these terms, acidogenic

fermentation becomes a tool to exploit discarded biomass (Wang et al., 2017) as a non-virgin feedstock, save limited natural resources, and reduce greenhouse gases emissions by lowering the amount of bio-waste to be discarded and row material to be processed.

With a focus on the environmental problems related to plastic disposal, which are due to its non-degradable behaviour (Tripathi et al., 2013), attention is now paid on the development of completely biodegradable plastics such as bio-polyethylene (Bio-PE) (Isikgor and Becer, 2015; Önal et al., 2014), poly-lactic acid (PLA) (Daniels et al., 2014; Guo et al., 2014; Liang et al., 2015), polyvinyl acetate (PVA) (Chen et al., 2016b; Sawatdeenarunat et al., 2017; Singh and Wahid, 2015), polyhydroxyalkanoates (PHA) (Amache et al., 2013). They all are polymers of biological metabolites namely ethanol, lactate, acetate, and VFAs (in general), respectively. In this sense, direct biological production of hydrogen through dark fermentation appears to be only a pre-treatment step within a larger bioenergy and biochemical production concept (Angenent et al., 2004). Monomers recovery for bio-polymers production can be achieved starting both from agro-industrial and household organic wastes (Table 1) either through fermentation of carbohydrate-rich feedstocks by microbes, often genetically modified, or by chemical processing of animal fats and vegetable oils (Fuessl et al., 2012; Girotto et al., 2015).

Table 5.4.1: Literature review of waste biomass tested as a substrate for bioplastic production.

Substrate	Biological metabolites after fermentation	Potential biopolymer	Reference
cheese whey	acetic, butyric and lactic acids	PVA, PHA, PLA	(Colombo et al., 2016; Oliveira et al., 2016; Patel et al., 2016; Valentino et al., 2015)
corn stover	acetic acid	PVA	(Zhao et al., 2014)
dried sugar beet pulp, wheat bran and distiller's dried grains	propionic and butyric acids	PHA	(Aarle et al., 2015)
food waste	acetic, propionic, butyric, and valeric acids	PVA, PHA	(Reddy and Mohan, 2012; Shen et al., 2016; Tang et al., 2017; Yin et al., 2016)
food waste and rice straw	lactic, butyric and acetic acids	PVA, PHA, PLA	(Chen et al., 2015)
fruit and vegetable waste	lactic acid and ethanol	PLA, Bio-PE	(Wu et al., 2016b, 2015)
fruit pomace and waste frying oil	acetic, propionic, butyric, and valeric acids	PVA, PHA	(Follonier et al., 2014)
spent coffee grounds oil	acetic, propionic, butyric, and valeric acids	PVA, PHA	(Cruz et al., 2014; Obruca et al., 2015, 2014)
tofu and egg white	acetic, propionic, butyric, and valeric acids	PVA, PHA	(Shen et al., 2016)

At present, amongst the various types of biodegradable plastics, PHA is one of the most promising (Girotto et al., 2015) and the waste biomass tested as potential non-virgin feedstock after their acidogenic fermentation pre-treatment have been many.

According to the characteristic of the fermented substrate and the process parameters settings, the yield of produced metabolites can vary also in terms of component percentage and quality. Acetate is mainly obtained when hydrogen partial pressure is maintained at low levels, while propionate and butyrate are formed during proteins hydrolysis and subsequent fermentation of amino acids, regardless hydrogen partial pressure (Agler et al., 2011; Nagase and Matsuo, 1982). Propionate is, instead, mainly produced when hydrogen partial pressure is quite high (Agler et al., 2011) and lactate fermentation dominates when applying mixed cultures for treating high concentrations of easily degradable substrates (Agler et al., 2011; Russell and Hino, 1985). Notwithstanding there is not an accurate outline of the best acidogenic fermentation conditions yet which may vary from one substrate to another in order to recover the highest amount of biological metabolites. Among the several parameters affecting the process outputs (Aarle et al., 2015) there are pH (Chen et al., 2015; Wu et al., 2016a), organic load (Arroja et al., n.d.; Rincón et al., 2008), temperature, inoculum as a single or mixed culture (Dahiya et al., 2015; Jun Yin et al., 2016). Moreover, stepping from batch (Rajagopal et al., 2014; Shen et al., 2016; Tang et al., 2017; Yin et al., 2016) to CSTR (Chen et al., 2016b; Krishnan et al., 2017; Wu et al., 2015) reactors highly influences the tests outcomes as well as the following full scale-up which has been put in operation nowhere yet. Indeed, theoretical bio-polymers production is much different from the product output potentially obtainable in reality. Many studies have reported that an acidic initial pH around 5 is the best value to reach the highest yield (Kuruti et al., 2015; Wu et al., 2015; Zhao et al., 2014) in terms of acidogenic fermentation process performances but this data is not definitive since many other researches have recognized an initial basic pH as best (Lavagnolo et al., 2009; Rajagopal et al., 2014).

This study aimed to fill the gap in the knowledge of the influence of the substrate to inoculum ratio and initial pH on acidogenic fermentation of two high volumes generated biomass, namely food waste and cheese whey, specifically addressed for producing bio-plastic precursors.

5.1.2 MATERIALS AND METHODS

The research program investigated the effect of three S/I ratios, namely 2, 4, and 6 gVS/gVS, and three initial pH values, namely 5, 7, and 9 on acidogenic fermentation performances during dark fermentation batch tests using a representative blend of food fractions (Favaro et al., 2013) and cheese whey as substrates. The choice of cheese whey was made on the basis that around 100

million tons/year of cheese whey are generated worldwide (Davila-Vazquez et al., 2009) and 8.5 million tons/year only in Italy (Troiani, 2015).

Bio-hydrogen production during acidogenic fermentation, and the fermented broth composition in terms of ethanol, VFAs, and lactate were evaluated.

Substrate and inoculum

A representative blend of food waste fractions (FW) was prepared on the basis of the composition of the organic fraction of municipal solid waste (OFMSW) in Padova municipality, Italy (Favaro et al., 2013). The representative FW was prepared as shown in Table 5.4.2 on the basis of the data reported by Favaro et al. (2013) which were mediated over the year of investigation.

Table 5.4.2: Representative blend of food waste fractions (FW) used as a substrate for the fermentation tests. Percentages adapted from Favaro et al. (2013)

OFMSW categories	Representative FW composition	Percentage
Meat/Fish/Cheese	Chicken breast	15.6%
	Tuna	
	Butter	
Fruit	Apple	30.2%
	Banana	
Vegetables	Minestrone (lyophilized)	40.8%
Pasta/Bread	Pasta	13.4%
	Bread	

Table 5.4.3: Chemical characteristics of food waste (FW) and cheese whey (CW) used in the tests.

Parameter	FW	CW
TS (%)	26.6	7.5
VS (%$_{TS}$)	96.8	91.9
TOC (% $_{TS}$)	46	78.9
TKN (% $_{TS}$)	3.6	2.1
lipids (% $_{TS}$)	24.3	8.7
proteins (% $_{TS}$)	15.6	12.5
glucose (% $_{TS}$)	4.3	nd
fructose (% $_{TS}$)	7.9	nd
sucrose (% $_{TS}$)	9.9	nd
lactose (% $_{TS}$)	nd	61.2

nd=not detected

Fresh crude (unskimmed) cheese whey (CW) was obtained from a dairy-products factory located in Casale di Selvazzano, Italy.

FW and CW were characterized for the following parameters: Total Solids (TS), Volatile Solids (VS), Total Organic Carbon (TOC), Total Kjeldahl Nitrogen (TKN), lipids, proteins, and sugars (Table 5.4.3).

Granular sludge (121.6 gVS/L) collected from a full-scale Upflow Anaerobic Sludge Blanket (UASB) digester of a brewery located in Padova, was used as inoculum for the acidogenic fermentation tests. The use of undefined mixed cultures was preferred because the synergetic effect between the microorganisms can improve acidogenic fermentation (Yin et al., 2016) and because they can tolerate the complexity and variability of substrates and process conditions owing to the metabolic flexibility conferred by the many members of the community (Agler et al., 2011).

Fermentation tests

Experimental design was performed in order to study the effects of different combinations of substrate to inoculum (S/I) ratio and initial pH on acidogenic fermentation of FW and CW (Table 5.4.4). Investigated S/I ratios were 2, 4, and 6 gVS/gVS; while the initial pH values were 5, 7, and 9.

S/I ratio was adjusted changing the amount of inoculum in each test and keeping the substrate quantity constant in the reactors.

Acidogenic fermentation batch tests were carried out using 500 mL glass bottles sealed with a silicon plug and a working volume of 250 mL. Different S/I ratios were achieved changing the amount of inoculum in each test while substrate concentration was kept constant at 10gVS/L in each bottle. Hydrochloric acid (HCl) was used to obtain an initial pH of 5, while sodium hydroxide (NaOH) was used to reach an initial pH value of 9. No buffer was used for the initial tests at neutral pH. pH was adjusted after inoculum addition.

In order to stop the anaerobic digestion process to the fermentation step, the inoculum was thermally pre-treated for 30 minutes at 80 °C to inhibit methanogenic archaea (Alibardi et al., 2012). The bottles were flushed with N_2 gas for 3 minutes to ensure anaerobic conditions and incubated at a temperature of 35 ± 1 °C. Liquid samples were collected at the end of the tests and analysed for the concentration of ethanol, VFAs and lactate. All tests were performed in triplicate. In particular, replicates for each condition were three for CW and six for FW. Since, following literature results, batch scale acidogenic fermentation tests should last 2 days for CW (Ferchichi et al., 2005; Yang et al., 2007) and between 2 and 4 days for FW (Lavagnolo et al., 2009; Giordano et al., 2014; Kuruti et al., 2017), the investigation was planned in order to stop all the CW replicates and three out of six FW replicates after 2 days; the other 3 FW replicates were stopped after 4 days. For a better comprehension of the metabolites production process, the composition of the fermented broths after 2 and 4 days FW tests were then detected and compared.

Blank tests using inoculum alone (without substrate) were also prepared to measure the quantity of biogas produced only by the biomass.

Table 5.4.4: Initial operative conditions of acidogenic fermentation batch tests both for food waste (FW) and cheese whey (CW).

Run	**S/I (gVS/gVS)** **T**wo-**F**our-**S**ix	**Initial pH** **F**ive-**S**even-**N**ine
TF	2.0	5.0
TS	2.0	7.0
TN	2.0	9.0
FF	4.0	5.0
FS	4.0	7.0
FN	4.0	9.0
SF	6.0	5.0
SS	6.0	7.0
SN	6.0	9.0

Note: The codex of the samples (consisting of two letters) refers to the initial letter of the S/I ratio value followed by the initial letter of the pH value.

5.1.3 ANALYTICAL METHODS

TS, VS, TOC, TKN, and alkalinity were analysed according to standard methods (APHA/AWWA/WEF, 2012).

Concentrations of proteins, lipids and sugars were obtained according to official methods (Chemists, 1920).

The volume of the gas produced during fermentation was measured by means of the water displacement method (Alibardi et al., 2012). H_2 and CO_2 concentrations in the gas were measured using a gas chromatograph HP5890 (Hewlett Packard, USA) equipped with thermal conductivity detector (TCD), HP-MOLSIV and HP-PLOT U columns, and nitrogen as carrier gas.

Volumes of H_2 produced in the time interval between each measurement [t – (t-1)] were calculated using a model taking into consideration the gas concentration at time t and time t-1, together with the total volume of the gas produced at time t, the concentration of specific gas at times t and t-1, and the volume of head space of reactors (Van Ginkel et al., 2005).

Data on H_2 production are expressed net of the inoculum contribution (subtracting the volumes measured from the blank tests) at a temperature of 0 °C and pressure of 1 atm (Normal conditions).

At the end of fermentation tests, liquid samples were taken for ethanol, VFAs (acetate, propionate, butyrate, and valerate), lactate and dissolved organic carbon (DOC) analyses. Samples were centrifuged at 6000 rpm for 10min, the supernatants were filtered using 0.45 μm Phenex-RC filters (Phenomenex, Castel Maggiore, Italy), and stored at −20 °C until analysis. Ethanol, VFAs, and

lactate concentrations were analysed by injection into a high-performance liquid chromatography system (Shimadzu, Tokyo, Japan) complete with an LC 9A Shimadzu pump, a SIL 10A auto-sampler, and a RID-model Shimadzu 10A detector. Analytes separation was performed at 40 °C using an Aminex HPX-87H column (300 × 7.8 mm) and one pre-column (Bio-Rad, Hercules, CA, USA). DOC values in filtered samples after fermentation were evaluated using a TOC analyser (TOC-V CSN, Shimadzu).

5.1.4 STATISTICAL ANALYSIS

Statistical analyses were performed using the Statgraphics Centurion XVII software program (Wilkinson, 1992) to find the intensity of correlation between the production of the main metabolites and the investigated variables, namely pH and S/I ratio, both for FW and CW. The different parameters were correlated in accordance with the general linear model procedure (Steel and Torrie, 1980).

5.1.5 RESULTS AND DISCUSSION

Effect of different combinations of S/I ratio and pH on FW hydrogen production yields

Hydrogen production behaviours during acidogenic fermentation tests are shown in Figure 5.4.2. The produced gas quality, tested with the gas chromatograph, enabled to verify that the thermal pre-treatment of the inoculum was succeeding in inhibiting the archaea activity being the methane concentration lower than 3% throughout the test.

As already mentioned in the previous paragraph, tests lasted two and four days before evaluating the fermented broths compositions, but three days were enough to reach the plateau in hydrogen production. The highest hydrogen yield of 93.1 m^3H_2/tVS was recorded from sample SF characterised by a S/I ratio of 6 and initial pH of 5. This result is in line with the outcomes reported in a previous study (Lavagnolo et al., 2009) where the S/I ratio of 6 and pH 5.5 were the best conditions during dark fermentation of FW. Pan et al. (2008) also reported a S/I ratio of 6 as the best condition to gain the highest hydrogen yield from mesophilic fermentation of FW. Previous studies dealing with the OFMSW reached a maximum yield of 61 m^3H_2/tVS with an initial pH of 7 (Shah et al., 2016) and values around 50 m^3H_2/tVS by setting the initial pH at around 5 (Castillo-Hernández and Mar-Alvarez, 2015; Elsamadony and Tawfik, 2015). From the graphs shown in Figure 5.4.2 the general behaviour was a sharp hydrogen production initial increase when initial pH was set at 7 and 9, while a much longer lag phase was reported from the samples characterized by an initial pH of 5. S/I ratios of 4 and 6 were the best performing demonstrating that a certain quantity of inoculum should be better fed with a high quantity of substrate.

The relationship between S/I ratio and hydrogen production was similar independently of initial pH values (see Figure 5.4.2). Batch scale tests characterized by S/I ratios of 4 and 6 corresponded to the highest hydrogen yields when the initial pH was 9 and 5, respectively. However, when S/I ratio was 6 the initial pH of 9 was also showing a yield (90.6 m^3H_2/tVS) very close to the maximum one obtained at pH 5.

As reported by Elbeshbishy et al. (2017), higher organic loading rate is preferred for energy-efficient operation of the fermentation process, however, high substrate concentrations may be unfavorable to H_2 production, since the activity of hydrogen-producing microbes may be inhibited in several ways including accumulation of VFAs, lower intracellular pH, and high H_2 partial pressure. In this specific case, a S/I ratio of 6 was not yet excessive.

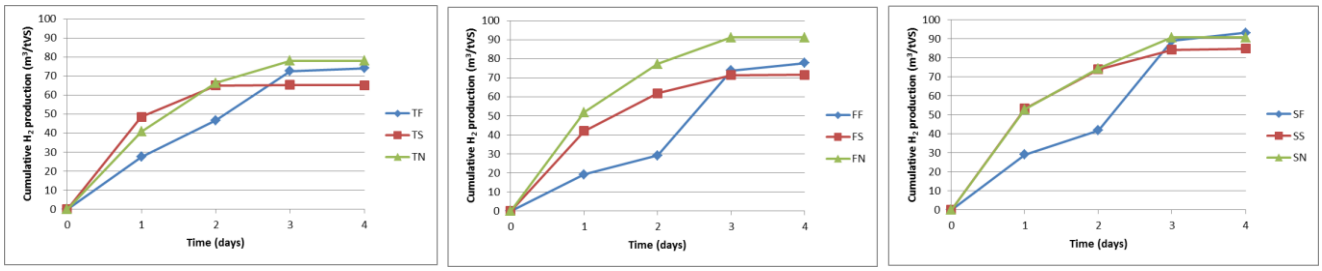


Figure 5.4.2: Food waste hydrogen production trends according to different S/I ratios and initial pH. TF (S/I=2; pH=5) - TS (S/I=2; pH=7) - TN (S/I=2; pH=9) - FF (S/I=4; pH=5) - FS (S/I=4; pH=7) - FN (S/I=4; pH=9) - SF (S/I=6; pH=5) - SS (S/I=6; pH=7) - SN (S/I=6; pH=9)

From Figure 5.4.3 it is possible to assert that the S/I ratio equal to 2 yielded a lower hydrogen production in respect of all tested initial pH (5, 7, and 9) settings. When S/I ratio was 6 the best initial pH was 5 leading to a yield of 93.1 m^3H_2/tVS. 9 was, instead, the best initial pH in correspondence of a S/I ratio equal to 4. In the latter case the hydrogen yield was 90 m^3H_2/tVS, similarly to that obtained with a S/I ratio of 6 at pH 9 as well.

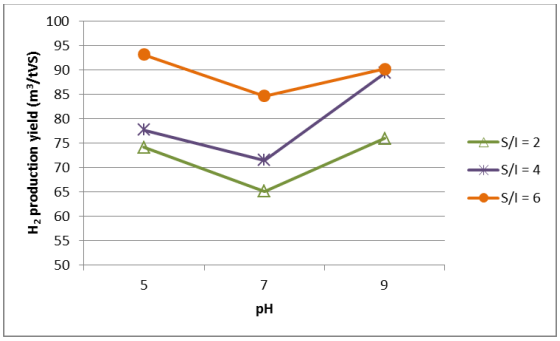


Figure 5.4.3: Food waste hydrogen production trends (after 4-days fermentation) according to different S/I ratios and initial pH.

Effect of different combinations of S/I ratio and pH on CW hydrogen production yields

One day was enough to reach the maximum hydrogen production yield as reported also by Davila et al. (2008), Venetsaneas et al. (2009), and De Gioannis et al. (2014).

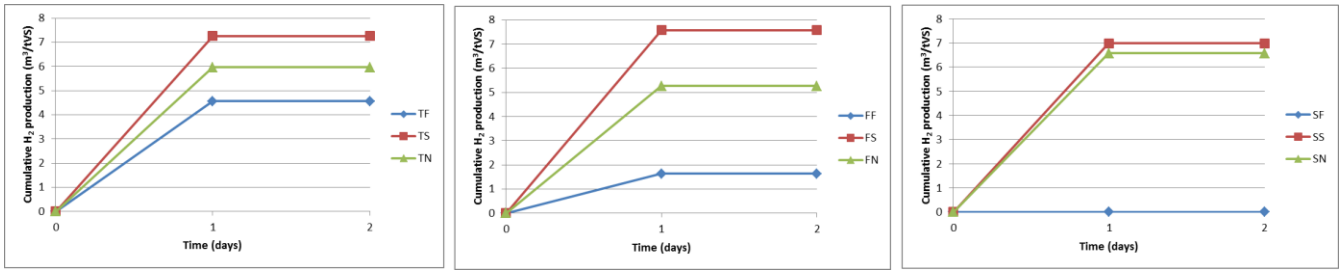


Figure 5.4.4: Cheese whey hydrogen production trends according to different S/I ratios and initial pH.

TF (S/I=2; pH=5) - TS (S/I=2; pH=7) - TN (S/I=2; pH=9) - FF (S/I=4; pH=5) - FS (S/I=4; pH=7) - FN (S/I=4; pH=9) - SF (S/I=6; pH=5) - SS (S/I=6; pH=7) - SN (S/I=6; pH=9)

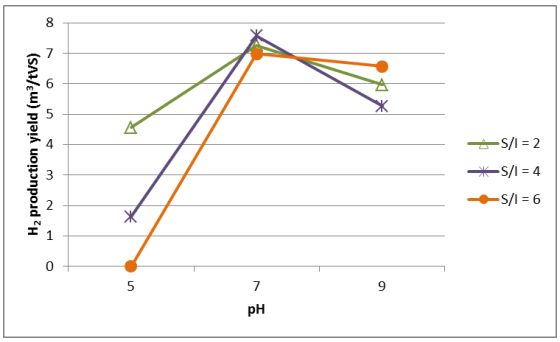


Figure 5.4.5: Cheese whey hydrogen production trends (after 2-days fermentation) according to different S/I ratios and initial pH. T (S/I=2) - F (S/I=4) - S (S/I=6)

Acidogenic fermentation results are shown in Figure 5.4.3. After two days the process was stopped and liquid samples were taken for ethanol, VFAs and lactate analysis.

The highest hydrogen yield of 7.6 m^3H_2/tVS was recorded from sample FS characterised by a S/I ratio of 4 and initial pH of 7. These results, assessing a lower hydrogen production yield in correspondence of acid and basic pH values, are in accordance with what reported in the literature (Davila-Vazquez et al., 2008; De Gioannis et al., 2014; Mongi Ferchichi et al., 2005). In particular, De Gioannis et al. (2014) attained a similarly significant fermentative hydrogen production from CW at pH 7.

From the graphs shown in Figure 5.4.4 outputs from samples characterized by a pH of 7 are the best performing ones in terms of hydrogen production and do not vary much in respect of the various S/I ratios. Differently from what achieved using FW as a substrate, pH 5 was the worst initial setting

which implied a decreasing hydrogen production as the S/I ratio was increasing; the yield was even null when the S/I ratio was 6. These results highlight the importance of optimising the initial pH to facilitate maximum hydrogen production in accordance with the specific treated substrate. Initial pH affects the hydrogen potential and yield, lag phase and fermentation times (Mongi Ferchichi et al., 2005).

From Figure 5.4.5 again the very low hydrogen production at pH 5 is clearly visible. An initial pH of 7 stands out as the best initial pH for CW acidogenic fermentation with a yield of 7.0, 7.3, and 7.6 m^3H_2/tVS when the S/I ratio was 6, 2, and 4, respectively. Differently from the outputs referred to FW, a S/I ratio equal to 2 was generally performing well in correspondence of all tested initial pH settings. Hydrogen production yield was generally quite low probably due to the bacterial inhibition caused by the excretion of bacteriocins (Noike et al., 2002), defined as a polypeptide antibiotic (Jack et al., 1995), and largely applied in food preservation, especially by dairy products industries (Jung and Sahl, 1991).

Even though heat pre-treatment is very effective in suppressing the activity of lactic acid bacteria which can partially or completely inhibit dark fermentation (Elbeshbishy et al., 2017; Enitan and Adeyemo, 2011; Fadda et al., 2010; Gomes et al., 2015), this results ineffective when those microorganisms are present inside the treated organic waste itself such as in the case of CW. This is also a reason why CW alone, is not the optimal substrate to be fermented if the final goal is to obtain high hydrogen volumes.

The average pH value measured at the end of the tests was 3.8, a very low value that clearly justifies such a low hydrogen production. When the starting point was pH equal to 5, the drop was faster and faster alongside with the increasing amount of substrate to microorganism ratio. In fact, it was demonstrated that gradual pH decrease inhibits hydrogen production since pH affects the activity of iron containing hydrogenase enzymes (Dabrock et al., 1992; Kapdan and Kargi, 2006).

Indeed, both during FW and CW acidogenic fermentation, the pH dropped significantly to an average of 4.7 and 3.8, respectively, showing that FW has a certain buffer capacity that is clearly missing in the case of CW. VFAs production is combined with the release of CO_2, which is soluble in water, reacts with hydroxide ions, and forms HCO_3^- which tends to restore the neutrality of the process pH (Elbeshbishy et al., 2017; Elsamadony and Tawfik, 2015; Igoni et al., 2008). Notwithstanding, the use of the water displacement method for measuring the volumes of produced gas implied a CO_2 removal from the reactors.

Effect of different combinations of S/I ratio and pH on biological metabolites production – food waste fermented 4 days

As reported in Figure 5.4.6 the most abundant products deriving from four days acidogenic fermentation of FW were acetic and butyric acids which is in line with what reported by Rafieenia et al. (2017) and Aarle et al. (2015). pH also affects the type of organic acids produced (Horiuchi et al., 2002; Kapdan and Kargi, 2006). As reported by Kapdan and Kargi (2006), when dealing with substrates rich in carbohydrates, more butyrate is produced at initial pH 4.0–6.0. Concentration of acetate and butyrate could be almost equal at pH 6.5–7.0 (Fang and Liu, 2002). Data shown in Figure 5.4.6 reveal that butyrate concentration ranged between 58 and 70% at pH 5 while it decreased to values between 57 and 60% at pH 7.

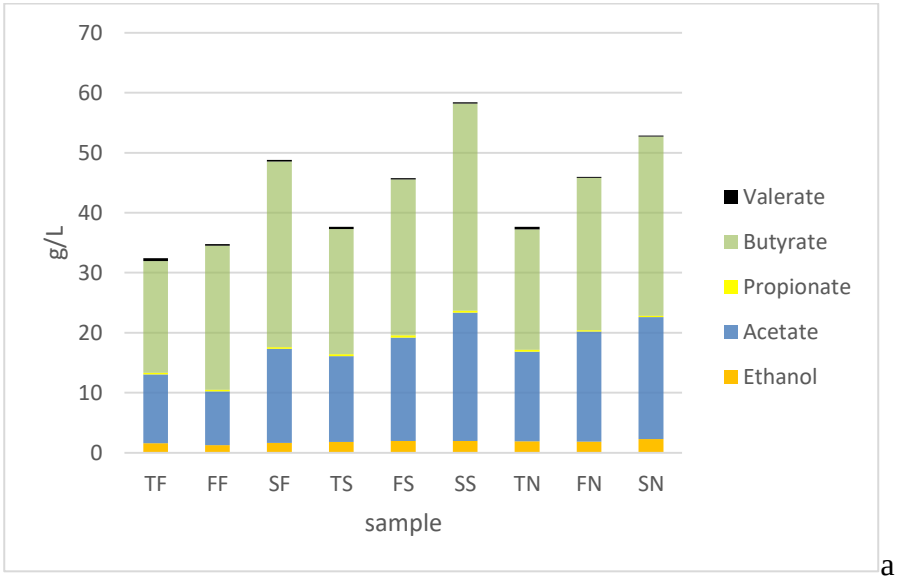
Propionate and valerate productions were almost negligible, while a low ethanol production ranged between 1.2 and 2.3 g/L in samples FF and SN, respectively. Lactate was not detectable in any of the samples. Acetate and butyrate production trends were increasing in parallel with the increase of the S/I ratio when initial pH was set equal to 7 and 9. Sample SS, characterized by a S/I ratio of 6 and a pH of 7, revealed the highest acetate and butyrate productions which were 21.4 and 34.5 g/L, respectively. Figure 5.4.6 also reveals that pH 5 is the less favourable in terms of metabolites production for each S/I ratio. The interesting trend that was clearly detectable for each initial pH, was the increasing amount of metabolites produced in correspondence of increasing S/I ratios.

In accordance with what reported by Zheng et al. (2015) and Wu et al. (2016), butyrate-type fermentation was observed at initial pH 5 for all the investigated S/I ratios. The highest concentration of acetate was achieved at initial pH 9 for each S/I ratio.

The opposite trend between the H_2 yield behaviour (Figure 5.4.3) for the same S/I ratio at different initial pH (downward concavity from pH 5 to 9) and the amount of metabolites produced (upward convexity from pH 5 to 9) was very interesting. This trend was mainly recognised from samples with a S/I ratio equal to 6. The reason can be encountered in the hydrogen partial pressure effect; the higher the pressure, the higher the amount of longer chain VFAs. In fact, an increase of the hydrogen partial pressure can lead to inhibition of VFAs (propionate and butyrate) degradation (Fukuzaki et al., 1990; Luo et al., 2011; Siriwongrungson et al., 2007). Ahring and Westermann (1988) were the first to prove that the higher was the hydrogen partial pressure in their reactors, the higher was the butyrate consumption inhibition.

As reported by Elbeshbishy et al. (2017), acetate and butyrate are the major VFAs deriving from glucose fermentation, thus, the production of these VFAs may indirectly reflect the H_2 productivity in dark fermentation. Accumulation of these soluble metabolites can inhibit the metabolic activity of H_2 producing bacteria, possibly due to the increase in ionic strength or inhibition by un-dissociated acids which may penetrate the microbial cell wall at low extracellular pH, and releasing the protons inside the cell at higher intracellular pH with consequent net adverse effect on the

microbial metabolism and growth (Ciranna et al., 2014; Elbeshbishy et al., 2017; Khanal et al., 2004; Santos et al., 2014; Srikanth and Mohan, 2014; Zhao and Ruan, 2014).



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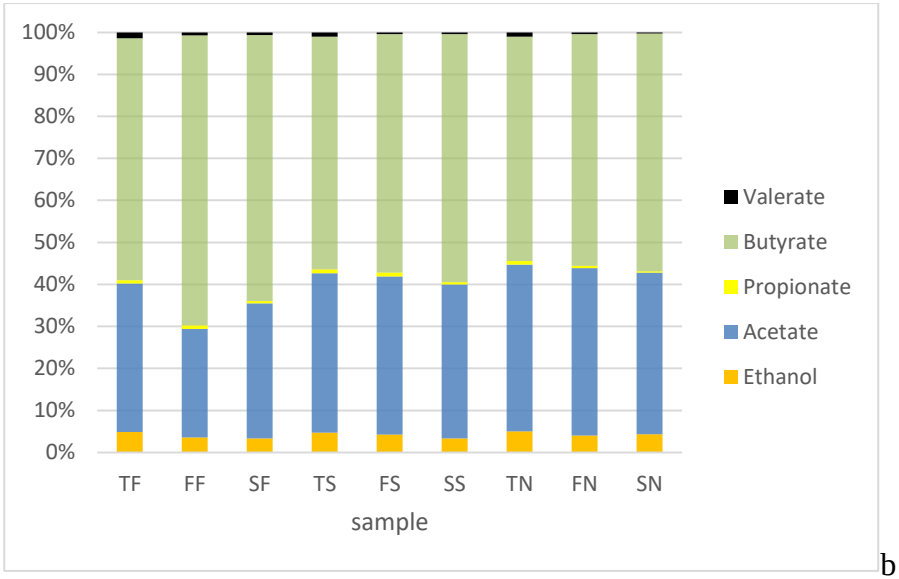


b

Figure 5.4.6: Actual values (a) and percentage distribution (b) of biological metabolites generated after 4 days acidogenic fermentation of food waste.

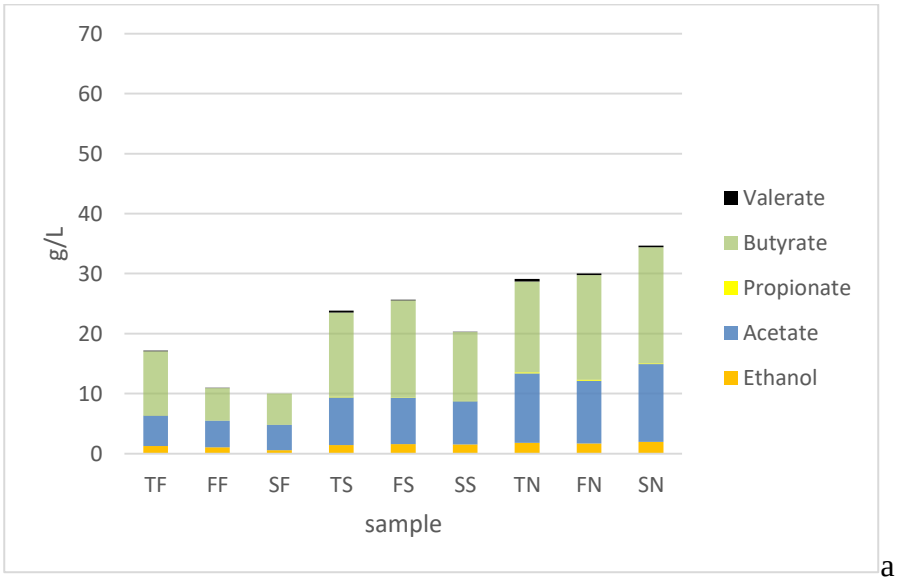
TF (S/I=2; pH=5) - TS (S/I=2; pH=7) - TN (S/I=2; pH=9) - FF (S/I=4; pH=5) - FS (S/I=4; pH=7) - FN (S/I=4; pH=9) - SF (S/I=6; pH=5) - SS (S/I=6; pH=7) - SN (S/I=6; pH=9)

Effect of different combinations of S/I ratio and pH on biological metabolites production – food waste fermented 2 days

After two days acidogenic fermentation of FW, again the most abundant products were acetate and butyrate (Fig 5.4.7). Propionate and valerate productions were almost negligible, while ethanol production ranged between 0.6 and 1.9 g/L in samples SF and SN, respectively. Lactate was not

detected in any of the samples. The peak of metabolites was reached in sample SN (34.6 g/L, 55% butyrate and 37% acetate). The results obtained after 2 and 4 days of acidogenic fermentation tests are in agreement with those obtained by Stein at al. (2017) who recorded a VFAs production peak at pH 9 after 2 days and at pH 7 after 6 days of food waste treatment.

In this case, the trend showed an increasing production of metabolites in correspondence of increasing initial pH values for each S/I ratio.



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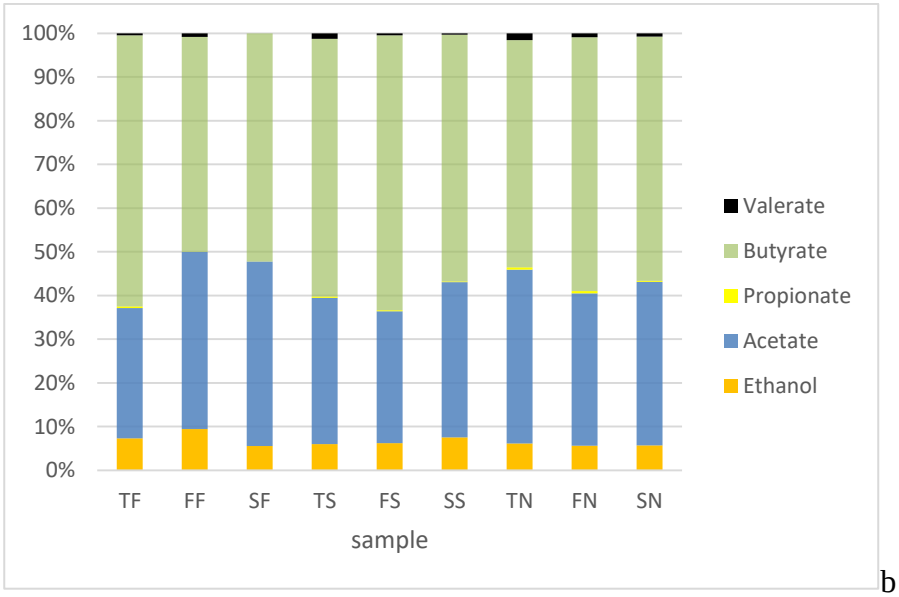


b

Figure 5.4.7: Actual values (a) and percentage distribution (b) of biological metabolites generated after 2 days acidogenic fermentation of food waste.

TF (S/I=2; pH=5) - TS (S/I=2; pH=7) - TN (S/I=2; pH=9) - FF (S/I=4; pH=5) - FS (S/I=4; pH=7) - FN (S/I=4; pH=9) - SF (S/I=6; pH=5) - SS (S/I=6; pH=7) - SN (S/I=6; pH=9)

In general, for none of the initial conditions (S/I ratio and pH) two days resulted to lead to higher metabolites production yields in comparison with the yields after four days. This was most probably due to the lack of time for a sufficiently enhanced biological fermentation. Indeed, Kuruti et al. (2017) assessed that four days is the best hydraulic retention time to reach the highest metabolites production yield during acidogenic fermentation of FW. Notwithstanding, it was interesting to point out that the percentage of ethanol within the produced metabolites after two days acidogenic fermentation was higher than the percentage after four days, with a peak of 10% in sample FF.

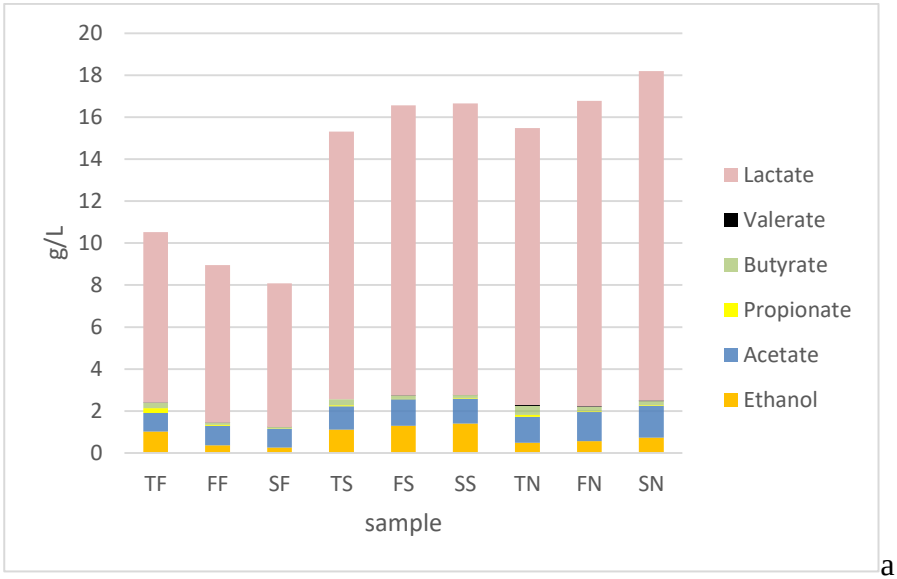
Clearly, as already detected during the elaboration of hydrogen production data for which the longer lag phase was related to initial acidic pH conditions, pH 5 results to be the value at which the production of metabolites is much lower in proportion to what it is possible to reach after 4 days of acidogenic fermentation. This means that an initial value of 5 represents an unfavourable beginning pH condition both for H_2 (long lag phase) and VFAs (low total volume) productions.

Effect of different combinations of S/I ratio and pH on biological metabolites production - cheese whey fermented 2 days

In relation to what reported by Shen et al. (2016), a large amount of amino acids inside cheese whey was converted into lactate and not VFAs thanks to lactic acid bacteria, naturally present inside cheese whey. With respect to the totality of produced metabolites, the percentage of lactate ranged between 78% (sample TF) and 87% (sample SN).

In contrast with Tang et al. (2017) who obtained the highest lactate yield at pH 5, outputs displaced in Figure 5.4.8 revealed the highest yields at pH 7 and 9. In particular, the amount of lactate increased in parallel with the S/I ratio from 12.8 g/L (sample TS) to 13.9 g/L (sample SS) and from 13.2 g/L (sample TN) to 15.7 g/L (sample SN) at pH 7 and 9, respectively. A S/I ratio of 6 and a initial pH of 9 appeared to be the best conditions in order to reach the highest lactate production.

The amount of produced metabolites increased in correspondence to increasing initial pH values for each S/I ratio, similarly to the outputs obtained in the case of two days-fermented FW.



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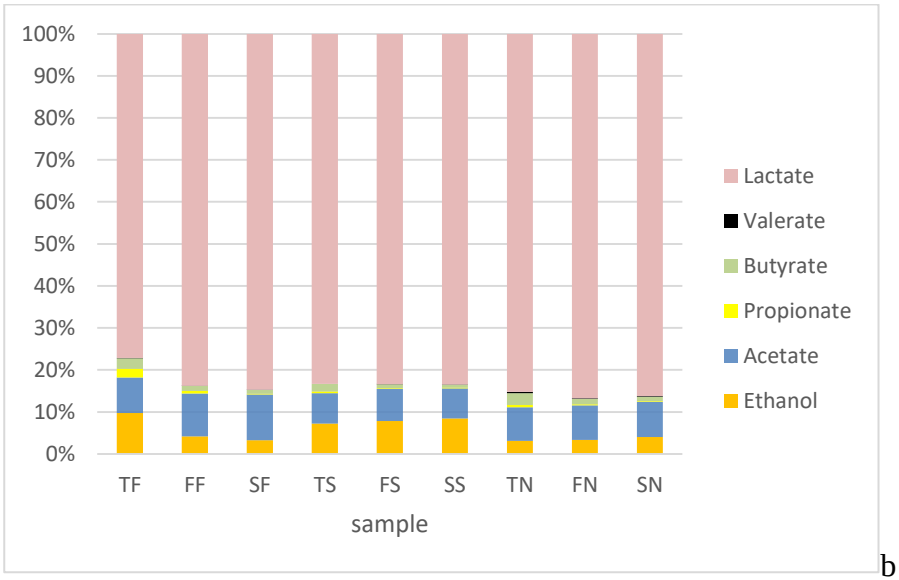

b

Figure 5.4.8: Actual values (a) and percentage distribution (b) of biological metabolites generated after 2 days acidogenic fermentation of cheese whey.
TF (S/I=2; pH=5) - TS (S/I=2; pH=7) - TN (S/I=2; pH=9) - FF (S/I=4; pH=5) - FS (S/I=4; pH=7) - FN (S/I=4; pH=9) - SF (S/I=6; pH=5) - SS (S/I=6; pH=7) - SN (S/I=6; pH=9)

With respect to the produced VFAs and alcohols, acetate and ethanol are the most abundant. Propionate, butyrate and valerate productions were negligible. When excluding lactate, ethanol was the most abundant metabolite at pH 7 for each S/I ratio; the highest yield (1.4 g/L) was recorded from sample SS. Acetate was instead the most abundant at pH 9 for each S/I ratio; the highest yield (1.5 g/L) was recorded from sample SN.
In parallel with hydrogen production yields recorded from samples characterized by an initial pH of 5, also the amount of metabolites measured from those samples was generally the lowest.

Statistical analysis

The calculation of the variation percentages, both when keeping S/I ratio and pH constant with respect to the maximum metabolites production, was performed both for FW (4-days acidogenic fermentation) and CW. The interesting conclusion is that S/I ratio seems to be the dominant variable when dealing with FW (the variation calculated over the maximum output ranges between 27 and 49%), while initial pH is the key parameter that needs to be properly set when treating CW (the variation calculated over the maximum output ranges between 4 and 83%).

In order to enforce the above mentioned findings, results were statistically elaborated with Statgraphics Centurion XVII software program to find the significant (p-value < 0.05) strong (correlation coefficient absolute value > 0.6) correlations between the main produced metabolites (acetate and butyrate for 4-days fermented FW, and lactate in case of fermented CW) and both S/I ratio and pH (Table 5.4.5). Correlations between the initial conditions (S/I ratio and pH) and hydrogen production were also addressed. Significant and strong proportional correlations were those between S/I ratio and butyrate (Table 5.4.5) and S/I ratio and hydrogen production (p-value=0.0104 and correlation coefficient=0.8) if dealing with FW, and between pH and lactate if dealing with CW (Table 5.4.5).

On equal terms of pH, there is also a significant proportional correlation between butyrate and H_2 (p-value=0.0358 and correlation coefficient=0.7), and a inversely proportional one between lactate and H_2 (p-value=0.0047 and correlation coefficient=-0.84), which is again in line with what reported in the literature (Ahring and Westermann, 1988; Elbeshbishy et al., 2017).

Table 5.4.5: Correlation outputs between the main produced metabolites and both S/I ratio and pH using Statgraphics Centurion XVII software.

	FW				**CW**	
	acetate		**butyrate**		**lactate**	
	correlation	**p-value**	**correlation**	**p-value**	**correlation**	**p-value**
S/I ratio	0.6035 (strong)	0.0853 (not significant)	0.9619 (strong)	0.0000 (significant)	0.0981 (weak)	0.8017 (not significant)
pH	0.6252 (strong)	0.0718 (not significant)	0.0458 (weak)	0.9068 (not significant)	0.900 (strong)	0.0009 (significant)

Note: food waste – FW; cheese whey – CW.

5.1.6 DISCUSSION

Within the carboxylate platform framework, with the specific focus on bio-plastics production, optimization of anaerobic processes depends on the desired final products. For example, in the context of PHA production, it may be interesting to maximize the VFAs yield (Kok et al., 2013; Tamis and Joosse, 2015) fermenting municipal food waste at a high S/I ratio. On the other side, if polylactic acid is the target product, substrates rich in lactose, such as cheese whey, should be investigated and treated through fermentation preferably at alkaline pH. In any case, a reaction time

of two days confirmed to be too short for a successful FW acidogenic fermentation (Kuruti et al., 2017) whether the focus is to produce H_2 or metabolites for bio-plastic production.

5.1.7 CONCLUSIONS

Results showed that the most abundant metabolites derived from FW fermentation were butyrate and acetate, mainly influenced by the S/I ratio (acetate and butyrate maximum productions of 21.4 and 34.5 g/L, respectively, at S/I=6). Instead, when dealing with CW, lactate was the dominant metabolite significantly correlated with pH (lactate maximum production of 15.7 g/L at pH=9).

The outputs obtained from these fermentation batch tests could be used for the optimisation of S/I ratio and pH when using multi criteria analysis tools for deciding case-specific organic waste management strategies. A further development could be the profiling of a standard method to perform laboratory tests aimed at plastic-monomers recovery. In this sense, many other trials should be performed keeping in mind the variability of the process performances and optimal conditions on the basis of the chemical characteristics of the specific fermented substrate.

Finally, the batch test upgrade to semi-continuous and continuous tests would enable to monitor another important variable, the operating pH.

6. GENERAL CONCLUSIONS

The development of sustainable solutions for food waste management could lead to social, economical and environmental benefits. Avoidance, or better minimization, of food waste generation could be ideally obtained by a proper equilibrium between food production and consumption, but such an optimal arranging is still far from being attained. A feasible management of excess production of edible food consists in its redistribution to feed poor people, even though many are still the legal and health issues that need to be solved. The reality is that still huge volumes of food waste need to be handled and managed.

(1) In Countries where separate collection has not started yet, the problem of gaseous and liquid emissions from landfilled organic waste must be contained. In these terms, the first part of the research lead to the conclusion that pre-aeration could be an appropriate treatment to be applied to MSW rich in organic compounds to enhance their anaerobic degradation and to limit environmental problems deriving from its subsequent landfilling.

(2) & (3) In order to divert the tendency of missing the opportunity to exploit agro-industrial and household food waste, the production of bio-fuels together with the extraction of high-value components (i.e. bio-plastics monomers) was also addressed and developed during the Ph.D. activity.

The interconnection of biotechnological processes in the co-production of bio-fuels and bio-products represents a key strategy aimed at maximising the utilisation of food waste and raising the potential income of the entire bioprocess chain. Therefore, when considering properly segregated food waste streams, both from decentralized and not-decentralized realities, these become sustainable non-virgin feedstock.

The several outputs obtained from the different anaerobic digestion (one and two-stage) and fermentation experimental batch tests could be used to calibrate parameters like S/I ratio and initial pH to be applied into multi criteria analysis tools when deciding case-specific organic waste management strategies. The carbon footprint is arguably one of the most important issues in improving the environmental responsibility of the food chain especially when analysing its waste streams. Therefore, primary challenge for science is to provide the necessary information about several management options also on the basis of software tools reproducing realistic models calibrated with real experimental data like the one reported in this thesis.

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*"**Thanks, sir; all the rest is mute.**"* - William Shakespeare
(From: *All's Well That Ends Well*, Act II, Scene 3)

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….well, just to strike a blow for me, I add the obviousness '*Nobody is perfect*'. Could you imagine how boring the world would be? And there would not be any more hilarious topics to tattle about during the unmissable coffee breaks in the lab.

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*"**Grazie, signore; il seguito è silenzio.**"* - William Shakespeare
(Da: *Tutto è bene quel che finisce bene*, Atto II, Scena 3)

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