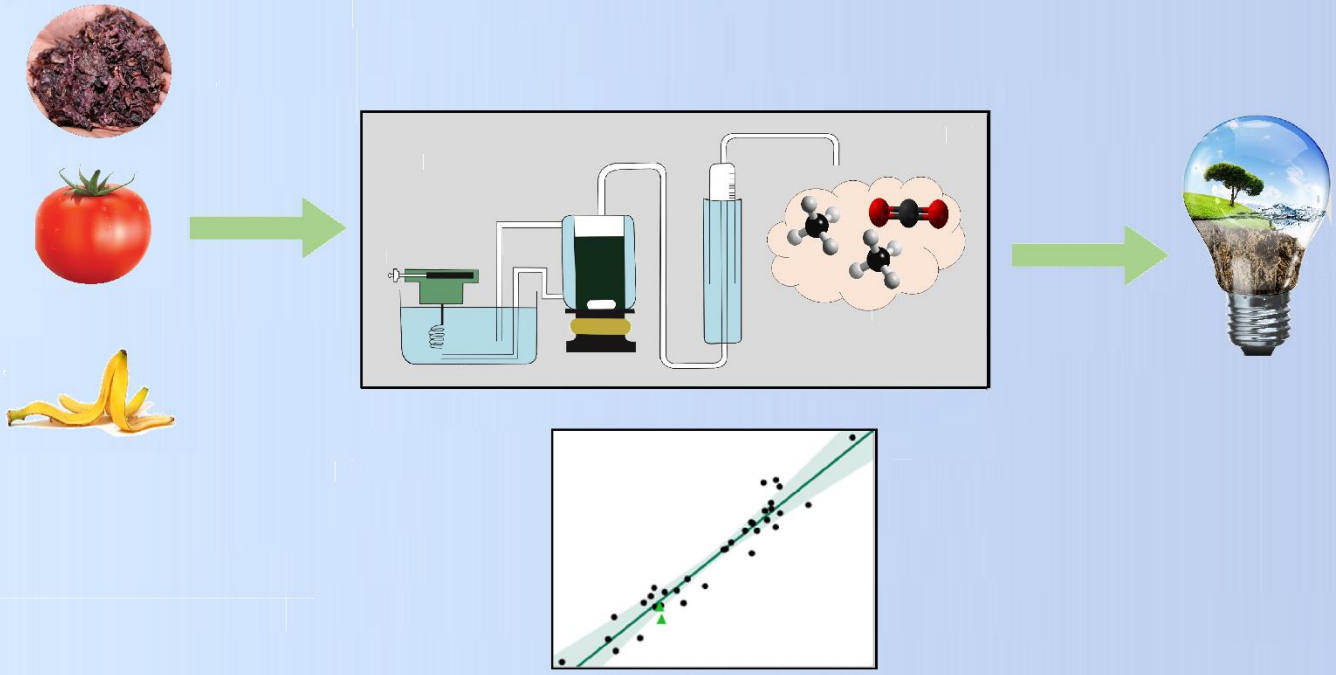

Rafaela Pereira Rodrigues

Anaerobic digestion process for agro-industrial wastes valorization: experimental and theoretical biochemical methane potential prediction

Master's Dissertation in Chemical Engineering, submitted to the Department of Chemical Engineering, Faculty of Science and Technology, University of Coimbra

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Thesis Project in the scientific area of Chemical Engineering, submitted to the Department of Chemical Engineering, Faculty of Science and Technology, University of Coimbra

Supervisors:
Prof. Dr. Margarida Maria João de Quina
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“Try to make sense of what you see and wonder about what makes the universe exist. Be curious, and however difficult life may seem, there is always something you can do, and succeed at. It matters that you don’t just give up.”

Stephen Hawking

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ABSTRACT

Among all environmental problems faced nowadays, climate change is undoubtedly the most imminent. Biofuels such as biogas may have a major role in the replacement of fossil fuels. Biogas is the main product of anaerobic digestion (AD) and can be used for producing energy in an efficient and eco-friendly way.

The main objective of this work is the study of the AD process to assess the potential valorization of agro-industrial wastes through experimental and theoretical biochemical methane potential (BMP) prediction. In a first phase, 40 scientific papers were analyzed and was possible to obtain the BMP for 149 substrates. Then, an exhaustive analysis of these data was carried out through simple linear and multivariate polynomial regressions. Moreover, experimental tests of BMP and AD batch tests were used to evaluate the methane production of three substrates, namely winery wastewater (WW), tomato waste (TW) and banana peel waste (BW). Substrate-inoculum (S/I) ratios were optimized for WW and then applied to other substrates.

Through the explorative analysis of the literature data, it was possible to verify that the BMP is a very complex parameter, making its prediction very hard. In fact, the methods for predicting this parameter referred in several papers are unsatisfactory since large differences are found between the predicted and the experimental BMP values. With the multivariate polynomial regressions performed in this study, it was possible to develop two models that present great potential for BMP prediction. From experimental tests, it was possible to verify that TW is the substrate with the highest potential for AD, followed by BW and lastly, with a lower potential, WW. The batch AD of WW revealed that the optimal S/I ratio is 0.5, which is in accordance with the literature. By using this S/I ratio it was possible to obtain 358.6 NmL gVS^{-1} of biogas for WW, 453.4 NmL gVS^{-1} for TW and 574.1 NmL gVS^{-1} for BW.

This study demonstrated that BMP plays a fundamental role in the evaluation of a substrate potential for AD. It was possible to conclude that this parameter may be assessed in a simpler and faster way by theoretical models when compared to the experimental laborious methodologies. It was also possible to show that the substrates explored in this study can be valorized through an AD process.

Key-words: Biochemical methane potential; Anaerobic digestion; Biogas; Methane; Biodegradability; Agro-industrial wastes.

RESUMO

De entre todos os problemas ambientais enfrentados hoje em dia, as alterações climáticas são, sem dúvida, as mais preocupantes. Os biocombustíveis, como o biogás, começam a ter um papel importante na substituição dos combustíveis fósseis. Este gás é o principal produto da digestão anaeróbia (AD) e pode ser usado para produzir energia de forma eficiente e ecológica. Existem diversos tipos de substratos orgânicos biodegradáveis que podem ser utilizados como substrato para na AD. Portanto, é de grande importância avaliar a biodegradabilidade e o potencial bioquímico de metano (BMP) destes.

Este trabalho tem como principal objetivo o estudo do processo de AD, a fim de avaliar a possibilidade de valorização de resíduos agroindustriais. Através da análise de 40 artigos científicos, foi possível obter o BMP para 149 substratos diferentes. Após esta coleta foi realizada uma análise exaustiva desses dados através de regressões lineares simples e regressões polinomiais multivariadas. Foram realizados testes experimentais de BMP e de AD para avaliar o potencial de produção de metano de três substratos, nomeadamente de um efluente vinícola (WW), resíduo de tomate (TW) e resíduo de cascas de banana (BW). A razão substrato-inoculo (S/I) foi otimizada através da digestão do substrato WW, sendo depois aplicada aos restantes.

Através da análise exploratória dos dados da literatura, foi possível verificar se o BMP é um parâmetro bastante complexo, fazendo com que a sua previsão seja difícil. Daí, os métodos para prever esse parâmetro referidos na literatura serem insatisfatórios. Através das regressões polinomiais multivariadas realizadas neste estudo foi possível desenvolver dois modelos que apresentam grande potencial no que toca à previsão do BMP. A partir dos testes experimentais BMP, foi possível verificar que o TW é o substrato com o maior potencial de AD, seguido do BW e, por último, com menor potencial, o WW. A AD do WW revelou que a razão S/I ideal, dentro dos valores testados, é de 0.5. Através desta razão S/I, foi possível obter 358.6 NmL gVS^{-1} de biogás para WW, 453.4 NmL gVS^{-1} para TW e 574.1 NmL gVS^{-1} para o BW.

Este estudo demonstrou que o BMP desempenha um papel fundamental na avaliação do potencial de um substrato para AD e que é possível prever este parâmetro de forma mais simples e rápida em comparação com o procedimento experimental. Foi possível também mostrar que os substratos explorados neste estudo podem ser valorizados através de um processo AD.

Palavras-chave: Potencial bioquímico de metano; Digestão anaeróbia; Biogás; Metano; Biodegradabilidade; Resíduos agroindustriais.

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ACRONYMS

AcoD – Anaerobic co-digestion

AD – Anaerobic digestion

ADF – Acid detergent fiber

BD – Biodegradability

BD_{VS} – Biodegradable fraction of volatile solids

BMP – Biochemical methane potential

BMP_{EXP} – Experimental biochemical methane potential

BMP_{PRED} – Predicted biochemical methane potential

BW – Banana peel waste

CEL – Cellulose

COD – Chemical oxygen demand

CRB – Carbohydrates

EU – European Union

FOKM. – First order model

GHG – Greenhouse gases

HMC – Hemicelluloses

HRT – Hydraulic retention time

LG – Lignin

LP – Lipids

NIR – Near-infrared spectroscopy

OLR – Organic load rate

OFC – Organic fraction composition

PA – Partial alkalinity

PT – Proteins

SBP – Specific biogas production

sCOD – Chemical oxygen demand on soluble fraction

S/I ratio – Substrate- Inoculum ratio

SMP – Specific methane production

SRB – Sulphate-reducing bacteria

SRT – Solids retention time

SS – Suspended solids

TA – Total alkalinity

tCOD – Total chemical oxygen demand

TOC – Total organic carbon

TW – Tomato waste

VFA – Volatile fatty acids

VS – Volatile solids of substrate

VS_0 – Volatile solids of inoculum

VSS – Volatile suspended solids

WW – Winery wastewater

1. INTRODUCTION

1.1. WORK MOTIVATION

Climate change is the most imminent environmental problem faced nowadays by mankind. The increase of anthropogenic activity has been leading to a high production of wastes and emission of greenhouse gases, which are mainly produced in the generation of power and heat (Appels et al., 2011).

In order to minimize these effects, a global effort has been made to find and implement eco-friendly alternatives towards energy generation. Biofuels, such as biogas (resulting from anaerobic digestion), begin to have a major role in the replacement of fossil fuels (Divya et al., 2015).

The anaerobic digestion (AD) is a biological process, where organic molecules are broken down by specific microorganisms in the absence of oxygen. Thus, AD has been suggested for liquid and waste treatment in the case high load of the biodegradable matter is present. Biogas is the main product and can be used for producing energy in an efficient and eco-friendly way thanks to the low emission of hazardous pollutants. In addition to the biogas produced, slurry (digestate) is also formed, which can be used as a fertilizer in agriculture due to its richness in nitrogen and other nutrients (Appels et al., 2011).

There are many types of biodegradable organic substrates that can be used as feedstock for the production of biogas through AD or co-digestion, such as municipal solid wastes, animal manure, fruits and vegetables, etc. For practical applications, it is very important to assess the biodegradability and the biochemical methane potential (BMP) of the feedstocks due to their different characteristics (Lesteur et al., 2010). In this work, two categories of subtracts were selected, namely wine and fruit wastes.

Wine production is an important sector of the Portuguese economy. However, it requires a large amount of resources and produces an equally large amount of organic wastes and wastewater. Since the wine industry plays an important role in European Union (EU) countries economy, with a total production of about 150 million hectolitres, it is crucial to make this a sustainable industry by reducing the potential negative impacts (Ruggieri et al., 2009). Also, fruit and vegetable wastes are easily degraded by microorganisms because they are readily biodegradable. Thus, important negative environmental impacts may arise, even for short-term disposal (Efisio et al., 2014). So, these types of substrates represent a potential source of energy

and at the same time, this management strategy diverts this biodegradable waste from landfill (Gunaseelan, 2004).

Nowadays in EU, an intense discussion is in progress related to how "circular economy" can help climate action and be a part of the solution to the global climate challenge. In fact, the circular economy may represent a crucial role to sustain human life in the Earth, since it allows keeping materials circulating in the technosphere. By promoting AD, it is expected to contribute to cut down GHG (greenhouse gases) emissions, by reducing energy from fossil fuels

1.2. OBJECTIVE

The main objective of this work is the study of the AD process for the valorization of agro-industrial wastes through experimental and theoretical biochemical methane potential prediction. Through the analysis of the substrate characteristics, this work aims to develop models able to predict the biochemical methane potential, in order to evaluate this parameter faster than the experimental method. Even so, the BMP test at lab scale was implemented to assess the methane capacity of three substrates: winery waste (WW), tomato waste (TW) and banana peel waste (BW). Taking into account the results obtained in the BMP tests, the performance of an anaerobic digestion reactor of 5 L was optimized in terms of specific operating parameters, namely the ratio substrate to inoculum.

1.3. THESIS STRUCTURE

This thesis is organized into six chapters. The work motivation and objectives are presented in Chapter 1. Chapter 2 summarizes the theoretical background essential to understanding all work. In Chapter 3 a bibliographic review on this topic is presented. Chapter 4 describes the materials and methods used in the course of the work. Chapter 5 presents the results as well as the critical analysis of the results. Finally, in Chapter 6 the main conclusions are summarized and some suggestions of future work are indicated.

2. THEORETICAL BACKGROUND

2.1. ANAEROBIC DIGESTION

Anaerobic digestion (AD) is a fermentation process that occurs in the absence of oxygen in which organic compounds are degraded and biogas (mainly methane and carbon dioxide) is generated. This process is very effective when it is used for the removal of the biodegradable organic compound and it can be applied at laboratory or industrial scale (Lier et al., 2008).

In 2011 there were about 12000 biogas plants in Europe and this number has increased over the years as shown in Fig. 2.1.

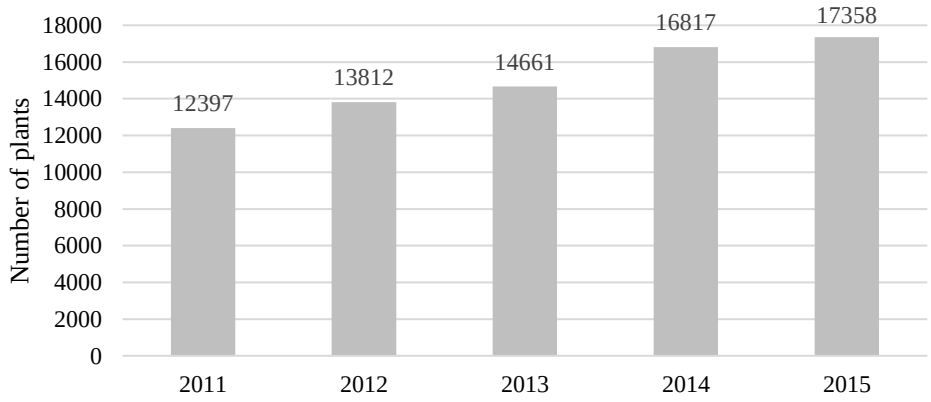

Fig. 2. 1. Number of biogas plants installed in Europe over last years (adapted from European Biogas Association (2015)).

The AD of organic materials is a multi-step biological process characterized by four main and successive phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis as shown in Fig. 2.2, which are briefly described below.

Hydrolysis: This process is mainly a surface phenomenon in which polymers like proteins and carbohydrates are degraded through the action of exo-enzymes to origin low molecular weight compounds (monomers) (Zhang et al. 2014). This is a crucial step since bacteria are unable to destroy complex organic matter. Once in this phase, large amounts of fatty acids are produced. Microorganisms are very sensitive to pH and temperature fluctuations, hydrolysis is considered rate-limiting in most AD processes (Lier et al., 2008).

Acidogenesis: In this step, low molecular weight compounds (produced in the previous step) are degraded through fermentative bacteria resulting mainly in VFA (volatile fatty acids). Once at this stage bacteria have a high growth rate, acidogenesis is considered the quickest of all AD phases (Lier et al., 2008).

Acetogenesis: The products of acidogenesis (mainly butyrate and propionate) are further converted, through the action of acetogenic bacteria, mainly into acetate, hydrogen and carbon dioxide (Zhang et al., 2014).

Methanogenesis: This is the final stage of AD and the one where methane is generated. The methanogenic bacteria reduce the products of acetogenesis mainly into methane, carbon dioxide and water (Molino et al., 2013). This stage can be described by the Eqs. (2.1) and (2.2).

$$CH_3COOH \longrightarrow CH_4 + CO_2 \quad (2.1)$$

$$CO_2 + 4H_2 \longrightarrow CH_4 + H_2O \quad (2.2)$$

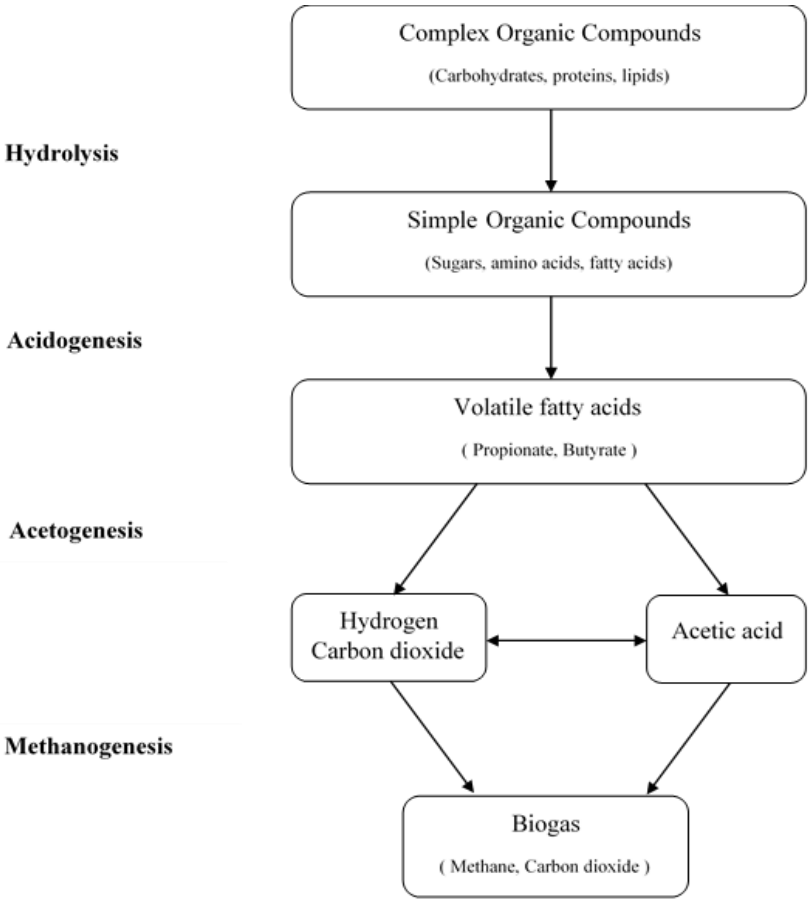

Fig.2.2. Steps of AD (adapted from Zhang et al. (2014)).

2.2. OPERATIONAL PARAMETERS

AD is a complex and sensitive biological process so it is quite important to have a control over all factors that can influence this technology. Some of the most important parameters can be found in Fig.2.3.

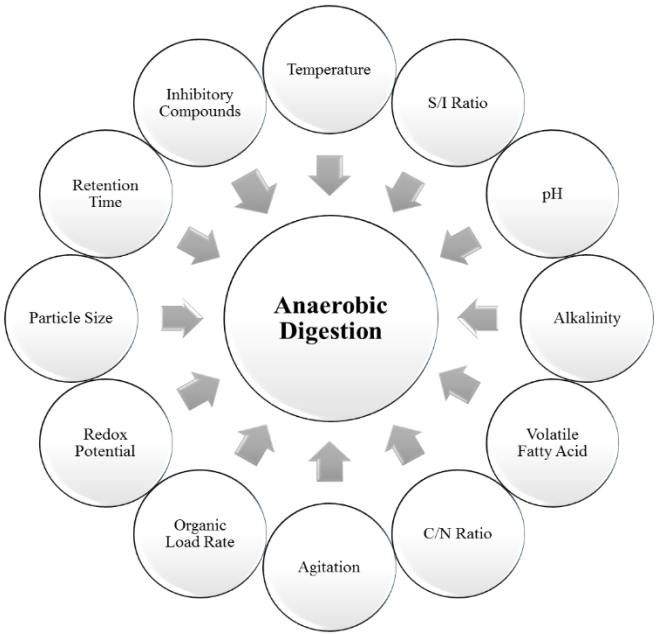

Fig.2. 3. Operational parameters affecting AD.

Temperature

The operating temperature is not only important to the survival of microorganisms but also to the overall performance of AD. In general, AD can occur under mesophilic (25-40°C) or thermophilic (50-65°C) conditions.

In the thermophilic range, the metabolic and specific growth rates are higher, pathogenic destruction is possible and biogas productions are larger due to the acceleration of hydrolysis step. However, in this range, the gas producing bacteria are very sensitive to small environmental changes and may even die (Mir et al., 2016).The control of the systems is harder.

On the other hand, the mesophilic system operates with microorganisms that tolerate environmental changes. So, digesters are easier to operate and maintain. The main disadvantages are in this case the higher retention time and the lower specific biogas production(Mir et al., 2016).

S/I Ratio

Besides the characteristics of substrates, their proportions to inoculum have also a great influence in the AD operation (Zhou et al., 2011). If the microorganisms in the inoculum are more or less than the required amount, methane production can be reduced or even inhibited. This proportion between substrate and inoculum is referred as S/I ratio and is usually expressed as the amount of substrate volatile solids (VS) added per VS of inoculum (Liu et al., 2009).

pH, alkalinity and VFA

The micro-organisms involved in this system have different ideal ranges of pH. For example, the fermentative bacteria may live well for pH between 4.0 and 8.5, while methanogenic bacteria require pH between 6.5 and 7.5 (Jain et al., 2015).

So, in order to maximize the overall efficiency of the digester, it is necessary to properly control pH since this affects the enzymatic activity and the metabolism of microorganisms. According to Jain et al. (2015), the ideal pH for AD is between 6.5 and 7.5 because outside this range the methanogenic bacteria growth is strongly hindered.

The alkalinity results mainly from the balance between carbon dioxide and bicarbonate ions in the digester, which ensures significant resistance to pH changes in the growth medium. Maintaining this buffering capacity is important to ensure the stability of the digester. Its value is proportional to the concentration of bicarbonate present in the digester and can be expressed as partial alkalinity (PA) or total alkalinity (TA) in terms of mg $CaCO3\ L^{-1}$ (Ward et al. 2008). PA and TA are often determined by titration with 0.1 N HCl until two equivalent points are reached: pH 5.75 for PA and pH 5.00 for TA

The volatile fatty acids (VFA) are short-chain acids (such as acetic, propionic, formic acids), intermediates of the first stage of AD (hydrolysis). However, the accumulation of VFA leads to a reduction of pH and consequently into the process instability. Concentrations of VFA lower than 1-4 $g\ L^{-1}$ generally guarantee process stability. When the concentration of acetic or propionic acid is greater than 4 $g\ L^{-1}$ and 1 $g\ L^{-1}$, respectively, there is instability in the digester or even failure.

In practice, the control of VFA to TA ratio is widely used in industrial processes to determine the stability of the system. If this ratio is lower than 0.3, AD is considered in the stability region; between 0.3 and 0.8 some signs of instability are observed and greater than 0.8 the process becomes unstable and biogas production can be inhibited (Drosg, 2013).

C/N ratio

The concentration of nutrients in the digester has a determinant role in its performance since the microbial growth and the synthesis of enzymes are essential to the biochemical and metabolic reactions. AD is significantly affected by the carbon/nitrogen ratio (C/N), since carbon constitutes a source of energy for microorganisms, while nitrogen stimulates microbial growth (Igoni et al., 2008). For reduced C/N ratios, ammonia production is favored and, consequently, methane production is inhibited. However, for high C/N ratios, there is a lack of nitrogen, making it difficult to produce proteins essential for the metabolism of microorganisms. According to Jain et al.(2015), in AD is preferable to have C/N ratios between 20 and 30, with the optimal ratio being about 25.

Agitation

Agitation must ensure a perfect mixture, because this is a key aspect for achieving uniformity in the substrate concentration, temperature, and the medium conditions, and to reduce the risk of solid deposition and foam formation as well (Mir et al., 2016). Ward et al. (2008), referred that excessive mixing can diminish biogas production, while low-speed mixing allows the digester to absorb more efficiently the disturbance of shock loading.

Organic loading rate

The organic loading rate (OLR) refers to the mass of organic biodegradable solids loaded per volume of the reactor and per time and is often expressed as kg VS m^{-3} d^{-1}.

OLR depends on several factors, namely the quantity and activity of biomass in the reactor, temperature, inhibitors or toxic compounds, degradability of substrate, and presence of suspended solids. To reduce the cost of the digester is preferred to have high OLR so that the size of the equipment can be low. But if the digester is overloaded with raw material, acids will accumulate and digestion can stop. Additionally, the OLR affects the ratio between organic matter and the microorganisms ($VS:VS_0$). When this ratio is higher than the optimal value, there is an organic overload, and therefore organic matter is only partially degraded (Jain et al., 2015).

Redox potential

In opposite to aerobic microorganisms, anaerobic bacteria need a negative redox potential for their metabolism. In the case of AD, the redox potential should be lower than -300 mV (Drosg, 2013).

Particle size

The size of the particles of the substrate influences the rate of AD as decomposition occurs on the surface of the particles. For large particles, the decomposition happens quite slowly and, consequently, the production of biogas is slower. Thus, it is advisable to increase the total accessible surface area by reducing their average size (Mir et al, 2016).

Retention time

There are two types of retention time: the solid retention time (SRT) and the hydraulic retention time (HRT). The first one refers to the average time that solids stay in the digester. If SRT is less than the regeneration time of the slowest growing microbial organisms in the system, it is not possible to ensure enough suitable bacteria. The temperature of the digester influences the SRT, once with the increase of temperature the times of regeneration of the bacteria decrease. Thus, the higher the digester temperature, the lower SRT required (Jain et al., 2015).

HRT is the average number of time a given volume of liquid remains in the digester, which is usually enough time for efficient degradation. This parameter is typically a few weeks but depends on parameters such as OLR or substrate composition. The reduction of HRT favors the accumulation of VFA. So, the best strategy for maximizing methane yield results from combining short OLR and long HRT (Mir et al., 2016).

Inhibitory compounds

Table 2.1 summarizes the main inhibitory compounds to a good performance of AD.

Table 2. 1. Inhibitory compound to the methane formation.

Compound	**Effect**	**Inhibitory concentration (mg L^{-1}) [a]**	
Ammonia	Ammonia comes from the breakdown of N-rich protein and organic substrates and appears mostly in the form of ammonium (NH_4^+) and free ammonia (NH_3) (Zhang et al., 2014). Depending on the concentration it could be either an important nutrient for bacterial growth or at high concentrations could be toxic. Previous studies show that ammonia is able to neutralize VFAs formed during de AD process, giving the system some balance (Zhang et al., 2014).	Ammonia (NH_3)	80
		Ammonium (NH_4^+)	1500-10000
Heavy metals	Metal elements (light and heavy metal ions) are required by anaerobic bacteria because they play a significant part in enzyme synthesis and activity (Schattauer et al., 2010). Still, inhibition could be caused by both of light and heavy metal elements depending on their concentration. Heavy metals, unlike many toxic substances, are not biodegradable, and thus they can be accumulated to inhibitory concentrations (Chen et al., 2008). This inhibition is caused by the disruption of enzymatic function and structure (Zhang et al., 2014).	Chromium	
		Cr (VI)	3.0 [b]
			200-250 [c]
		Cr^{3+}	2.0 [b]
			180-420 [c]
		Nickel (Ni^{2+})	30 [c]
		Zinc (Zn^{2+})	1.0 [b]
		Copper (Cu^{2+})	0.5 [b]
			200-250 [c]
		Cadmium (Cd^{2+})	70-600 [c]
		Lead (Pb^{2+})	8-340 [c]
Sulphide	Sulphate, under anaerobic conditions, is reduced to sulfide through the action of sulfate-reducing bacteria (SRB). SBR are able to metabolize substrates like alcohols, organic acids, and VFAs. So, they compete for the same substrates with fermentative, acetogenic and methanogenic bacteria. On the other hand, non-dissociated hydrogen sulfide can freely diffuse through the cell membrane of methanogens and sulfate reducers causing the denaturation of proteins (Appels et al., 2011). Therefore, the Inhibition occurs at two different stages: firstly is caused by the competition for substrates from SRB and secondly is due to the toxicity of sulfides to the different microorganisms.	Hydrogen sulfide (H_2S)	68 – 102
Oxygen	Unlike acidifying bacteria (facultative anaerobic), methanogenic bacteria lack an oxygen-free environment, and small amounts of oxygen can cause their death stopping the production of methane (Deublein and Steinhauser, 2010).	Oxygen (O_2)	0.1

[a] Adapted from Appels et al. (2008), Deublein and Steinhauser (2010) and Turovskiy and Mathai (2006)
[b] Soluble
[c] Total

2.3. ANAEROBIC REACTORS

There is a variety of anaerobic reactors design for the treatment of diverse feedstocks. For a correct selection of the digester type is essential to consider the characteristics of the substrate.

Table 2.2 shows the different types of digesters according to their different classifications.

Table 2. 2. Types of digesters according to their different classifications

Feed type	Continuous	Organic matter is constantly added or added in stages to the reactor. The biogas production is constant because the end products and organic matter are constantly removed and added, respectively. It allows the continuous and steady growth of microorganisms. However, there is no guarantee that the substrate removed is completely degraded(Igoni et al., 2008).
	Batch	Initially, the biomass is added and then the reactor is sealed for the duration of the process. Typically, biogas production will be formed with a normal distribution pattern over time. Is a cheaper operation due to a lower requirement on equipment and design level
	Semi-batch	The organic matter feed is intermittent and the end products are constantly removed. As in the continuous mode, this allows the continuous and steady growth of microorganisms. However, there is no guarantee that the substrate is completely degraded (Igoni et al., 2008).
Stages number	Single stage	All biological reactions occur within a single sealed reactor. The different biological reactions can be in direct competition with each other, leading to less control of the system. However, the construction costs are strongly reduced (Ahring, 2003)
	Multi-stage	In this process, different digestion vessels are optimised to bring maximum control over the bacterial communities in the digesters. Normally, hydrolysis, acetogenesis, and acidogenesis occur within the first reaction vessel. The organic material is then heated to the required operating temperature prior to being introduced into the methanogenic reactor (Griffin et al., 1998).
Temperature	Mesophilic	AD takes place optimally around 30 to 38 °C, but broaden ranges can be used (20- 45°C).
	Thermophilic	AD takes place optimally around 49 to 57 °C, or at elevated temperatures up to 70 °C.
Biomass retention system	Suspended	These systems lack the continuous development of anaerobic bacteria due to their constant removal.
	Fixed	Since there is no removal of biomass, it is possible to produce biogas in a constant and efficient way.

2.4. PRE-TREATMENTS

Although with high organic matter content, not all substrates are suitable for biodegradation. In order to increase the bioavailability of a substrate for the anaerobic bacteria, specific pre-treatments may be used. As the hydrolysis step is usually the limiting one, the accomplishment of a pre-treatment to accelerate this stage is often desirable. Thus, certain pre-treatments can lead to an increase in the biogas production as a result of the higher degradation yield of volatile solids. However, it is important to carefully evaluate the suitability of pre-treatments because it can lead to high costs and the benefits may not be sufficient to compensate them (Zhang et al., 2014). Different types of pre-treatments are summarized in Table 2.3.

Table 2. 3. Pre-treatments often used in AD.

Pre-treatment	**Main characteristics**	**Examples**
Thermal	The substrate is subjected to high temperature and high pressure in order to avoid evaporation of compounds. High molecular weight components are solubilized or degraded and becoming more biodegradable. Furthermore, the dehydration of the materials reduces the presence of pathogenic microorganisms. Industrially, thermal pre-treatments are the most used and those that ensure better results (Ariunbaatar et al., 2014).	High temperature (>100ºC) Low temperature (<100ºC)
Chemical	Strong acids, alkalis or oxidants can be used to achieve the destruction of some organic compounds. Acidic and oxidative methods are also used to enhance the biogas production and improve the hydrolysis rate. These treatments are not appropriate for substrates containing high amounts of carbohydrates, due to their accelerated degradation and following accumulation of VFA. However, it can have a positive effect on substrates rich in lignin (Ariunbaatar et al., 2014)	Acid Alkaline Oxidative
Biological	Biological pre-treatment is slower than the others making it less attractive on an industrial level. Aerobic degradation previous to AD is a possibility, which allows the growth of specific microorganisms by the acceleration of the hydrolysis step (Jain et al., 2015).	Composting
Mechanical	These treatments allow the increase of the specific area, enhancing the contact with the anaerobic bacteria, through the disintegration the particles of the substrate, leading to an increase in the efficiency of the process (Ariunbaatar et al., 2014).	Milling Maceration Extrusion Ultrasound High-pressure homogenizer
Combined	Substrates used in AD may differ considerably in terms of their composition, and sometimes it is necessary to combine different mechanisms to solubilize organic matter.	Thermochemical Thermomechanical

2.5. ANAEROBIC DIGESTION PRODUCTS

In AD process, biogas is the main and desirable product but there is also the formation of digestion sludge.

Biogas

Biogas is a clean and an eco-friendly fuel, usually considered as a binary mixture of methane (50-75%) and carbon dioxide (25-50%). However, there are other constituents, such as water vapor, traces of H_2S and H_2, depending on biodegradability of organic matter (Turovskiy and Mathai, 2006). In the biogas, it is important to control specific compounds even in trace concentrations, since they behave as impurities. In most cases, this biofuel needs a purification step, which varies depending on the end use for which it is intended.

Biogas has a relative density between 0.80 and 1.04 and the lower heating value ranges from 21-25 MJ m^{-3} (30 to 40% lower than that of natural gas: 37.3 MJ m^{-3}) (Appels et al. 2008). Indeed, biogas can be used in almost every application that was developed for natural gas. It can be used for the production of heat and steam, electricity generation or co-generation, use as vehicle fuel, and for the production of chemicals (Appels et al. 2008).

Worldwide, biogas is mainly used in combined heat and power applications, while various EU countries have invested on programs to use a portion of the biogas in the transport sector, especially due to the constant increase of fossil fuels cost (Appels et al. 2008).

Digestion Sludge

Digestion sludge consists of the solid biomass and the liquid fraction rich in nutrients such as phosphorus and nitrogen, having applicability as a fertilizer in agriculture. Digestion sludge is considered partially treated and it is important to control the presence of pathogens. The solid fraction is composed mainly of fibrous material and biomass that has a slow digestion and the application to soil is simple and cheap. In order to improve the value of the digestion sludge, it can be composted to generate a more stable, nutritious and commercially attractive product (Turovskiy and Mathai, 2006).

The clarified digestion liquid has a wide diversity of nutrients and a high water content. So, it can be used for irrigation of agricultural fields if it is guaranteed that it will not have a negative impact on the ecosystems (Turovskiy and Mathai, 2006).

2.6. CO-DIGESTION

Anaerobic co-digestion (AcoD) is the combined degradation of two or more substrates in the same digester. Mixing different types of residues can have positive effects on the anaerobic degradation yield and economy as it allows to increase the stability of the process. Co-digestion also allows, in many cases, the treatment of residues that would be difficult to digest individually. AcoD involves several advantages such as the balance of macro and micronutrient, C/N ratio, the content of inhibitory/toxic compounds and biodegradable organic matter content. The process should create synergies and increased methane yield per unit of digested mass (Shah et al. 2015).

2.7. BIOGAS IN EUROPE

About 80% of energy consumption in the world is derived from fossil fuels, which creates severe problems to the environment, namely due to its impact on the climate change. Therefore, alternative energy resources must be developed and applied worldwide (Hijazi et al., 2016). In 2012 European countries were able to produce approximately 13379 ktoe of biogas, where Germany was the major producer with a contribution of approximately 6717 ktoe. Portugal produced 65.3 ktoe of biogas as the primary production, as can be observed in Fig.2.4.

In Portugal, only in 2007, with the publication of Decreto-Lei Nº. 225/2007, of May 31, AD of organic matter was officially recognized. In fact, this law came to materialize a set of measures related to renewable energies which were already foreseen in the "National Strategy for Energy" of 2005. Possibly, for this reason, there are still few industrial units that treat effluents or solid wastes by AD. So, there is still a high unexploited potential for obtaining biogas, given the enormous availability of resources.

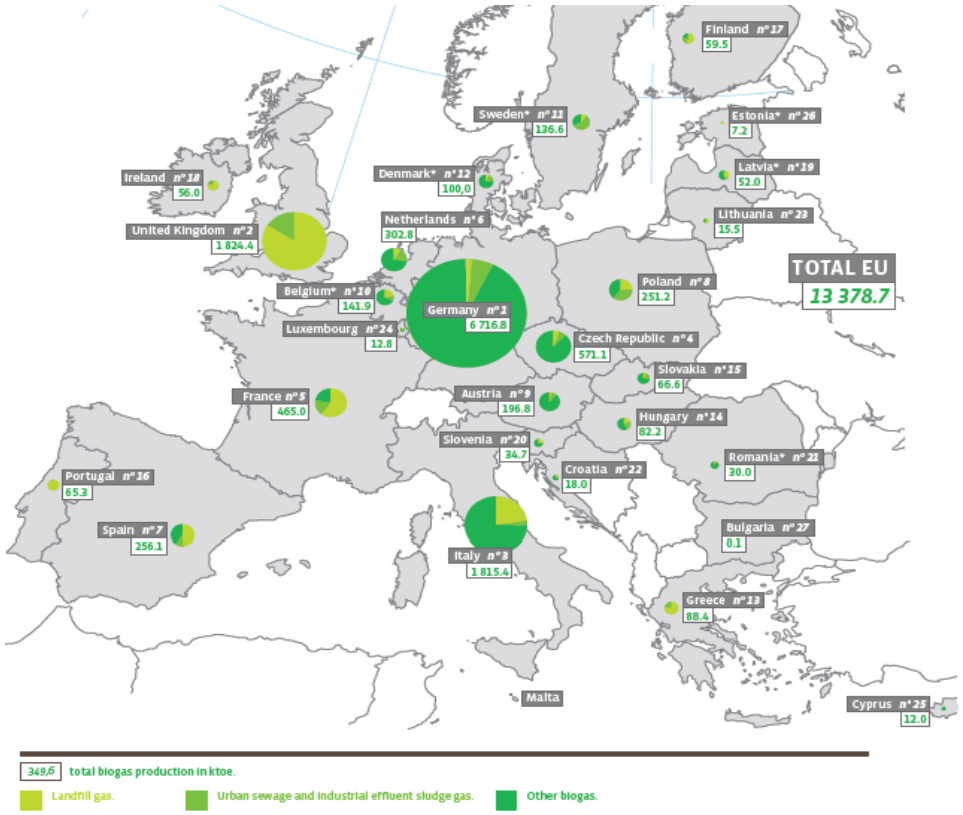

Fig.2. 4.Primary production of biogas in the European Union in 2012 (adapted from EurObser'vER (2014)).

2.8. BIOCHEMICAL METHANE POTENTIAL

Biochemical methane potential (BMP) is referred as the ultimate methane yield or the maximum methane production at infinite digestion time (Wang et al. 2017). This assay allows to evaluate the biodegradability, the suitability of a substrate to produce methane and to determine the optimum ratios between co-substrates if AcoD is needed (Labatut et al., 2011).

This parameter is usually determined through experimental bioassays, but there are other alternatives such as theoretical and analytical methods that can be used to predict the BMP value in order to save time and costs (Nielfa et al., 2015).

The experimental method will be described in section 4.2. and some the theoretical and analytical methods are described below:

Method I (Met_I) – Elemental composition analysis

This method was developed in 1933 by Simons and Buswell for the determination of the theoretical potential of methane production (BMP_{MET_I}) and is based on the atomic

composition of the substrate material, (Eq. 2.3), by taking into account the elements C, H, O, and N (Nielfa et al., 2015). The Buswell's formula (Eq. 2.4) does not consider the biodegradability of the substrate, meaning that the value obtained is the maximum production in case of all organic matter was converted into methane. So, this method normally overestimates the real BMP value (Lesteur et al., 2010),

$$C_nH_aO_bN_c + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{8}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right)CO_2 + cNH_3 \quad (2.3)$$

$$BMP_{MET\_I} = \frac{22400\left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)}{12n + a + 16b + 14c} \quad (2.4)$$

where BMP_{MET_I} is expressed in NmL CH_4 gVS^{-1}.

Method II (Met_II) – Organic fraction composition

This method is based on Buswell's formula (previously described), but only takes into account the really biodegradable fractions of organic matter such as lipids, carbohydrates, and proteins (Lesteur et al., 2010).

Lipids (LP), carbohydrates (CRB) and proteins (PT) can be defined by the following generic formulas $C_{57}H_{104}O_6$, $C_6H_{10}O_5$ and $C_5H_7O_2N$, respectively (Raposo et al., 2008). Accordingly, through the Met_I it is possible to conclude that LP have the biggest contribution to the production of methane ($NmLCH_4$ gLP^{-1}), followed by PT (496 $NmLCH_4$ gPT^{-1}) and CRB (415 $NmLCH_4$ $gCRB^{-1}$). Thus, the BMP predicted by the organic fraction composition (BMP_{MET_II}) is described by Eq. (2.5),

$$BMP_{MET\_II} = 1014 \times LP + 496 \times PT + 415 \times CRB \quad (2.5)$$

where LP, PT and CRB are fractions values expressed in (g gVS^{-1}).

Method III (Met_III) – Chemical oxygen demand

The chemical oxygen demand (COD) is a parameter that indirectly corresponds to the amount of organic matter present on the substrate. For this reason, also COD can be used to

predict BMP (Jingura and Kamusoko, 2017). This method is based on the assumption that to oxidize carbon to carbon dioxide, two moles of oxygen are needed per mole of methane (according to Eq. (2.6)). Therefore, by the definition of COD, 4 g of COD corresponds to 1 g of CH_4 and based on the ideal gas law 1 g of methane is equivalent to 1.4 L. So, it can be assumed that 1 g of COD is able to produce 350 mL of methane, meaning that the theoretical BMP based on COD (BMP_{MET_III}) can be defined by Eq. (2.7),

$$CH_4+2O_2 \rightarrow CO_2+2H_2O \quad (2.6)$$

$$BMP_{MET\_III}=COD \times 350 \quad (2.7)$$

where BMP_{MET_III} is expressed in (NmL CH_4 gVS^{-1}) and COD in (g gVS^{-1}).

Method IV (Met_IV) – Near-infrared spectroscopy

Near-infrared spectroscopy (NIR) is a non-destructive method since the substrate is irradiated by near-infrared light in order to obtain the spectrum of absorbance with the "fingerprint" of the composition. For this, a special halogen lamp is used, that does not modify the substrate. This is a fast and economical method, but to determine the BMP it is necessary to calibrate first a multivariate model. This method allows also to determine the biodegradability using a calibration curve, or the composition of the substrate may be determined and then the BMP is deduced (BMP_{MET_IV}) (Doublet et al., 2013).

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3. STATE OF THE ART

3.1. BIOCHEMICAL METHANE POTENTIAL

According to European legislation (Directive 2008/98/EC and Directive 2009/28/EC), the biological treatment of organic household waste is required due to both waste management and energy production strategies. Therefore, AD has been recognized as the main technology suggested for application in this respect.

To manage full-scale AD, the proper characterization of the feedstock is required, in order to optimize the process as well as predict methane production. Among the properties of the feedstock commonly monitored for AD, BMP is the major requirement (Naroznova et al., 2016).

In fact, BMP has been used as the most relevant indicator for predicting biodegradability (Triolo et al., 2011). Though, BMP should not be directly related to biodegradability (BD). BMP is the methane yield and reflects the biological destruction of organic materials, and the methane potential of each organic component in the volatile solids (VS) pool varies widely (Triolo et al., 2011). Thus, BMP test is used to understand which types of substrates, from a variety of possibilities, have the highest biochemical potential (Nielfa et al., 2015).

The theoretical methane potential has been widely recognized to give an indication of the maximum methane production expected from a specific waste. Although, the experimental methane yield is often much lower than theoretical yield due to difficulties in degrading the tightly lignocellulosic material.

Examples of approaches for obtaining quick BMP results include the use of empirical relationships based on the chemical and biochemical composition of the material, by measuring organic composition in the form of volatile fatty acids, proteins, lipids and carbohydrates (Naroznova et al., 2016). Moreover, there are other theoretical methods, as described in section 2.8. However, these methods do not provide any information about the kinetic parameters involved in the process (Nielfa et al., 2015).

Regardless of the theoretical method used, its accuracy will largely depend on the knowledge of the substrate composition, and particularly, on its biodegradable fraction. Thus, the need for a simple, quick, and accurate method to estimate biomethane yields and biodegradability of organic substrates is obvious. In this study, data from 40 papers were collected and the BMP values for various substrates are summarized in Table 3.1. More detailed information about these data, namely about nomenclature used, can be found in Appendix A.

Table 3. 1. BMP ranges for different substrates reported in the literature.

Reference	Substrates	BMP ($mLCH_4\ gVS^{-1}$)	Reference	Substrates	BMP ($mLCH_4\ gVS^{-1}$)
Bolado-Rodríguez et al. (2016)	P23	222.0	Kafle et al. (2013)	C1, D1, D4, D7	304.0 – 539.0
Buffiere et al. (2006)	V10	294.0	Kafle and Chen (2016)	M3, M4, M5	155.0 – 259.0
Buffière et al. (2008)	F10_2	291.7	Labatut et al. (2011)	B1, C5_2, D3, D5, D8,	171.0 – 648.5
Calabrò et al. (2015)	F7_3	330.0		D11, P18, V15_2	
Cho et al. (1995)	D2	294.0	Li et al. (2013)	D10, D12, P5_1, P5_2	171.0 – 776.0
Davidsson et al (2007)	W1, W2, W3, W4, W5, W6, W7, W8, W9,	298.0 – 573.0	Möller et al. (2004)	M2_2	148.0
	W10, W11, W13, W14, W15		Naroznova et al. (2016)	O3, O5, O6, P0, V0	202.0 – 425.0
Edward et al. (2015)	P14, P15	113.3 – 141.5	Nielfa et al. (2015)	S0, W18	164.5 – 201.5
Gunaseelan (2004)	F1_1, F1_2, F7_1, F11_1, F14_1,	180.0 – 374.0	Nieto et al. (2012)	B0, D6, D13	425.0 – 584.0
	F14_2, V3, V4_2, V5_1, V5_2, V6,		Pecorini et al. (2016)	W16, W17	119.6 – 172.1
	V7_1, V7_2, V8_2, V14		Pellera and Gidarakos (2016)	B2_2	446.2
Gunaseelan (2007)	F2, F4, F9_1, F9_2, F9_3, F11_2, P17,	240.0 – 523.0	Pesce et al. (2017)	P8	221.8
	V1, V2, V4_1, V8_1,		Qiao et al. (2011)	D0	531.3
	V11, V12		Qin et al. (2017)	C2_1, D9, F8, F12, V13	310.8 – 331.6
Gunaseelan (2009)	F6, P13	237.0 – 306.0	Raposo et al. (2008)	P12	227.0
Gunaseelan (2014)	P21_1, P21_2	205.0 – 322.0	Rico et al. (2014)	F13	223.0
Gunaseelan (2016)	P1_1, P1_2, P4_1, P4_2, P9, P10_1,	114.0 – 382.0	Rincón et al. (2013)	F15_2	373.0
	P10_2, P22_1, P22_2, P24_1,		Sambusiti et al. (2012)	C3	271.0
	P24_2,		Shen et al. (2014)	P2	225.0
	P25_1, P25_2		Strömberg et al. (2014)	O1	380.0
Gurung et al. (2012)	P6, P19	179.0 – 256.0	Sun et al. (2015)	C4, C5_1, P3, P11	174.0 – 280.0
Hansen et al. (2004)	W12	495.0	Thygesen et al. (2014)	M6, M7	166.0 – 182.0
Hidalgo and Martín-Marroquín (2015)	M2_1, M8, M9, O2, S1, S2, S3, S4, S5, S6, S7	164.5 – 706.0	Triolo et al. (2012)	P20	332.7
			Zheng et al. (2013)	F3, P7_1, P16, V9	118.0 – 445.0
Jokela et al. (2005)	O4	217.0			

3.2. ANAEROBIC DIGESTION PROCESS

The high relevance of AD has led to the evolution of the technology and optimization of the operating conditions that allow increasing the profitability of the processes. The most commonly used mode of operation is still digestion in only one digester. Although, treatment units with more than one stage are increasing. AD processes can still be performed in three and four stages to separate the phases in order to intensify the digestion reactions. However, they are not very used because the yield obtained in biogas does not compensate the equipment investment (Ward et al., 2008).

The yield of biogas specific production and methane is strongly dependent on the operating conditions and pre-treatments that can be applied to the substrate. Table 3.2 summarizes the most common operating parameters used in AD.

Table 3. 2. Operational parameters in AD at lab scale.

Operational parameter	**Observations**
Reactor volume	At laboratory scale digester volumes can vary between 0.1-5 L with a working volume 70 and 80% of the total volume of the reactor (Mao et al., 2015).
Temperature	Although thermophilic has a rate-advantage over mesophilic digestion, processes in thermophilic conditions have lower solubilization rates compared to those obtained for mesophilic conditions, which can make this temperature range unfeasible (Zhang et al., 2014).
S/I ratio	According to Liu et al. (2009), it has been found that lower methane yield is obtained at S/I ratios higher than 4.0 and that maximum conversion of different feedstocks were obtained with S/I ratios of 0.5–1.0.
OLR	The biogas production yield increases with increasing of OLR. However, the process can be inhibited with the increase of OLR. To avoid this inhibition it is recommended to apply OLR in the range of 0.5-9.2 g VS L^{-1} d^{-1} (Mao et al., 2015).
Agitation	This parameter varies according to the volume of the digester. Agitation can be orbital, magnetic, mechanical or manual (in the case of small reactors).

4. MATERIALS AND METHODS

4.1. SUBSTRATE AND INOCULUM

Winery waste (WW) is a result of the production of wine spirits and was collected from a Portuguese winery located in Bairrada. The divided sample was frozen in smaller subsamples (0.5 and 1 L) until further use.

Tomato waste (TW) (*S. lycopersicum L.*) and banana peel waste (BW) (*Musa paradisiaca L.*) in a high state of maturation were collected in a local supermarket. In the case of TW, the whole fruit was smashed and the BW peels were milled in order to reduce their size to particles smaller than 10 mm. Both TW and BW were frozen in small fraction until further use.

The inoculum used in laboratory tests was collected from an anaerobic digester of an urban wastewater treatment plant (Choupal, Coimbra).

4.2. BMP ASSAY

The batch BMP test was developed in 300 mL Erlenmeyers, with a working volume of 75% of the total volume. The experimental setup can be seen in Fig. 4.1. The test lasted until no further production was observed, normally approximately 12 days. These vessels are placed in a thermostatic bath at 38 ° C and connected with a plastic tube to a glass vial containing a solution of NaOH (4 M) to solubilize CO_2 present in the biogas (Qin et al., 2017). After passing through the NaOH solution, the gas follows into a graduated gasometer with a sealing solution (60% NaCl at pH 2) which does not permit the gas solubilization (Owamah and Izinyon, 2015) but allowing the measurement of the volume of methane produced.

In each digester, the substrate and inoculum were placed with an S/I ratio of 0.5, which is the best proportion for the BMP indicated by the literature (Labatut et al., 2011).

Before starting each test, the medium pH was set at approximately 7.2 and the vessels were purged with nitrogen for 15 minutes to ensure anaerobic conditions and were immediately isolated.

Manual shaking was performed once a day to ensure homogenization of the mixture.

In each BMP trial, one blank flask containing just inoculum was included to account for background methane production.

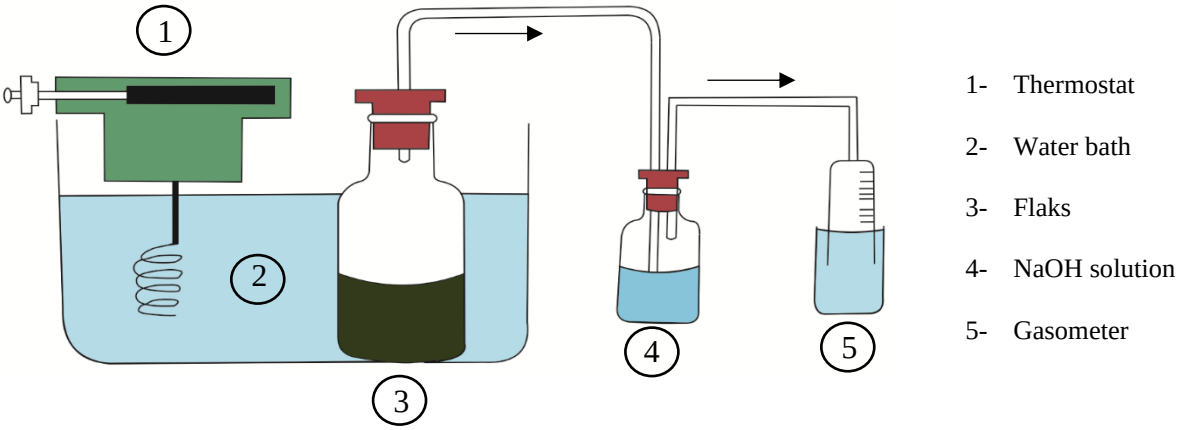


Fig.4. 1. BMP test experimental setup.

Table 4. 1. Different assay performed in BMP test.

Assay	Substrate	S/I	T (°C)	Objective
WW_{BMP1}	Winery waste	0.5	38 ± 0.1	Determine BMP of each substrate
WW_{BMP2}	Winery waste	0.5	38 ± 0.1	
TW_{BMP1}	Tomato waste	0.5	38 ± 0.1	
TW_{BMP2}	Tomato waste	0.5	38 ± 0.1	
BW_{BMP1}	Banana waste	0.5	38 ± 0.1	
BW_{BMP2}	Banana waste	0.5	38 ± 0.1	

In addition to the experimental assessment of BMP, in this work, this parameter was also obtained based on NIR (BMP_{NIR}) spectra. This determination was done in Lódz University of Technology, in Poland, through the spectrometer NIRFlex N500. To obtain NIR spectra, the substrates were freeze-dried and ground. This method requires a mathematical model that relates specific absorption bands of the spectrum and the value of interest (BMP) (Ward, 2016).

4.3. ANAEROBIC REACTOR

4.3.1. Setup and design

The batch AD tests were developed in a 5 L acrylic reactor, isolated from the light, with a working volume of 75% of the total (3.75 L). The experiments of AD were carried until no further biogas production was observed (one or two weeks).

The digester has a heating jacket, connected to a thermostatic bath at 38 °C. The biogas flows through a plastic tube to a graduated gasometer, with a sealing solution (60% NaCl at pH

2) for avoiding its solubilization (Owamah and Izinyon, 2015). The experimental setup can be seen in Fig. 4.2. Before starting each test, the medium pH was set at approximately 7.2 and the digester was purged with nitrogen for 30 minutes to ensure anaerobic conditions and was immediately isolated. for avoiding the entrance of air. Magnetic agitation was performed, at approximately 400 rpm, to ensure the homogenization of the mixture. The operational conditions of each assay can be observed in Table 4.2.

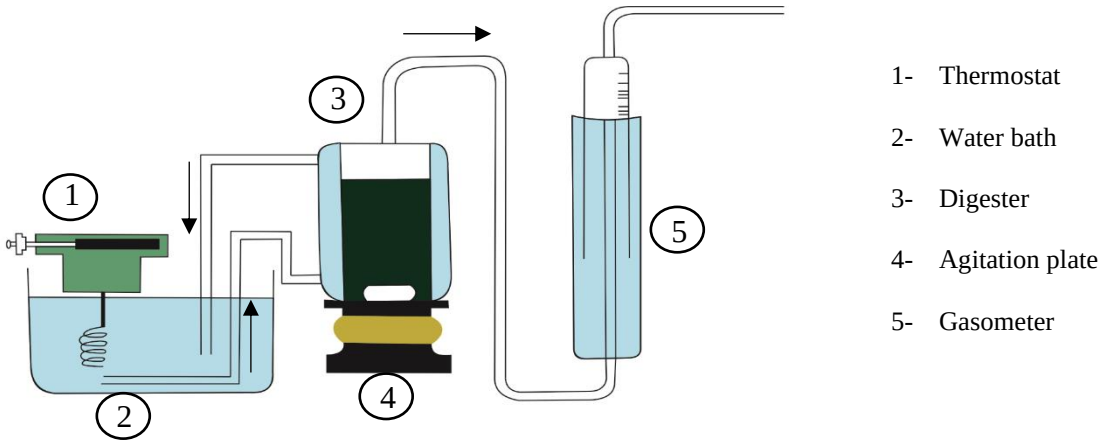


Fig.4. 3. AD experimental setup used at lab scale.

Table 4. 2. Different assay performed in AD test.

Assay	Substrate	S/I	T (°C)	Objective
WW1	Winery waste	0.5	38 ± 0.1	Determine the optimum S/I ratio and biogas production
WW2	Winery waste	1.0	38 ± 0.1	
WW3	Winery waste	1.5	38 ± 0.1	
TW	Tomato waste	0.5	38 ± 0.1	Determine the biogas production
BW	Banana waste	0.5	38 ± 0.1	

4.3.2. Biogas measurement and calculations

The methane content in the biogas was measured according to the procedure described in Abdel-Hadi (2008) and outlined in Fig. B1.1 of Appendix B. This method assumes that the composition of the biogas is a binary mixture of CH_4 and CO_2, therefore this is an approximate method.

In this measurement, a syringe is introduced into the gaseous sampling port of the reactor and 30 mL of biogas is normally collected. The syringe is then introduced into a NaOH solution (4 M) and biogas is discarded until only 10 mL remains inside. Afterwards, about 20

mL of NaOH are introduced in the syringe and vigorously stirred to promote the contact of the gas with the liquid, for 30 s. After this time, the plunger is withdrawn from the syringe until the volume of 10 mL (initial volume). The methane percentage can be determined through Eq. (4.1).

$$CH_4(\%) = \left(1 - \frac{V_{\text{liquid remains}}}{V_{\text{initial gas}}}\right) \times 100 \quad (4.1)$$

4.4. ANALYTICAL METHODS

All chemical or physical parameters were determined using procedures well described in the literature.

Moisture and total solids

A sample of fresh waste with known weight or volume was dried in an oven at 105 °C until constant weight (approximately 24 hours) according to APHA (1998). Moisture content (Moist.) corresponds to the difference between initial and final weights, according to Eq. (4.2).

Total solids (TS) represent the amount of dried solids in the sample and can be determined through Eq. (4.3),

$$\text{Moist.}(\%) = \frac{m_{fs} - m_{ds}}{m_{fs}} \times 100 \quad (4.2)$$

where m_{fs} is the mass of fresh sample (g) and m_{ds} is the mass of dried sample at 105ºC (g).

$$TS\ (\%) = 100 - \text{Moist.}\ (\%) \quad (4.3)$$

Volatile solids and total organic carbon

Volatile solids (VS) are determined using crucibles previously calcined. After the sample is dried at 105 ºC, it was calcined at 550 ºC, during approximately 2 h. VS was calculated by Eq. (4.4).

$$VS(\%TS) = \frac{m_{ds} - m_{cs}}{m_{ds}} \times 100 \quad (4.4)$$

where m_{cs} is the sample weight after calcination at 550 ºC (g).

Total suspended solids and suspended volatile solids

Total suspended solids (TSS) are determined through filtration of a known volume sample. The filter is dried in an oven at 105 ºC until constant weight. The % of SS can be calculated using the Eq. (4.5). After that, the filter is dried and weighed and placed in a crucible (previously calcinated) for calcination at 550 ºC for approximately 2 h. Thus, the content of volatile suspended solids (VSS) is calculated by Eq. (4.6),

$$TSS = \frac{m_{pfds} - m_{pf}}{V_{samp}} \quad (4.5)$$

$$VSS = \frac{m_{pfcs} - m_{pfds}}{V_{samp}} \quad (4.6)$$

where m_{pf}, m_{pfds} and m_{pfcs} are the mass of the dried paper filter before filtration (g), dried paper filter after filtration (g) and dried paper filter after calcination (g), respectively. V_{samp} is the volume of sample filtered (L), where TSS and SVS are both expressed in (g L^{-1}).

pH, alkalinity and VFA

For the determination of pH the equipment *Crison micro pH 2002* was used.

The determination of partial alkalinity (PA), total alkalinity (TA) and volatile fatty acids (VFA) was performed following the titration method of 3 pH points described in Purser et al. (2014).

After the titration method PA, TA and VFA can be determined by the Eq. (4.7), Eq. (4.8) and Eq. (4.9), respectively,

$$PA = \frac{V_{acid\ 5.75} \times N_{acid} \times 50000}{V_{sample}} \quad (4.7)$$

$$TA = \frac{V_{acid\ 5.00} \times N_{acid} \times 50000}{V_{sample}} \quad (4.8)$$

$$VFA = \frac{(V_{acid\ 4.30} - V_{acid\ 5.75}) \times N_{acid} \times 50000}{V_{sample}} \quad (4.9)$$

where N_{acid} is de normality (N) of the titrant, V_{sample} is the volume (mL) of the titrated sample; $V_{acid\ 4.30}$, $V_{acid\ 5.00}$ and $V_{acid\ 5.75}$ are the volume (mL) of titrant used to reach pH equal to 4.30, 5.00 and 5.75, respectively; PA and TA are expressed in (mg $CaCO_3$ L^{-1}) and VFA is expressed in (mg L^{-1}).

Chemical oxygen demand

- Liquid samples

Total Chemical oxygen demand (tCOD) was determined by the preparation of sample test vials and calibration vials, which were digested in the *ECO25 thermoreactor (VELP Scientifica)* for 2 h at 150 ºC. Then, after cool until room temperature, the absorbance of each solution in the vials was read in the *photometer PhotoLab S6 (WTW)* at 605 nm. For the sCOD the same procedure was used, but the liquid sample was previously filtered through a 1-3 µm filter.

Each test vial contains 1.2 mL of digestion solution, 2.8 mL of acid solution and 1 mL of sample.

- Solid samples

The COD of solid samples was assessed through the procedure referred in Noguerol-Arias et al. (2012). The adopted methodology involved preparation of sample test vials and calibration vials, which were digested in the *ECO25 thermoreactor (VELP Scientifica)* for 2 h at 150 ºC. Then, after cooling down until room temperature, the absorbance of each solution in the vials was read in the *photometer PhotoLab S6 (WTW)* at 605 nm. Each test vials contains 1 mg of sample (dried at 105 ºC), 399 mg of distilled water, 3.6 mL of acid solution, 3.6 mL of digestion solution.

Both COD of liquids and solids determination required the preparation of acid and digestion solutions as well as a calibration curve. These procedures are summarized in Appendix C.

Elemental composition analysis

The elemental composition of each residue, C, N, H and O was determined on the equipment Elemental Analyzer NA 2500 (Instruction Manual NA 2500) at Lódz University of Technology in Poland. The samples were dried and milled previously to analysis.

4.5. STATISTICAL METHODS

In order to evaluate the characteristics of the substrate that affect BMP and develop mathematical models that are able to predict this parameter, experimental data from different substrates were collected from about 40 articles from the literature. The data collected was organized in 10 substrates categories, namely: beverages (B), cereals (C), diet (D), fruit (F), manure (M), plants (P), sludge (S), vegetables (V), municipal wastes (W) and others (O). In each case all data potentially correlated with BMP was also collected, namely VS, lipids (LP), protein (PT), carbohydrate (CRB), lignin (LG), hemicellulose (HMC), cellulose (CEL), crude fibre (CF), acid detergent fibre (ADF) content and also the total chemical oxygen demand (tCOD), elemental composition (C, N, H, O) and carbon-nitrogen ratio (C/N).

In the model development process, a standard least squares regression method was used. Throughout this process, all relations between the different variables were explored, in order to obtain a statistically significant model, the terms without mathematical meaning were eliminated, which allowed to obtain simpler models. In order to obtain a model with a high predicting capability of BMP_{EXP}, the points identified as outliers were eliminated, resulting in a final model.

The statistical analyses, namely linear and standard least squares regressions, were performed using *JMP Pro software* and the kinetic analysis presented in this study were performed using *SigmaPlot software*.

5. RESULTS AND DISCUSSION

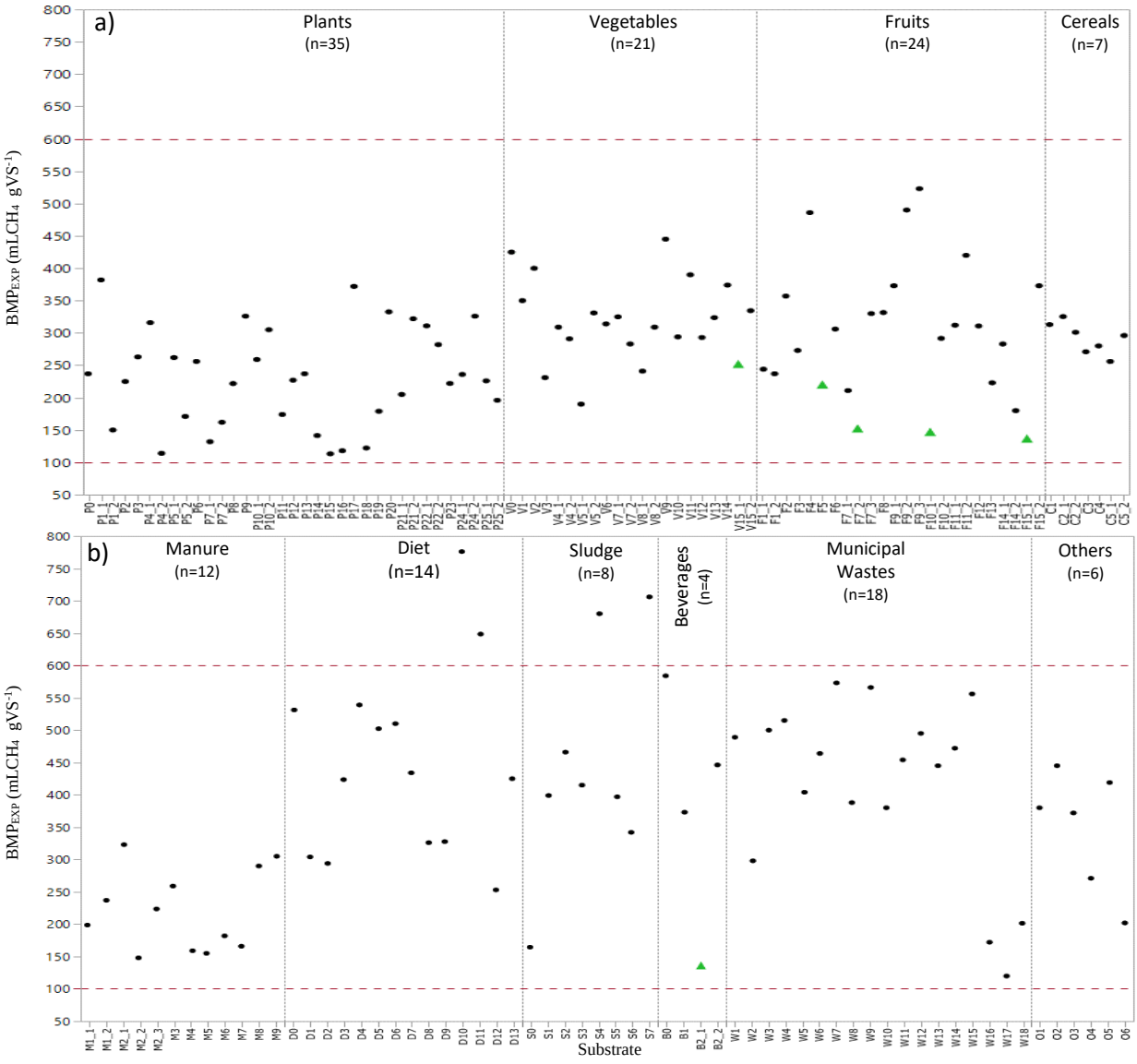
5.1. BIOCHEMICAL METHANE POTENTIAL STATISTICAL ANALYSIS

In order to evaluate the characteristics of the substrate that affect BMP experimental data from 40 scientific papers were collected. From these papers, it was possible to obtain BMP values for about 149 different substrates (detailed in Appendix A) grouped in 10 categories: beverages (B), cereals (C), diet (D), fruit (F), manure (M), plants (P), sludge (S), vegetables (V), municipal wastes (W) and others (O).

In Fig. 5.1 are represented the experimental BMP data for the different categories, where values marked with a (▲) were obtained in this study and will be discussed in detail in the following sections. It is possible to conclude that BMP from these 149 measurements can be mostly between 100-600 ($mLCH_4\ gVS^{-1}$). The exceptions are D_{10}, D_{11}, S_4 e S_7, that corresponds to used vegetable oil, used animal oil, oily waste sludge and vegetable oil sludge, with values extremely high.

Fig. 5.1 shows that plants, vegetables, cereals and manure categories typically can reach methane yields between 110-400 ($mLCH_4\ gVS^{-1}$), while in the other categories great dispersion can be observed, due to their intrinsic characteristics and complexity.

In general, these experimental BMP data reveal that based on a specific category is not possible to predict easily the value of this important parameter.



B0	Nieto et al., 2012	F2	Gunaseelan, 2007	M8 - M9	Hidalgo and Martín-marroquín, 2015	P21_1 - P21_2	Gunaseelan, 2014
B1	Labatut et al., 2011	F3	Zheng et al., 2013	O1	Strömberg et al., 2014	P22_1 - P22_2	Gunaseelan, 2016
B2_1	This study	F4	Gunaseelan, 2007	O2	Hidalgo and Martín-marroquín, 2015	P23	Bolado-rodríguez et al., 2016
B2_2	Pellera and Gidarakos, 2016	F5	This study	O3	Naroznova et al., 2016	P24_1 - P25_2	Gunaseelan, 2016
C1	Kafle et al., 2013	F6	Gunaseelan, 2009	O4	Jokela et al., 2005	S0	Nielfa et al., 2015
C2_1	Qin et al., 2017	F7_1	Gunaseelan, 2004	O5-P0	Naroznova et al., 2016	S1 - S7	Hidalgo and Martín-marroquín, 2015
C2_2	Sun et al., 2015	F7_2	This study	P1_1 - P1_2	Gunaseelan, 2016	V0	Naroznova et al., 2016
C3	Sambusiti et al., 2012	F7_3	Calabrò et al., 2015	P2	Shen et al., 2014	V1 - V2	Gunaseelan, 2007
C4 - C5_1	Sun et al., 2015	F8	Qin et al., 2017	P3	Sun et al., 2015	V3	Gunaseelan, 2004
C5_2	Labatut et al., 2011	F9_1 - F9_3	Gunaseelan, 2007	P4_1	Gunaseelan, 2016	V4_1 - V4_2	Gunaseelan, 2007
D0	Qiao et al., 2011	F10_1	This study	P4_2	Gunaseelan, 2016	V5_1 - V7_2	Gunaseelan, 2004
D1	Kafle et al., 2013	F10_2	Buffière et al., 2008	P5_1 - P5_2	Li et al., 2013	V8_1	Gunaseelan, 2007
D2	Cho et al., 1995	F11_1	Gunaseelan, 2004	P6	Gurung et al., 2012	V8_2	Gunaseelan, 2004
D3	Labatut et al., 2011	F11_2	Gunaseelan, 2007	P7_1 - P7_2	Zheng et al., 2013	V9	Zheng et al., 2013
D4	Kafle et al., 2013	F12	Qin et al., 2017	P8	Pesce et al., 2017	V10	Buffiere et al., 2006
D5	Labatut et al., 2011	F13	Rico et al., 2014	P9 - P10_2	Gunaseelan, 2016	V11 - V12	Gunaseelan, 2007
D6	Nieto et al., 2012	F14_2	Gunaseelan, 2004	P11	Sun et al., 2015	V13	Qin et al., 2017
D7	Kafle et al., 2013	F15_1	This study	P12	Raposo et al., 2008	V14	Gunaseelan, 2004
D8	Labatut et al., 2011	F15_2	Rincón et al., 2013	P13	Gunaseelan, 2009	V15_1	This study
D9	Qin et al., 2017	M1_1 - M1_2	Triolo et al., 2011	P14 - P15	Edward et al., 2015	V15_2	Labatut et al., 2011
D10	Li et al., 2013	M2_1	Hidalgo and Martín-marroquín, 2015	P16	Zheng et al., 2013	W1 – W11	Davidsson et al., 2007
D11	Labatut et al., 2011	M2_2	Möller et al.,2004	P17	Gunaseelan, 2007	W12	Hansen et al., 2004
D12	Li et al., 2013	M2_3	Triolo et al., 2011	P18	Labatut et al., 2011	W13 – W15	Davidsson et al., 2007
D13	Nieto et al., 2012	M3 - M5	Kafle and Chen, 2016	P19	Gurung et al., 2012	W16 - W17	Pecorini et al., 2016
F1_1 - F1_2	Gunaseelan, 2004	M6 - M7	Thygesen et al., 2014	P20	Triolo et al., 2012	W18	Nielfa et al., 2015

Fig.5. 1. Range of BMP measured in different substrate categories: a) plants, vegetables, fruits and cereals; b) manure, diet, sludge, beverages, municipal wastes and others**.**

5.1.1. Comparison between theoretical and experimental BMP

Based on the literature it was possible to identify four theoretical methods to predict BMP: Met_I – elemental analysis, Eq. (2.4); Met_II – organic composition fraction, Eq. (2.5); Met_III – chemical oxygen demand, Eq. (2.7); and Met_IV – Near-infrared spectroscopy.

A comparison between the values predicted by the first three methods and the experimental BMP reported in the literature was conducted as indicated in Fig 5.2. This comparison did not involve Met_IV, since this method was not mentioned in the scientific papers used in this study. However, it is worthy to emphasize that very recent papers in the literature shows very promise results with this method (Fitamo et al., 2017). In order to carry out the comparison of experimental BMP with the one predicted by the inherent characteristics of each substrate Eq. (2.4), (2.5) and (2.7) were used directly (results in Fig. 5.2). Moreover, these theoretical values were corrected, whenever possible, by the biodegradable fraction of volatile solids (BDVS) which can be estimated using Eq. (5.1) (Chandler et al.,1980).

$$BD_{VS}=0.83-0.028\times LG\ (\%VS) \tag{5.1}$$

where LG is the lignin content as % of VS (0<LG<20%). In fact, Eq. (5.1) shows that even if LG content is zero, the fraction of biodegradable VS does not exceed 0.83.

In Fig. 5.2 a)-c) shows the predicted BMP by Met_I to Met_III as a function of the experimental values is represented. Moreover, in the case where LG content was available, the theoretical BMP values were corrected by using Eq. (5.1) (data referred as Met_Ic, Met_IIc, Met_IIIc).

Through the analysis of Fig. 5.2 is possible to observe that, in general, the BMP obtained by the theoretical relations presented overestimate experimental BMP. This was expected since biodegradability of the substrate is not taken into account, assuming that all organic matter is converted to methane (Lesteur et al., 2010). Therefore, if the theoretical values are corrected with BD_{VS}, the data may become closer to the line that represents the identity function. In some cases, there is an underestimation of the actual value. In the case of Met_I, with the biodegradability correction, there is a significant approximation of the predicted to the experimental values.

In general, the theoretical BMP models do not fully represent the reality, due to the high complexity of this parameter. Nonetheless, these models may be valuable as they provide an

indication of BMP with much less effort and costs than by the experimental assessment. The correction with BD_{VS} seems to lead to improved predictions, mainly in the case of Met_I.

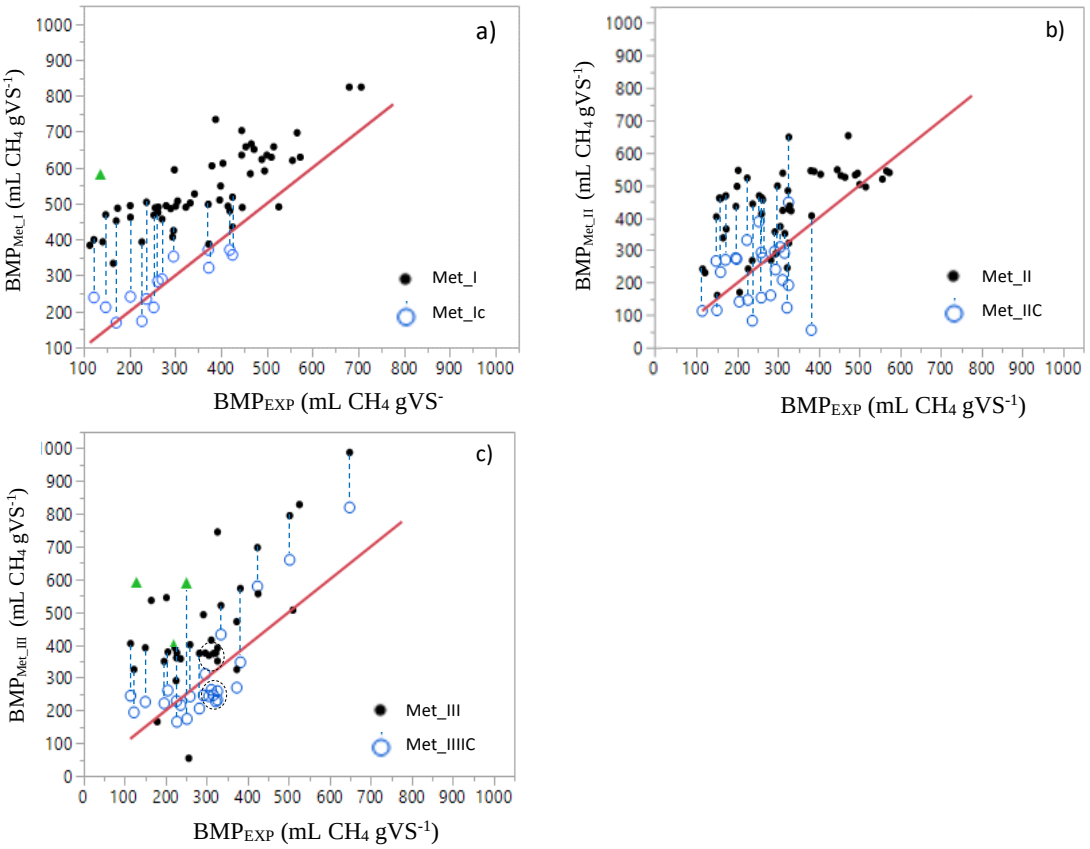


Fig.5. 2. Parity plot of experimental versus predicted and corrected BMP values. a) Met._I; b) Met_II; c) Met_III.

5.1.2. Influence of analytical parameters on BMP

Since no obvious dependence on the origin of the substrate and BMP was concluded, nor the theoretical methods were able to represent totally the reality, as seen in the previous sections, so a more detailed analysis is needed.

According to data from the literature there are several parameters often determined simultaneously to BMP: volatile solids (VS), lipids (LP), proteins (PT), carbohydrates (CRB), elemental composition (C, N, H, O), lignin (LG), hemicelluloses (HMC), celluloses (CEL), acid detergent fibre (ADF), total chemical oxygen demand (tCOD) and carbon-nitrogen ratio (C/N).

ADF corresponds to the fibrous component of substrates, and represents the least digestible fiber portion, composed mainly by LG and CEL.

In order to assess the influence of these 14 parameters over BMP value, a simple linear regression analysis was assessed, as shown in Fig.5.3. All these factors are related to the organic part of the substrates

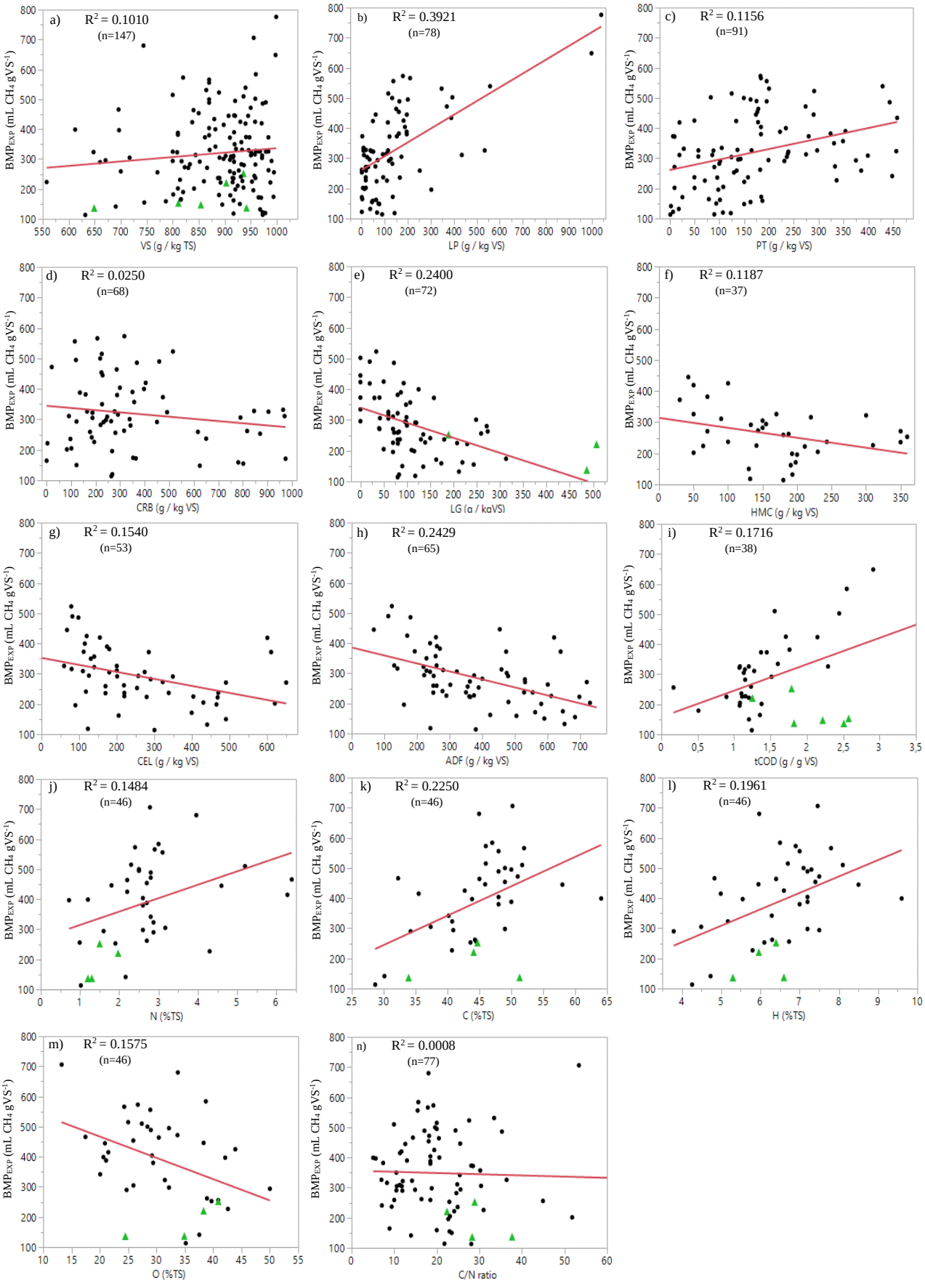


Fig.5. 3. Scatter plots of the experimental BMP with the different parameters characterizing organic matter of substrates: a) VS; b) LP; c) PT; d) CRB; e) LG; f) HMC; g) CEL; h) ADF; i) tCOD; j) %C; k) %N; l) %H; m) %O; n) C/N.

Fig. 5.3 shows that none of the factors is enough to explain the majority of the observed values of BMP since all R^2 are quite low ($R^2 < 0.40$). Even so, the variables with the highest coefficient of determination, albeit insufficient to explain BMP due to high dispersion, are LP ($R^2 = 0.39$), ADF ($R^2=0.2429$) and LG ($R^2 = 0.24$). The positive correlation between LP, PT, tCOD, %N, %C, %H and BMP_{EXP}, indicate that substrates with a larger value of these variables it is likely that BMP may be higher. On the other hand, there is a negative correlation of BMP with CRB, O%, LG, CEL, HMC and ADF. The negative correlation between LG, CEL and ADF with BMP was expected since, these structures represent the most complex and low biodegradable parts of the organic substrates (Appels et al., 2011). It is interesting to note that volatile solids are usually referred to as a good indicator of the amount of organic matter present in a substrate and therefore this parameter tends to be used as a primary indicator of methane production. Hence, the volume of CH_4 produced is usually normalized by the amount of VS in the AD system (Raposo et al., 2011). However, contrary to expectations that BMP_{EXP} increases with the increase of VS content, there is no evidence of the relationship of VS content to methane production. In fact, Fig. 5.3.a) shows that R^2 is 0.01 even using 147 points from the literature.

Also, C/N ratio is commonly referred as a key operational parameter when it comes to methane production (Igoni et al., 2008). However, as it can be observed through Fig. 5.3. h), there is no evidence of dependency between BMP_{EXP} and C/N ratio. Thus, this parameter should not be referred as a good indicator, at least by its own.

Since no linear relationship between single variables is observed (Table 5.1), a more complex analysis is necessary in order to model BMP. Analysing the Table 5.1 is possible to see that C/N, VS and CRB are the variables with less statistical significance (higher p-value) the remaining variables present a good level of significance and should be further explored.

Table 5. 1. Statistical parameters of the linear regressions.

Variables	Number of points	R^2	p-value
VS	147	0.1010	0.2265
LP	78	0.3921	<0.0001
PT	91	0.1156	0.0010
CRB	68	0.0250	0.1980
LG	72	0.2400	0.0010
HMC	37	0.1187	0.0368
CEL	53	0.1540	0.0037
ADF	65	0.2429	<0.0001
tCOD	38	0.1716	0.0087
% N	46	0.1484	0.0019
% C	46	0.2250	0.0020
% H	46	0.1961	0.0041
% O	46	0.1575	0.0009
C/N	77	0.0008	0.8025

5.1.3. BMP multiple regression

The principal component analysis (PCA) is an exploratory data analysis tool for the formulation of multivariate models. In this study, due to the number of variables that may influence BMP parameter, it would be helpful to use this tool. However, this approach was not possible in this case because the number of observations were very different for each parameter. In an attempt to model the BMP, a multiple regression was carried out. Before proceeding, a correlation analysis between the different variables was made, as indicated in the matrix present in Fig. D.1 of the Appendix D. It is possible to confirm a strong correlation between the CEL and ADF. This indicates that these variables should not be present in the same model since they depend on each other. This analysis was taken into account for the different regressions.

In the literature, there are three methods for predicting BMP, these methods are based on elemental composition, organic fraction composition and chemical oxygen demand. The models developed in this study are associated with the relationships underlying these methods, with the addition of the interaction between the lignocellulosic parts, and between the lignocellulosic parts and the organic fraction composition of the substrates. The predictor variables that characterize the different models are identified in Table 5.2

The regression models tested are polynomial with 2, 3 or 5 variables (N) that may include linear interactions and quadratic terms. The general equation is:

$$\hat{Y} = b_0 + b_i \sum_{i=1}^{N} x_i + b_{ii} \sum_{i=1}^{N} {x_i}^2 + b_{ij} \sum_{i \neq j}^{N} \sum_{j=1}^{N} x_i x_j \quad (5.2)$$

Table 5. 2. Independent variables considered in each model.

Model reference	Function	Number of predictor variables
Mod_I	BMP = f (C, H, N, O)	4
Mod_II	BMP = f (HMC, LG, ADF)	3
Mod_III	BMP = f (VS, tCOD)	2
Mod_IV	BMP = f (PT, CRB, LP)	3
Mod_V	BMP = f (CRB, LP, PT, LG, ADF)	5

As referred, the models developed in this study were based on the relationships presented in the theoretical literature: methods. Mod_I is based on elemental composition of the substrate; Mod_II is based on the lignocellulosic parts; Mod_II takes in account VS and total chemical oxygen demand; Mod_IV is related to proteins (PT), carbohydrates (CRB) and lipids (LP); And Mod_V takes into account the organic fraction composition content (CRB, LP and PT) and the lignocellulosic parts of the substrates (LG, HMC, ADF).

In order to develop these multiple regression models several steps were followed.

First, all the points available in the literature that simultaneously determine BMP and the properties required for each model were selected. All relations between the different BMP and predictor variables were explored, but limiting the model to a multiple quadratic with interactions of two or more variables. Thus, a very complex model could be obtained that requires further analysis. The parameters of each model were determined by least squares estimates, using *JMP Pro software.*

In a second phase, the terms without mathematical meaning were eliminated by testing for significance of regression coefficients. This will allow to obtain simpler models. With the simplification of the models there was a decrease in the value of the coefficient of determination (R^2), which allows to evaluate the adjustment of a statistical model in relation to the observed values. In order to obtain a model capable of determining the experimental BMP, the points identified as outliers were removed.

Table 5.3 summarizes the statistical parameters of each model, as well as the categories of the points included in the model and the mathematical expression. In Fig. 5.4 a)-c) is possible to observe the overall significance of each model developed.

Table 5. 3. Models to predict the experimental BMP.

Model	R^2	R^2_{adj}	RMSE	NP	Categories	Mathematical expression
Mod_I	0.93	0.91	42.75	30	All 10 categories	$BMP = 3160.439 - 19.293\ N + 1.834\ C - 40.199\ H - 9.566\ O + 1.993\times10^{-1}\ N \times H + 1.897\times10^{-2}\ N \times O + 1.157\times10^{-1}\ H \times O - 1.482\times10^{-3}$
Mod_II	0.94	0.91	16.61	24	Cereals, plants and vegetables	$BMP = 455.167 - 2.807\ LG + 7.200\times10^{-3}\ LG^2 + 2.900\times10^{-3}\ LG \times HMC - 7.140\times10^{-4}\ HMC \times ADF$
Mod_III	0.93	0.92	36.93	24	Plants, fruits diet and beverages	$BMP = -42.849 - 182.814\ tCOD + 5.432 \times 10^{-1}\ VS \times tCOD - 41.544\ tCOD^2$
Mod_IV	0.94	0.93	36.98	28	Fruits, plants, manure and diet	$BMP = 115.302 + 9.371\times10^{-1}\ PT + 2.379\times10^{-1}\ CRB + 5.706\times10^{-4}\ LP^2 - 1.505\times10^{-3}\ PT \times CRB$
Mod_V	0.98	0.96	13.82	18	Plants and vegetables	$BMP = 108.888 + 8.064\times10^{-1}\ PT + 5.248\times10^{-1}\ CRB + 2.469\times10^{-4}\ LP^2 - 1.483\times10^{-3}\ PT \times CRB - 9.440\times10^{-4}\ CRB \times ADF + 2.223\times10^{-3}\ LG \times ADF + 3.740\times10^{-4}\ ADF^2$

RMSE – Root mean square error

NP – Number of points

where, C, N, H, O, HMC, ADF, LG, LP, PT, CRB are expressed in g $kgVS^{-1}$; tCOD is expressed in g gVS^{-1}; VS is expressed in g $kgTS^{-1}$

Taking into account that BMP is a complex parameter the five models presented in Table 5.3 show some potential for prediction since their determination coefficients (R^2), and adjusted determination coefficients are high (R^2_{adj}). The main difference between these two determination coefficients is that R^2 assumes that every single variable explains the variation in the dependent variable, while R^2_{adj} translates the percentage of variation explained by only the independent variables that actually affect the dependent variable. In addition to R^2 and R^2_{adj}, the parameter RMSE also gives some information about the adjustment capacity of the model, since it allows to evaluate the deviation of the residuals in relation to the line of best fit.

From the models presented in Table 5.3, it is possible to verify that the one that presents the best capacity to predict the BMP, based on R^2 and RMSE, is Mod_V. This ability is due to the fact that this model combines the organic and lignocellulosic parts of the substrates. However, for the application of this model it is required a greater and harder determination of substrate characteristics since this one is composed of 5 predictive variables. Analysing the Table 5.3 it can be seen that Mod_II and IV have the same R^2, but Mod_II has a lower RMSE

value. Then, in theory, Mod_II has a greater predictive capacity than Mod_IV. The model that presents less complexity is Mod_III since it depends only on two parameters. However, this model combines the tCOD with the VS, and previously it was verified that this last parameter did not have great significance in relation to the BMP. Given this, this model may have weak predictive ability for points outside its construction. The Mod_I, although its R^2 value is not as high as the others, can become a model with high predictive capacity. Once, despite having a high number of terms (8), all of them are obtained with a single analysis (elemental analysis). Therefore, this model can be used more quickly and simply.

In order to validate the models Mod_I to Mod_V, additional points that were not used before were selected in the literature. For each point predicted BMP (BMP_{PRED}) was determined, when possible, and to evaluate the adequacy of the fitted model the deviation between the BMP_{EXP} ($mLCH_4\ gVS^{-1}$) and BMP_{PRED} ($mLCH_4\ gVS^{-1}$) was calculated to obtain the relative error according to Eq. (5.3). The results are summarized in Table 5.4.

$$E\ (\%) = \left| \frac{BMP_{EXP} - BMP_{PRED}}{BMP_{EXP}} \right| \times 100 \tag{5.3}$$

Table 5.4 shows the lowest relative error values for each substrate, meaning that for the substrate the model with the lowest value of E (%) can predict better the BMP value.

Through the analysis of Table 5.4, it is possible to observe that Mod_II is the model that can predict the BMP of most substrates, followed by Mod_V, Mod_IV and finally by Mod_I and III. Model II has the ability to predict the BMP of substrates of the different categories defined in this study, presenting errors comprised between 0.1 and 29%, with the exception of the substrate *Thespesia p. yellow leaf* and carrots. This means that there is an evident dependence between BMP and the least biodegradable parts of the substrate (ADF, LG and HMC). Mod_V presents an intermediate prediction capacity, with the ability to predict the BMP mainly of substrates belonging to the category of fruits and vegetables. This model adds to the Mod_II the OFC parts, giving it a greater predictability capacity in the categories indicated above. Although Mod_I does not stand out from the other models, in terms of the BMP forecast, it has to be noted that this model has relatively small prediction errors (9.3-32%) with the exception of rice straw and switchgrass.

In general, Mod_II has a great predictability. However, model I, due to its simplicity vis-a-vis Mod_II, may also constitute a high potential for prediction of BMP.

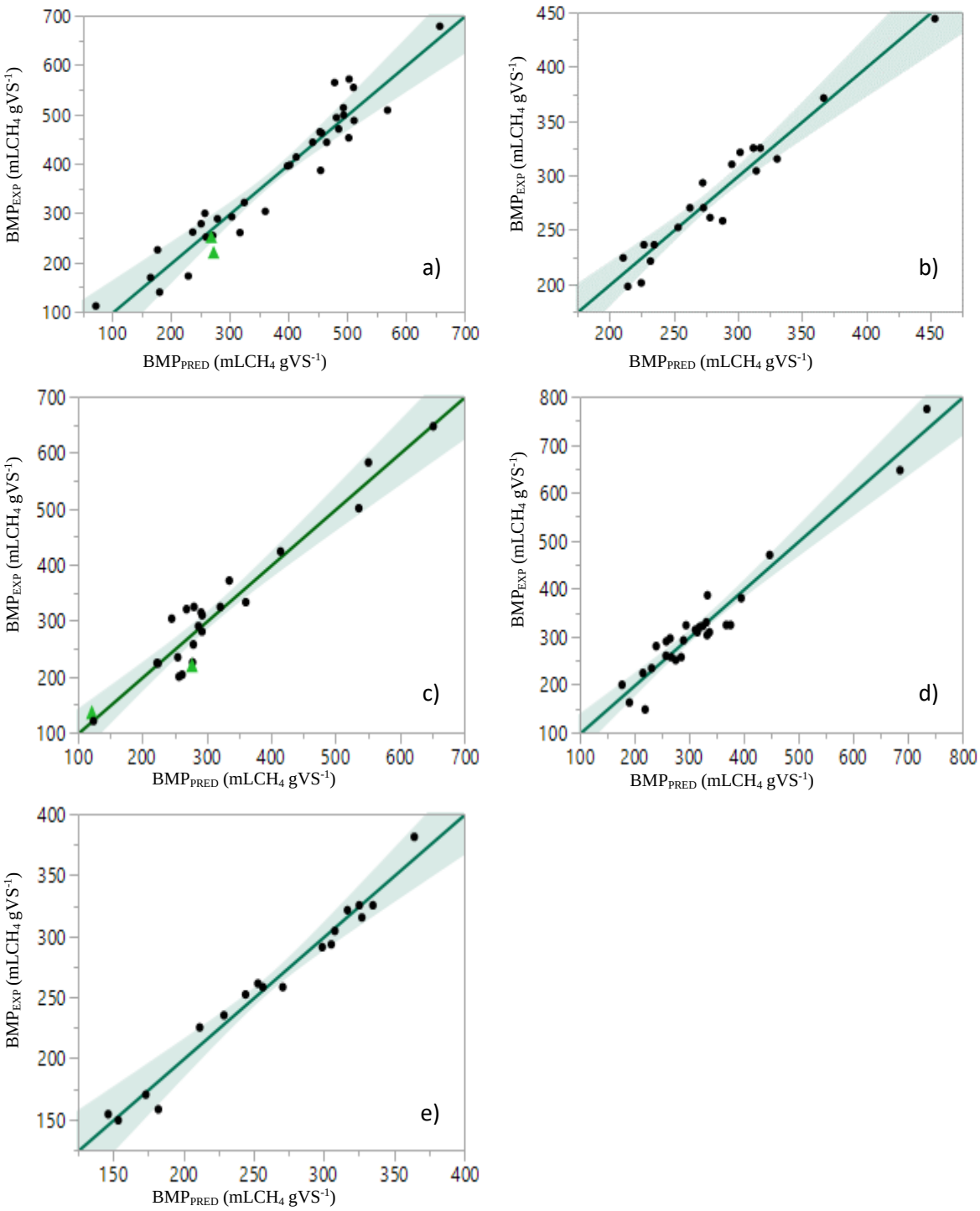


Fig.5. 4. Significance plot of the models: a) Mod_I; b) Mod_II; c) Mod_III; d) Mod_IV; e) Mod_V.

Table 5. 4. Relative error analysis for comparing the adequacy of the models.

Substrate category	Substrate	Mod_ I		Mod_II		Mod_III		Mod_ IV		Mod_V		BMP_{EXP}	Reference
		BMP_{PRED}	E(%)	BMP_{PRED}	E(%)	BMP_{PRED}	E(%)	BMP_{PRED}	E(%)	BMP_{PRED}	E(%)		
Cereals	Corn stover	197.1	18.2	204.7	**15.1**			313.3	30.0	75.0	68.9	241	Li et al., 2013
	Wheat straw	165.9	32.3	216.4	**11.7**			331.0	35.1	58.5	76.1	245	Li et al., 2013
	Rice straw	165.0	41.3	199.1	29.1			306.4	**9.0**	61.0	78.3	281	Li et al., 2013
Manure	Chicken	359.7	21.9	355.2	20.4			246.0	**16.6**	202.8	31.2	295	Li et al., 2013
	Swine	277.8	13.7	322.8	**0.3**			256.4	20.4	308.6	4.2	322	Li et al., 2013
Plants	Switchgrass	156.0	36.6	206.7	**16.0**			333.1	35.4	109.4	55.5	246	Li et al., 2013
	Grass			300.5	**22.5**	278.8	28.1	261.5	32.6	274.7	29.2	388	Buffière et al., 2006
	Gliricidia Maculata												
	yellow leaf			277.8	8.5	178.0	30.5	239.4	6.5	251.0	**1.9**	256	Gunaseelan, 2016
	inflorescence			284.3	**2.0**	241.2	16.8	269.6	7.0	250.0	13.8	290	Gunaseelan, 2016
	Thespesia p. yellow leaf			408.9	94.7	306.6	46.0	262.6	**25.0**	387.9	84.7	210	Gunaseelan, 201
Vegetables	Potato			317.9	18.5	303.4	22.2	276.2	29.2	329.5	**15.5**	390	Buffière et al., 2006
	Carrots			231.0	40.5	286.7	26.1	256,8	33.8	329.8	**15.0**	388	Buffière et al., 2006
Fruits	Banana			289.3	**0.1**	354.9	22.8	258.0	10.7	339.8	17.6	289	Buffière et al., 2006
	Apple			356.0	12.3	341.5	7.7	266.8	15.8	327.0	**3.2**	317	Buffière et al., 2006
	Orange			270.2	9.0			262.0	11.8	302.5	**1.9**	297	Buffière et al., 2006
	Fruit and Vegetable waste	324.7	9.3	331.9	**3.0**			370.5	8.3	319.5	6.6	342	Buffière et al., 2006
Others	Kitchen Waste	432.3	26.4	455.2	**15.9**			685.9	26.8	355.8	34.2	541	Li et al., 2013
	Used animal oil			204.7	**15.1**			313.3	30.0	75.0	90.3	776	Li et al., 2013

5.2. SUBSTRATE AND INOCULUM CHARACTERIZATION

This part of the work is devoted to the experimental analysis of three agro-industrial wastes, namely, winery waste (WW), tomato waste (TW) and banana waste (BW), which will be tested as substrates in AD process. It is important to note that the substrates referred as F5, F15_1 and V15_1 in the previous section were obtained within the scope of this project but in an previous study, whereby only B2_1 (WW), F7_2 (TW) and F10_1 (BW) will be discussed in this section.

The main characteristics of the residues WW, TW and BW are summarized in Table 5.5-5.7, respectively. The experimental results are represented by the mean value ± standard deviation, and whenever possible compared with literature.

Table 5. 5. Chemical characterization of WW.

	This study	**Pellera and Gidarakos (2016)**	**Akassou et al. (2010)**	**Borja et al. (1993)**
Residue	**WW**	**WW**	**WW**	**WW**
TS (%)	3.01 ± 0.05	28.1	3.6	3.2
VS (%TS)	64.86 ± 0.65	91.8	94.3	79.4
tCOD (mg O_2 gVS^{-1})	2509± 4	1398	3298,4	1574,3
sCOD (mg O_2 gVS^{-1})	1959 ± 6	nd	3291,1	nd
pH	4.15 ± 0.03	3.7	3.32	3.8
C (%TS)	33.9 ± 0.6	45.9	nd	nd
N (%TS)	1.2 ± 0.0	1.8	nd	nd
O (%TS)	24.5 ±0.7	38.3	nd	nd
H (%TS)	5.3 ± 0.0	5.95	nd	nd
C/N	28.3	25.5	nd	nd
Empirical formula	$C_{32.1}H_{60.1}O_{17.4}N$	$C_{29.8}H_{46.4}O_{18.7}N$	nd	nd

nd: not determined

Table 5. 6.Chemical characterization of TW.

	This study	**Ferrer et al. (2014)**	**Gil et al. (2015)**	**Calabrò et al. (2015)**
Residue	**TW**	**TW**	**TW**	**TW**
TS (%)	5.99 ± 0.46	6.25	5.4 ± 0.1	16.5
VS (%TS)	81.08 ± 1.19	89.9	84.2	94.6
tCOD (mg O_2 gVS^{-1})	2575 ± 10	nd	1870.13	nd
pH	4.35 ± 0.03	4.56	4.35 ± 0.01	4.3
C (%TS)	38.9 ± 0.1	nd	nd	nd
N (%TS)	1.8 ± 0.0	nd	nd	nd
O (%TS)	34.1 ± 0.0	nd	nd	nd
H (%TS)	6.2 ± 0.1	nd	nd	nd
C/N	21.6	nd	nd	nd
Empirical formula	$C_{25.2}H_{48.2}O_{16.6}N$	nd	nd	nd

nd: not determined

Table 5. 7. Chemical characterization of BW.

Residue	This study BW	Buffière et al. (2015) BW	Bardiya et al.(1996) BW	Tumutegyereize et al. (2011) BW
TS (%)	15.87 ± 0.46	12.8	10.68	16.84
VS (%TS)	85.41 ± 0.34	85.2	86.65	92.32
tCOD (mg O_2 gVS^{-1})	2218 ± 9	1515	nd	nd
pH	6.53 ± 0.05	nd	nd	nd
C (%TS)	42.2 ± 0.6	nd	62.4	nd
N (%TS)	1.4 ± 0.0	nd	1.6	nd
O (%TS)	36.1 ± 0.7	nd	nd	nd
H (%TS)	5.7 ± 0.1	nd	nd	nd
C/N	31.0	nd	39	34.06
Empirical formula	$C_{36.2}H_{59.2}O_{23.3}N$	nd	nd	nd

nd: not determined

In general, the characteristics of the substrates used in this work are consistent with the literature. Nevertheless, it is hard to find studies reporting all the parameters measured. The WW is the residue that shows more differences when compared to the literature namely in the VS and sCOD content. This can be explained by the different origins of the grapes that give rise to this residue since these are quite affected by the climate of the countries where they are cultivated. Moreover, the grapes processing methodology will also lead to wastes with different characteristics. In fact, sometimes in literature is not easy to understand how the waste was generated; therefore, the direct comparison is sometimes not possible. TW and BW chemical characterization is quite similar to what is reported in other studies, with the exception of tCOD. In the case of this study, both substrates present a higher chemical oxygen demand. All residues have a C/N ratio within the range considered as adequate for AD, according to Jain et al.(2015).

tCOD is considered as a good indicator of the amount of organic matter (Jingura and Kamusoko, 2017). Thus, according to that parameter, the most suitable substrate for AD is TW, followed by BW and lastly, with the least potential, WW.

The characteristics of the inoculum used for determine BMP and to AD of the wastes are summarized in Table 5.4. The results are represented by the mean value ± standard deviation, whenever possible.

Table 5. 8. Chemical characterization of the inoculum.

	This study	Arhoun et al. (2013)	Huang et al. (2016)	Fezzani and Cheikh (2010)
TS (%)	3.59 ± 0.16	1.97	5.01	4.00 ± 0.15
VS (%TS)	59.39 ± 0.05	59.00	79.61	67.50 ± 0.30
tCOD (mg O_2 gVS^{-1})	2541 ±15	542.97	1037.19	1314.81 ± 92.59
pH	7.30 ± 0.03	7.30	7.36	7.4 ± 0.03

Globally, the characteristics of the inoculum used in this work are consistent with the literature values, although the tCOD is higher than what is referred in other studies.

5.3. EXPERIMENTAL AND THEORETICAL BMP DETERMINATION

5.3.1. Experimental BMP

BMP experimental determination for each waste was carried out using the experimental setup described in Fig.4.1 (Chapter 4). The experiments were run in duplicate (BMP1 and BMP2), and each replicate contained two trials and a blank flask, but this experimental procedure must be improved in the future work. All the chemical parameters determined in the suspensions at the beginning (t0) and at the end (t∞) of each BMP test are presented in Table 5.9 to 5.11 for three substrates WW, TW and BW, respectively. The characterization included TS, VS, SS, SSV, pH, TA, VFA, tCOD and sCOD.

Table 5. 9. Characterization of suspensions in two replicates of BMP test for WW substrate.

	Replicate 1		**Replicate 2**	
Parameter	**WW1_t0**	**WW1_t∞**	**WW2_t0**	**WW2_t∞**
TS (g L^{-1})	31.56 ± 0.35	30.29 ± 0.47	30.61 ± 0.56	27.58 ± 0.19
VS (% TS)	49.78 ± 0.34	47.80 ± 0.44	48.26 ± 0.85	42.03 ± 0.26
SS (g L^{-1})	4.60 ± 0.13	4.11 ± 0.12	5.79 ± 0.21	3.90 ± 0.29
SSV (% SS)	62.75 ± 0.27	60.37 ± 1.18	70.59 ± 0.90	53.85 ± 0.64
pH	7.22 ± 0.01	6.56 ± 0.02	7.22 ± 0.02	7.11 ± 0.03
TA (g $CaCO_3$ L^{-1})	4.56 ± 0.08	4.67 ± 0.07	6.31 ± 0.25	6.49 ± 0.03
VFA (g L^{-1})	7.64 ± 0.10	8.58 ± 0.18	5.91 ± 0.11	6.69 ± 0.33
VFA/TA	1.68 ± 0.02	1.84 ± 0.03	0.94 ± 0.03	1.03 ± 0.05
tCOD (gO_2 L^{-1})	35 ± 1	32 ± 1	48 ± 1	31 ± 3
sCOD (gO_2 L^{-1})	30 ± 1	26 ± 1	24 ± 1	16 ± 1
SMP_{cumul} (NmL CH4 gVS^{-1})		157.2 ± 26.2		115.6 ± 22.3

Table 5. 10. Characterization of suspensions in two replicates of BMP test for TW substrate.

	Replicate 1		Replicate 2	
Parameter	**TW1_t0**	**TW1_t∞**	**TW1_t0**	**TW1_t∞**
TS (g L^{-1})	25.39 ± 0.22	23.09 ± 0.11	31.44 ± 0.28	28.15 ± 0.13
VS (% TS)	48.48 ± 0.96	46.80 ± 0.33	52.75 ± 0.79	46.30 ± 1.19
SS (g L^{-1})	5.38 ± 0.00	4.22 ± 0.35	6.36 ± 0.63	5.95 ± 0.11
SSV (% SS)	56.88 ± 0.00	64.20 ± 2.52	61.77 ± 1.56	67.76 ± 1.59
pH	7.27 ± 0.01	7.19 ± 0.02	7.12 ± 0.01	7.04 ± 0.00
TA ($gCaCO_3$ L^{-1})	2.47 ± 0.00	4.78 ± 0.30	4.42 ± 0.03	5.87 ± 0.06
VFA (g L^{-1})	2.47 ± 0.00	4.09 ± 0.23	3.91 ± 0.03	6.93 ± 0.05
VFA/TA	1.00 ± 0.00	0.86 ± 0.01	0.88 ± 0.01	1.20 ± 0.02
tCOD (gO_2 L^{-1})	54 ± 1	47 ± 1	55 ± 1	43 ± 1
sCOD (gO_2 L^{-1})	14 ± 3	18 ± 1	21 ± 1	20 ± 1
SMP_{cumul} (NmL CH4 gVS^{-1})		162.1 ± 10.7		142.0 ± 4.2

Table 5. 11. Characterization of suspensions in two replicates of BMP test for BW substrate.

	Replicate 1		Replicate 2	
Parameter	**BW1_t0**	**BW1_t∞**	**BW2_t0**	**BW2_t∞**
TS (g L^{-1})	33.86 ± 0.52	32.97 ± 0.28	33.76 ± 0.94	29.53 ± 0.73
VS (% TS)	50.91 ± 0.26	48.79 ± 0.45	52.34 ± 0.29	44.67 ± 0.19
SS (g L^{-1})	6.31 ± 0.14	6.58 ± 0.19	7.27 ± 0.29	6.58 ± 0.19
SSV (% SS)	73.61 ± 1.11	71.07 ± 1.64	73.06 ± 0.53	69.49 ± 0.87
pH	7.40 ± 0.05	6.97 ± 0.03	7.24 ± 0.00	6.98± 0.02
TA ($gCaCO_3$ L^{-1})	4.47 ± 0.09	3.64 ± 0.06	6.69 ± 0.11	6.40 ± 0.11
VFA (g L^{-1})	8.56 ± 0.13	6.11 ± 0.08	5.49 ± 0.03	6.13 ± 0.16
VFA/TA	1.92 ± 0.01	1.68 ± 0.02	0.82 ± 0.01	0.96 ± 0.02
tCOD (gO_2 L^{-1})	36 ± 1	27 ± 1	49 ± 2	39 ± 1
sCOD (gO_2 L^{-1})	21 ± 0	15 ± 1	36 ± 2	16 ± 1
SMP_{cumul} (NmL CH4 gVS^{-1})		157.3 ± 4.1		135.9 ± 3.6

According to the literature, in order to avoid instability in AD process and in BMP tests as well, particular attention should be given to the evolution of pH, TA and VFA concentration. From Table 5.9 to Table 5.11 it is possible to observe a small decrease in pH in both assays for all the substrates. For example, in the case of WW substrate, pH decrease from 7.22 to 6.56 in replicate 1, and from 7.22 to 7.11 in replicate 2. Thus, there is a real tendency of a decrease in the pH of the medium along the BMP tests. This pH decrease is due to the increase in VFA concentration, and thus it is very important to guarantee a certain level of alkalinity. In the

literature, it is often referred that a ratio VFA/TA<0.8 is required for a stable operation. Although the VFA/TA in the BMP tests may be in unstable zone, the pH at the end of the tests are acceptable in all cases. Indeed, the most important is to maintain the pH in the ideal range for optimal methanogenic bacteria activity (6.5-7.5) (Jain et al., 2015). Therefore, it was assumed that the BMP tests were not affected by the unfavorable ratio VFA/TA.

The results show that there was a reduction in both tCOD and sCOD for all assays, except in one test with TW. The COD reduction is an indication of the conversion of organic matter into methane and carbon dioxide. Due to the progressive hydrolysis of the organic matter, there is a progressive solubilization, causing in a first phase, an increase in sCOD. Later on, there is a decrease due to the conversion of these compounds into methane and carbon dioxide. sCOD at the end of the tests are indicative that there are available organic matter in the test flasks. Fig.5.5 a)-c) shows the specific production of cumulative methane (SMPcumul) for the substrates along time.

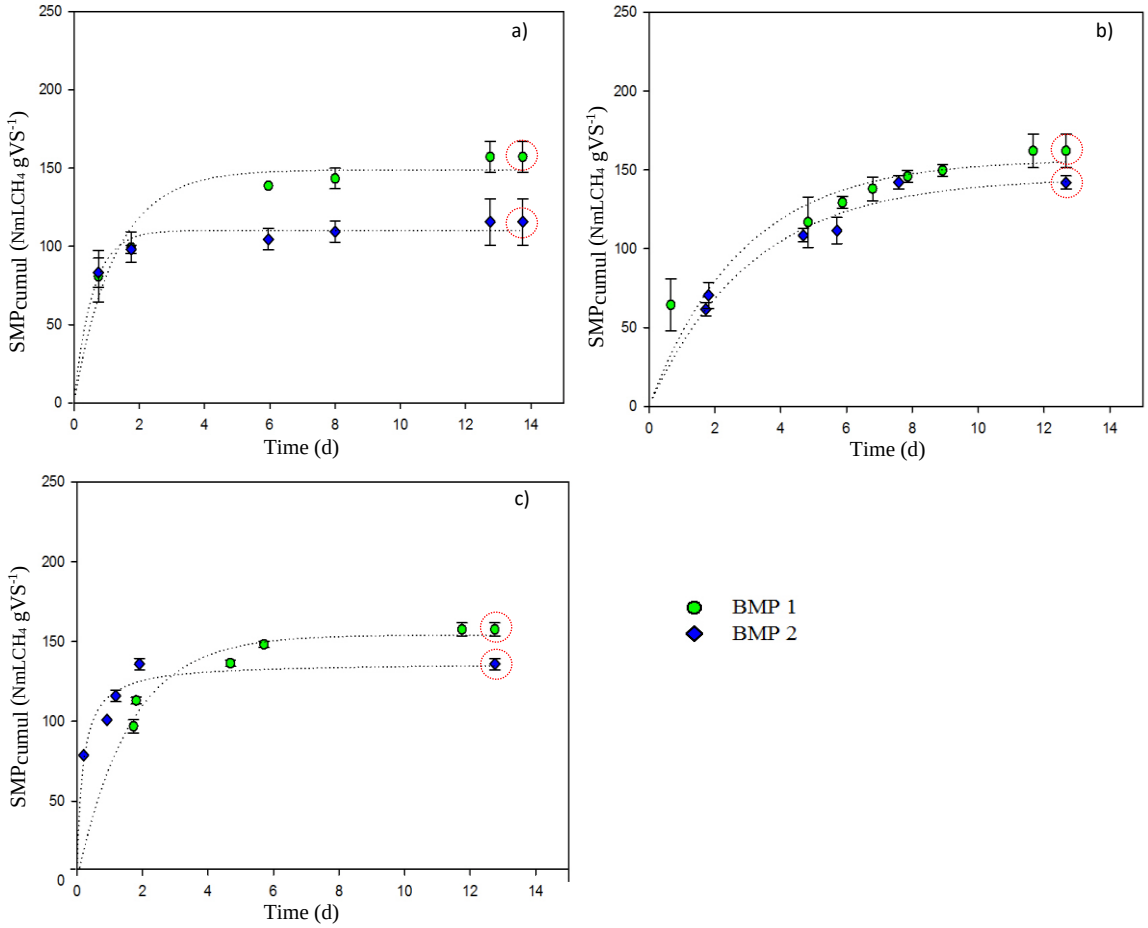


Fig.5. 5. Cumulative specific methane production measured during the BMP tests: a) WW; b) TW; c) BW.

Fig.5.5 a)-c) shows that for all substrates, the majority of the total methane production is reached in the first hours of the test, since the most biodegradable compounds are rapidly degraded. Then the production slows down until no measurable production is detected. After an initial period of time (140 hours for WW and BW; 200 hours for TW) the system presents a phase of low methane production, which is reflected in a stabilization of the SMPcumul. This is due to the accumulation of low biodegradable compounds, which leads to a decrease in CH_4 production. The maximum value of SMP for the three substrates was reached between the 12th and 13th day of production. Thus, the monitoring period of these tests lasted until the 15th day.

The maximum value of SMP is called BMP (biochemical methane potential). In the case of WW, an average value of about 136.40 NmL CH_4 $gVS^{-1}$was observed. However, the two replicates oscillating between 115.6 and 157.2 NmL CH_4 gVS^{-1}. Besides the BMP test did not replicate adequately, this value is quite low when compared to the reported by Pellera and Gidarakos (2016), where 446.23 NmL CH_4 gVS^{-1} is indicated. This means that this test must be repeated for WW, at least in triplicate.

In the case of TW substrate, the average BMP was 152.1 NmL CH_4 gVS^{-1}, Fig.5.5 b), but each test leads to 142.0 and 162.2 NmL CH_4 gVS^{-1}. Also, in this case, the value is low when compared to the one reported by Dinuccio et al. (2010) (218 NmL CH_4 gVS^{-1}) and Gunaseelan (2004) (211 mL CH_4 gVS^{-1}). For BW substrate, the average BMP value was 146.8 NmL CH_4 gVS^{-1} (mean value from 135.9 and 157.7 NmL CH_4 gVS^{-1}). The replicates are not consistent, and this test must be repeated, at least in triplicate. According to Clarke et al. (2008) and Buffière et al. (2015), BW can reach a BMP from 273 or 291 mL CH_4 gVS^{-1}, respectively.

Thus, since all the BMP obtained for the three substrates are low compared to the literature (but within the typical range 110-600 mL CH_4 gVS^{-1}) and because the replicates are not totally satisfactory (although this is a biochemical assessment and the tests are difficult to give accurate responses), the suggestion is that the experimental procedure must be improved in future work. Even so, according to the BMP assessment, it is expected a higher biogas production from TW, followed by BW and finally WW.

5.3.2. Comparison between experimental and theoretically estimated BMP values

For each substrate, besides BMP was determined experimentally, this value was assessed using the theoretical methods: Met_I - Buswell's formula; Met_III - chemical oxygen demand; Met_IV - near-infrared spectroscopy. Moreover, two models developed in this study in section 5 (Mod_I and Mod_III) were also used to predict BMP. The results are summarized in the Fig. 5.6 a)-c).

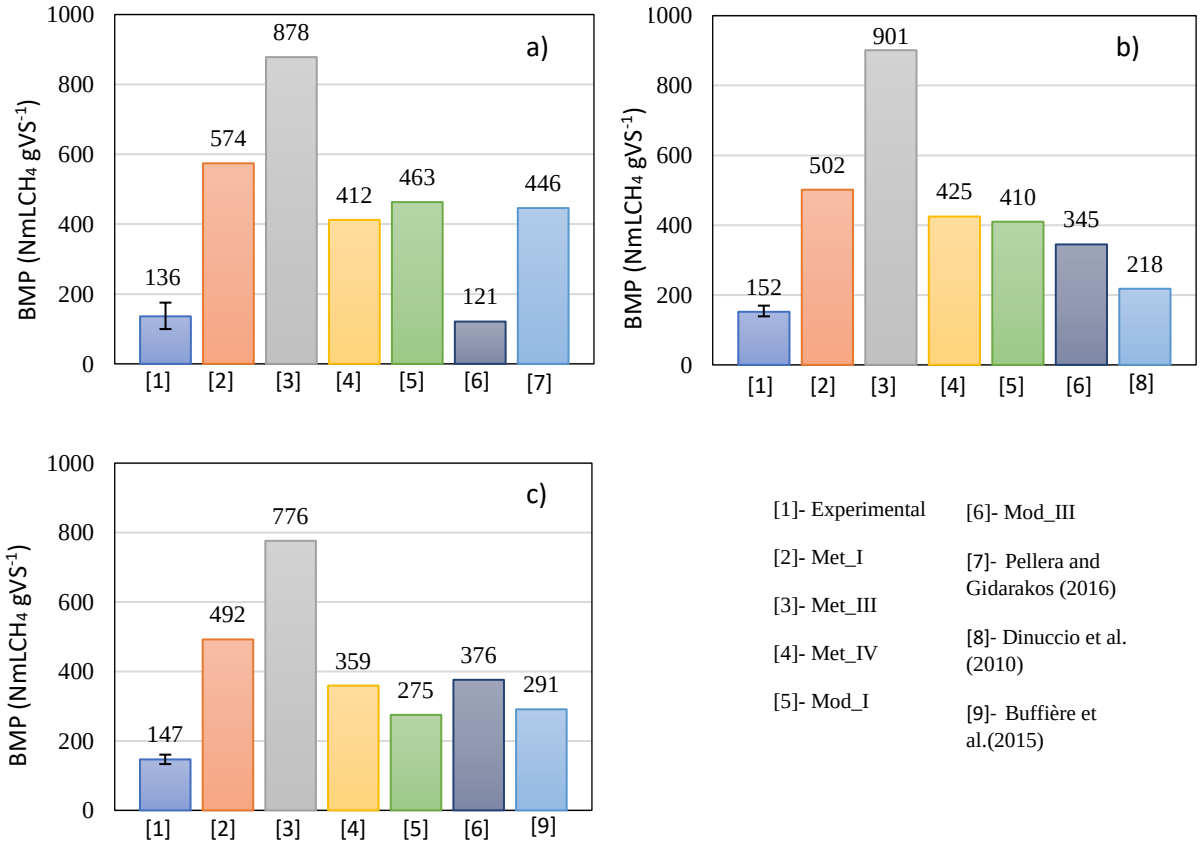


Fig.5. 6. BMP values determined through the various methods for each substrate: a) WW; b) TW; c) BW.

As discussed above and as can be seen from Fig.5.6 a)-c), the theoretical methods Met_I and Met_III tend to overestimate the experimental value of BMP. The Met_IV, which correspond to NIR prediction, also overestimated the experimental value found in this study. However, if compared with the literature, the predictions are very acceptable. Indeed, according to Doublet et al. (2013) NIR is one of the methods most able to approach the experimental BMP value.

When applying the models developed in this study, it is possible to verify a great BMP predictive capacity when compared with the values obtained by NIR. It should also be noted that the values predicted through Mod_I are very close to the values reported in the literature for each substrate. While the values foreseen by Mod_III deviate more from the reference values, with the exception of BW. Since Mod_I predictions are similar to the results of NIR analysis and the literature, it seems, as already mentioned in section 5.3.3, that the experimental tests must be improved it future work.

In general, Mod_I developed in this study reveals a good capacity to predict the value of BMP in relation to the most common methods reported in the literature, with the advantage that just requires to determine the elemental composition of a substrate.

5.4. BATCH ANAEROBIC DIGESTION PROCESS

In section 5.3 it was possible to verify that the BMP of the three substrates were within the range of the values usually found in other studies. Therefore, the substrates WW, TW and BW present a good potential for the transformation of organic matter into biogas through AD process. In this context, batch AD tests were performed to further evaluate their biogas production potential.

In a first step the optimum S/I ratio was optimized through the AD for WW. Then, the same ratio of S/I was used for TW and BW.

5.4.1. Experimental results

In order to monitor AD experiments, several parameters were determined at the beginning (t0) and at the end (t∞), namely TS, VS, SS, SSV, pH, TA, VFA, tCOD and sCOD. However, for time management reasons it was not possible to determine these parameters for the BW substrate. The results of three tests performed for WW substrate at different S/I ratio, are summarized in Table 5.13. The same was done for TW, but by testing only S/I ratio equal to 0.5, and the results are summarized in Table 5.14.

Table 5. 12. Characterization of suspensions at the beginning (t0) and at the end (t∞) of AD tests for WW substrate.

	S/I=0.5		S/I=1.0		S/I=1.5	
Parameter	**WW1_t0**	**WW1_t∞**	**WW2_t0**	**WW2_t∞**	**WW3_t0**	**WW3_t∞**
TS (g L^{-1})	32.49 ± 0.12	31.32 ± 0.19	36.94 ± 0.27	30.53± 0.07	30.65± 0.33	30.46± 0.20
VS (% TS)	50.44 ± 0.35	47.50 ± 0.23	50.62 ± 0.23	44.87 ± 0.56	51.28 ± 0.38	48.92 ± 0.43
SS (g L^{-1})	6.36 ± 0.09	7.14 ± 0.09	7.11 ± 0.19	6.33 ± 0.04	6.21 ± 0.01	7.32 ± 0.09
SSV (% SS)	71.82 ± 0.05	68.39 ± 1.91	76.59 ± 0.89	73.56 ± 0.72	73.05 ± 0.65	72.18 ± 0.62
pH	7.15 ± 0.03	7.18 ± 0.03	7.21 ± 0.03	7.44 ± 0.03	7.18 ± 0.03	6.64 ± 0.03
TA ($gCaCO_3$ L^{-1})	5.38 ± 0.06	6.24 ± 0.08	4.73 ± 0.00	7.29 ± 0.08	4.20 ± 0.00	4.73 ± 0.05
VFA (g L^{-1})	7.60 ± 0.09	6.78 ± 0.06	6.69 ± 0.03	7.29 ± 0.30	5.80 ± 0.05	7.47± 0.11
VFA/TA	1.41 ± 0.00	1.09 ± 0.02	1.41 ± 0.01	1.00 ± 0.03	1.38 ± 0.01	1.58 ± 0.00
tCOD (gO_2 L^{-1})	35 ± 1	34 ± 1	42 ± 1	39 ± 1	31 ± 1	30 ± 1
sCOD (gO_2 L^{-1})	30 ± 1	24.± 1	30 ± 1	25 ± 2	26 ± 1	21 ± 1
SBP_{cumul} (NmL gVS^{-1})		358.6		306.5		171.1
% CH_4		66		63		65

Table 5. 13. Characterization of suspensions at the beginning (t0) and at the end (t∞) of AD tests for TW.

	S/I=0.5	
Parameter	TW1_t0	TW1_t∞
TS (g L^{-1})	31.55 ± 0.20	29.10 ± 0.38
VS (% TS)	51.16 ± 1.26	44.10 ± 0.03
SS (g L^{-1})	8.22 ± 1.16	6.85 ± 0.34
SSV (% SS)	73.50 ± 1.86	69.25 ± 0.56
pH	7.35 ± 0.03	7.26 ± 0.03
TA ($gCaCO_3$ L^{-1})	5.64 ± 0.03	5.56 ± 0.08
VFA (g L^{-1})	6.71 ± 0.03	5.93 ± 0.19
VFA/TA	1.19 ± 0.01	1.07 ± 0.05
tCOD (gO_2 L^{-1})	59± 2	29 ± 2
sCOD (gO_2 L^{-1})	28 ± 1	18 ± 4
SBP_{cumul} (NmL gVS^{-1})		453.4
% CH_4		67

From Table 5.12 and Table 5.13 is possible to observe that, in similarity to what happened in BMP tests, even if VFA/TA > 0.8 in all tests, pH was maintained within the optimal range for methanogenic bacteria during the process.

The behavior of both tCOD and sCOD throughout the digestion is similar to what was observed in the BMP assays.

The Fig.5.7 a)-e) shows the specific production of accumulated biogas (SBP_{cumul}) and the differential biogas specific production (SBP_{dif}) for WW and for TW.

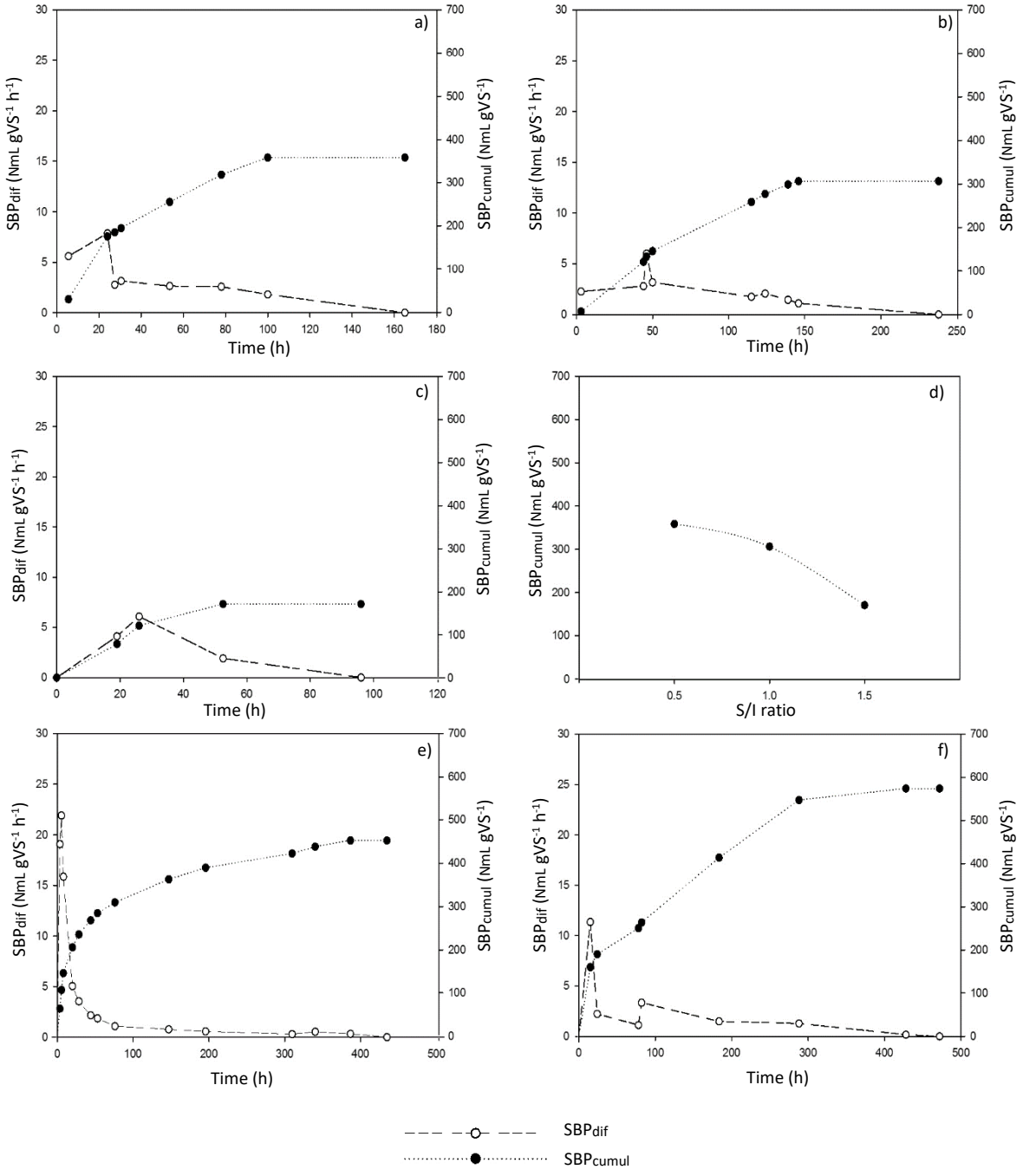


Fig.5. 7. Specific biogas production (differential and cumulative) for WW AD test: a) WW1; b) WW2; c) WW3; d) SBP for each S/I ratio; e) for TW AD test; f) for BW AD test.

From the Fig.5.7 a)-e) it is possible to observe that the biogas production is maximum in the first hours of digestion. According to Huang et al. (2016) this is due to the rapid biodegradation of specific substrates, such as carbohydrates and crude proteins. For WW1, WW3 and TW the maximum biogas production was observed in the first 20 h, and for WW2

the maximum was recorded after 50 h. It is also possible to verify that the WW3 test is very short when compared to the others. Indeed, the cessation of biogas production is due to inhibition caused by the excess of organic load (Jain et al., 2015). In all assays, on average, about 65% of methane in biogas was observed.

Throughout the analysis of Fig.5.7 d) it is possible to conclude that the SBP for WW is maximum when S/I ration equal to 0.5 was used. In this case, SBP_{cumul} was 358.6 NmL gVS^{-1}, which corresponds to about 232 NmL CH_4 gVS^{-1}. On the contrary, when S/I ratio was set equal to 1.5, SBP_{cumul} was 171.1 NmL gVS^{-1}. So, it is concluded that the S/I ratio equal to 0.5 is the best for the AD of the WW. This ratio was then used for the other substrates.

In the case of WW, it was not possible a direct comparison between SBP and the values reported in the literature, since data were not reported in this case. However, a comparison was made with reported SBP values for substrates from the same industry, namely grape marcs and grape stalks. Fabbri et al. (2015) obtained an SBP of 405.7 and 296.9 NmL gVS^{-1} for grape stalks and marcs, respectively. While Dinuccio et al. (2010) reported for the same substrates SBP values of 225 and 250 NmL gVS^{-1}. It can then be concluded that the biogas production obtained in this study for the WW substrate is in agreement with the literature.

In the case of TW, the SBP obtained in this study was 453.4 NmL gVS^{-1} which is in agreement the value 424 NmL gVS^{-1} referred by Dinuccio et al. (2010). While for BW a SBP of 574.1 NmL gVS^{-1} was observed, which is higher compared with the value obtained by Tumutegyereize et al. (2011) (484 NmL gVS^{-1}).

5.4.2. Kinetic analysis

The cumulative curves presented in Fig.5.7 appear to have a first-order exponential behavior, and thus a kinetic model of the 1st order was used to fit the data of SBP_{cumul} for each substrate. The prediction of biogas production of a specific substrate at industrial level requires the development of kinetic models (Nielfa et al.,2015).

The first-order kinetic model (FOKM) is a simplified model that assumes that the biogas production follows a first-order kinetics in which biogas accumulation was modeled with an exponential rise to a maximum (Banks and Heaven, 2013). The FOKM allows the prediction of SBP ($mLCH_4$ gVS^{-1}) and can be described by the Eq. (5.4).

$$SBP(t)=SBP_{\infty}[1-\exp(-\mu t)] \quad (5.4)$$

where SBP_{∞} in the predicted maximum volume of biogas accumulated at an infinite digestion time ($mLCH_4\ gVS^{-1}$), μ is the kinetic constant of biogas production (d^{-1}) and t is the digestion time (d). The results of the FOKM fitting are summarized in Fig.5.8 (a)-(d) and Table 5.15.

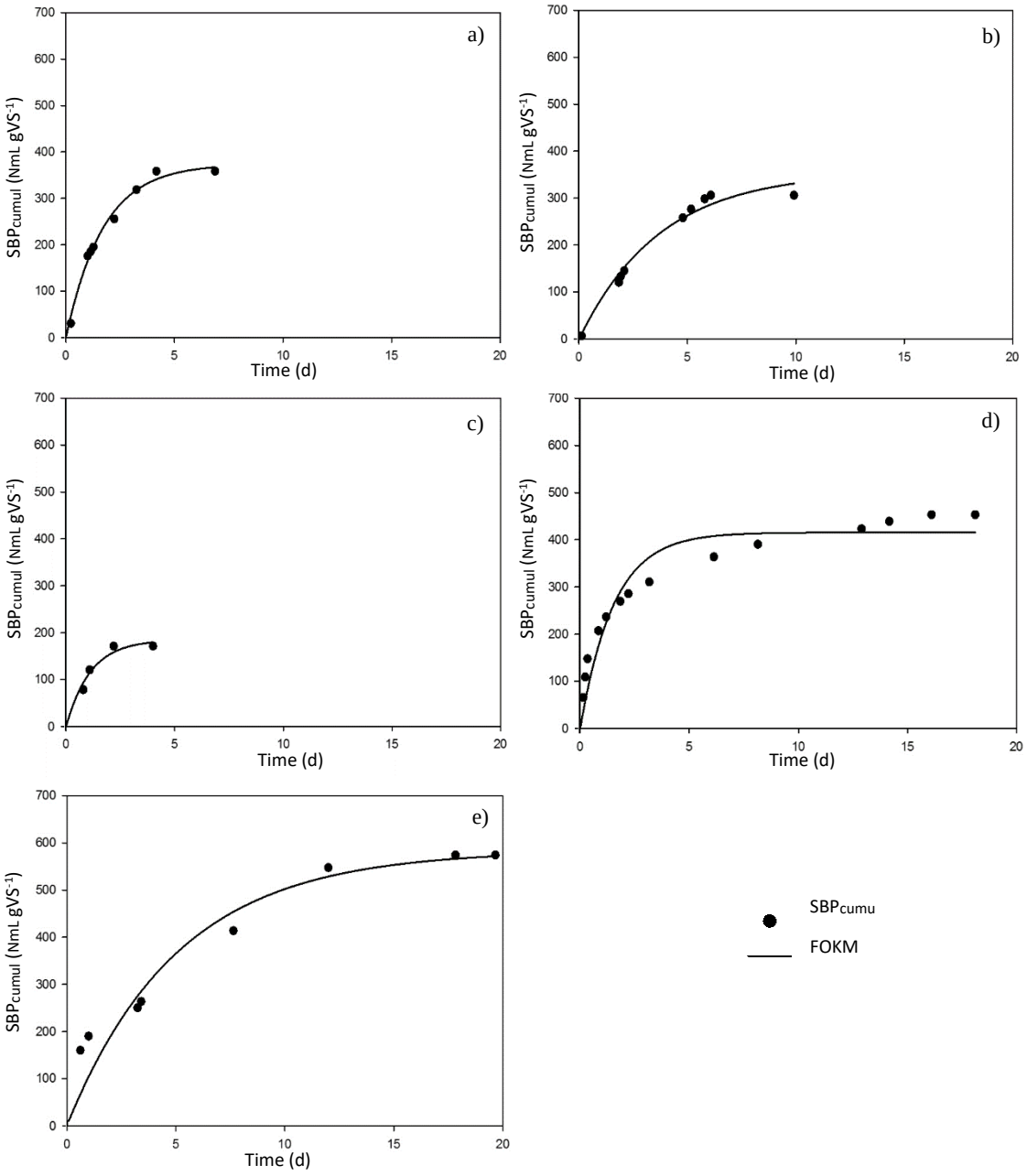


Fig.5. 8. Fitting of FOKM to the SBPcumul results: a) WW1; b) WW2; c) WW3; d) TW; e) BW

Table 5. 14. Parameters of FOKM obtained by non-linear regression.

		First order model (F.O.)		
Substrate	**Assay**	**SBP_∞ (NmL gVS^{-1})**	**μ (d^{-1})**	**R^2**
WW	WW1	374.4	0.58	0.992
	WW2	356.0	0.27	0.985
	WW3	184.3	0.89	0.975
TW	TW	415.6	0.65	0.937
BW	BW	583.8	0.19	0.944

Analysing the Fig.5.8 a)-c) and Table 5.15 it is possible to verify that the biogas production from WW, is very well described by a FOKM. Indeed, the coefficient of determination, R^2, vary from 0.975 to 0.992. However, for TW, the experimental points are not fully described by this type of model, and thus R^2 is 0.937 in this case. The poor adjustment may be due to the substrate heterogeneity, i.e., TW substrates consist of both rapidly and more slowly degrading fractions (Banks and Heaven 2013), namely seeds, peels and pulp.

WW1, WW2 and WW3 tests show a very different kinetic constant, μ, meaning that the velocity of organic matter degradation varies with the S/I ratio. WW3 is the assay with the highest degradation velocity, but AD stops suddenly due to the above-mentioned inhibition. For the S/I ratio of 0.5 and 1.0, a μ of 0.58 and 0.89 d^{-1} is observed, this value is close to the μ obtained in BMP determination. For TW, the values obtained in this study are similar to the degradation velocity reported by Banks and Heaven (2013) for food waste (0.19 d^{-1}). While BW has the slowest degradation velocity (0.17 d^{-1}) as reported by Tumutegyereize et al. (2011) (0.20 d^{-1}).

Through the studies of AD of WW and TW substrates, it was possible to verify that, as foreseen in section 5.3.1, the TW shows a higher biogas production compared to the WW production. So, it can be concluded that, in general, the BMP is a very important parameter in order to evaluate the potential that a substrate presents for anaerobic digestion.

6. CONCLUSIONS AND FUTURE WORK

The main objective of this work was the study of the AD process for the valorization of agro-industrial wastes. One of the main goals was the development of models for biochemical methane potential prediction. Moreover, several experimental methods were compared for the determination of this key parameter.

In a first phase, 40 scientific papers were analyzed. From the data collected it was possible to obtain the biochemical potential of methane (BMP) for 149 different substrates. These materials were later organized into 10 categories to facilitate their analysis. Through these results, it was possible to verify that there is no evident dependence between the BMP and the category in which the substrate is inserted.

From these papers, it was also possible to collect some physicochemical characteristics of the different substrates. However, it was observed that there was no standardized characterization, i.e., the characteristics determined for the wastes characterization varied from author to author. Through the physicochemical parameters collected for each substrate, it was possible to determine the theoretical BMP using three methods reported in the literature, namely elemental analysis (Met_I), organic fraction composition (Met_II) and chemical oxygen demand (Met_III). The results obtained were compared with those determined experimentally during AD. It was concluded that all the theoretical methods overestimated the experimentally observed BMP value. This occurs because these methods do not take into account the biodegradability of the substrate. With this in mind, a correction was made to BMP predicted values, whenever possible, using a biodegradability factor (B_{VS}). Through this correction, it was found that Met_I was able to reasonably predict the experimental BMP value. Therefore, whenever possible, this corrective factor should be applied.

However, this correction factor has some limitations. Thus, a more detailed analysis was carried by considering parameters that would influence the BMP value. In this analysis, linear regressions were performed between the experimental BMP and some physical-chemical characteristics of the substrate. From this test, it was verified that BMP is not a simple linear relationship of any of those characteristics. Based on this, a multivariate polynomial regression analysis, encompassing several physical-chemical parameters, was performed. The two models with higher ability for BMP prediction (Mod_I and Mod_II) are based, respectively, on the elemental composition and on the lignocellulosic parts of the substrates.

The results obtained through the laboratory-scale BMP test revealed that tomato waste (TW) is the substrate with the highest potential for AD with a BMP of 152 $NmLCH_4\ gVS^{-1}$, followed by banana waste (BW) (147 $NmLCH_4\ gVS^{-1}$) and lastly, with a lower potential, the winery wastewater (WW) (136 $NmLCH_4\ gVS^{-1}$). However, these values are below expectation. In fact, literature reports higher BMP for similar wastes. Besides, also the models developed in this thesis predict higher BMP. In this regard, the experimental methodology developed must be further improved and optimized in the future. By means of a kinetic analysis, it was found out that, in general, the three substrates degradations follow first-order kinetics.

To verify the suitability of the substrates valorization by AD, five assays were performed (WW1, WW2, WW3, TW and BW). The first three tests had, as the main objective, the optimization of the S/I ratio for the winery waste. Thus, different S/I ratios were used 0.5 (WW1), 1.0 (WW2) and 1.5 (WW3). From the results, it was concluded that the highest biogas production was obtained for an S/I ratio of 0.5 (359 NmL gVS^{-1}) and the lowest production was obtained for S/I of 1.5 (171 NmL gVS^{-1}). Thus, it was concluded that the optimum S/I ratio was 0.5.

Based on the results obtained for WW, the optimal S/I ratio was applied for the AD of TW and BW. With those conditions, a biogas production of 453 NmL gVS^{-1} was reached for TW and a production of 574 NmL gVS^{-1} for BW.

Finally, it should be pointed out that BMP plays a fundamental role in the evaluation of a substrate potential for AD and that it is possible to predict this parameter in a simple and fast way (without resorting to its experimental determination). It is also important to mention that the substrates evaluated in this study can be valorized through an anaerobic digestion process.

Future works on this subject may possibly contemplate the following topics:

- Optimize the experimental method for the BMP determination;
- Evaluate the BMP value for several substrates in order to validate the models developed in this study;
- Define the most relevant parameters in the characterization of a substrate in order to be possible a more uniform and transversal characterization of the wastes defining this way a procedure that should be followed in all related research;
- Evaluate the performance of anaerobic co-digestion of the substrates

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APPENDIX A – SUBSTRATES ANALYZED FROM LITERATURE

Experimental BMP data were collected from 40 scientific papers. From these papers, it was possible to obtain BMP values for about 149 different substrates, these substrates are detailed in Table A.1.

Table A. 1. Substrates designation and reference.

Subs.	Reference	Designation	Subs.	Reference	Designation
B0	Nieto et al., 2012	Beverage	D12	Li et al., 2013	Vinegar
B1	Labatut et al., 2011	Beverage (Cola)	D13	Nieto et al., 2012	Yogurt
B2_1	This study	Winery wastewater	F1_1	Gunaseelan, 2004	*Achras sapota L.* Peels
B2_2	Pellera and Gidarakos, 2016	*Oryza sativa L.*	F1_2	Gunaseelan, 2004	*Achras sapota L.* (Rotten)
C1	Kafle et al., 2013	Brewery grain	F2	Gunaseelan, 2007	*Ananas sativus L.* Peels
C2_1	Qin et al., 2017	*Oryza sativa L.*	F3	Zheng et al., 2013	*Citrullus lanatus*
C2_2	Sun et al., 2015	*Oryza sativa L.* stalk	F4	Gunaseelan, 2007	*Citrus reticulata Blanco Peels*
C3	Sambusiti et al., 2012	*Sorghum* forage	F6	Gunaseelan, 2009	*Jatropha curcas fruit* peel
C4 -	Sun et al., 2015	*Triticum aestivum L* stalk	F7_1	Gunaseelan, 2004	*Lycopersicon esculentum Var. Pyriforme*
C5_1	Sun et al., 2015	*Zea mays L.* stalk	F7_2	This study	*Lycopersicon esculentum Var. Pyriforme*
C5_2	Labatut et al., 2011	*Zea mays L.* silage	F7_3	Calabrò et al., 2015	*Lycopersicon esculentum Var. Commune*
D0	Qiao et al., 2011	Diet Waste	F8	Qin et al., 2017	*Malus domestica*
D1	Kafle et al., 2013	Bread	F9_1	Gunaseelan, 2007	*Mangifera indica L. var. Neelum* peels
D2	Cho et al., 1995	Boiled Rice	F9_2	Gunaseelan, 2007	*Mangifera indica L. var. Chenthuram* peels
D3	Labatut et al., 2011	Cheese Whey	F9_3	Gunaseelan, 2007	*Mangifera indica L. var. Mulgoa* peels
D4	Kafle et al., 2013	Cuttlefish	F10_1	This study	*Musa paradisiaca L. var. Cavendish* peels
D5	Labatut et al., 2011	Ice cream	F10_2	Buffière et al., 2008	*Musa paradisiaca L. Var. Red* peels
D6	Nieto et al., 2012	Milk	F11_1	Gunaseelan, 2004	*Punica granatum L.* Peels
D7	Kafle et al., 2013	Pacific Saury	F11_2	Gunaseelan, 2007	*Punica granatum L.* Pressings
D8	Labatut et al., 2011	Pasta	F12	Qin et al., 2017	*Pyrus L.*
D9	Qin et al., 2017	Steamed Bread	F13	Rico et al., 2014	*Theobroma cacao bean* shell
D10	Li et al., 2013	Used vegetable oil	F14_1	Gunaseelan, 2004	*Vitis vinifera L.* Pressings
D11	Labatut et al., 2011	Used animal oil	F14_2	Gunaseelan, 2004	*Vitis vinifera L.* Peduncle

Table A. 1. (continuation)

Subs.	Reference	Designation	Subs.	Reference	Designation
F15_1	This study	OMSW	P2	Shen et al., 2014	*Bambusa oldhamii*
F15_2	Rincón et al., 2013	OMSW	P3	Sun et al., 2015	*Brassica napus* stalk
M1_1	Triolo et al., 2011	Calf Manure	P4_1	Gunaseelan, 2016	*Cassia Fistula* seeds
M1_2	Triolo et al., 2011	Calf Manure	P4_2	Gunaseelan, 2016	*Cassia Fistula* pod husk
M2_1	Hidalgo and Martín-marroquín, 2015	Cattle Manure	P5_1	Li et al., 2013	*Chenopodium album L.* leaf and seed
M2_2	Möller et al.,2004	Cattle Manure	P5_2	Li et al., 2013	*Chenopodium album L.* stalk
M2_3	Triolo et al., 2011	Cattle Manure	P6	Gurung et al., 2012	*Chlorophyta*
M3	Kafle and Chen, 2016	Chicken Manure	P7_1	Zheng et al., 2013	*Cinnamomum Camphora* branch
M4	Kafle and Chen, 2016	Goat Manure	P7_2	Zheng et al., 2013	Cinnamomum Camphora leaf
M5	Kafle and Chen, 2016	Horse Manure	P8	Pesce et al., 2017	Cynara cardunculus
M6	Thygesen et al., 2014	Industrial Manure	P9	Gunaseelan, 2016	Delonix Regia seeds
M7	Thygesen et al., 2014	Pig Manure	P10_1	Gunaseelan, 2016	Gliricidia Maculata mature leaf
M8	Hidalgo and Martín-marroquín, 2015	Poultry Manure	P10_2	Gunaseelan, 2016	Gliricidia Maculata petals
M9	Hidalgo and Martín-marroquín, 2015	Sheep Manure	P11	Sun et al., 2015	*Gossypium spp L.* stalk
O1	Strömberg et al., 2014	Cellulose	P12	Raposo et al., 2008	*Helianthus annuus*
O2	Hidalgo and Martín-marroquín, 2015	Crude glycerol	P13	Gunaseelan, 2009	*Jatropha curcas* leaf
O3	Naroznova et al., 2016	Dirty paper	P14	Edward et al., 2015	*Laminaria digitata*
O4	Jokela et al., 2005	Carboard	P15	Edward et al., 2015	*Laminaria hyperborea*
O5	Naroznova et al., 2016	Kitchen tissue	P16	Zheng et al., 2013	*Metasequoia* leaf
O6	Naroznova et al., 2016	Moulded fibres	P17	Gunaseelan, 2007	*Pennisetum purpureum* lamina
P0	Naroznova et al., 2016	Plant waste	P18	Labatut et al., 2011	*Panicum virgatum*
P1_1	Gunaseelan, 2016	*Albizia Procera* seeds	P19	Gurung et al., 2012	*Phaeophyceae*
P1_2	Gunaseelan, 2016	*Albizia Procera* pod husk	P20	Triolo et al., 2012	*Poa pratensis & abbreviata* cuttings

Table A. 1. (continuation).

Subs.	Reference	Designation	Subs.	Reference	Designation
P21_1	Gunaseelan, 2014	*Pongamia pinnata* leaf	V4_2	Gunaseelan, 2004	*Brassica oleracea L var. capitata*
P21_2	Gunaseelan, 2014	*Pongamia pinnata* petals	V5_1	Gunaseelan, 2004	*Brassica oleracea L. var. botrytis* leaves
P22_1	Gunaseelan, 2016	*Prosopis Juliflora* mature leaf	V5_2	Gunaseelan, 2004	*Brassica oleracea L. var. botrytis* stem
P22_2	Gunaseelan, 2016	*Prosopis Juliflora* pod	V6	Gunaseelan, 2004	*Brassica rapa L.* leaves
P23	Bolado-rodríguez et al., 2016	*Saccharum officinarum* bagasse	V7_1	Gunaseelan, 2004	*Coriandrum sativum L.* leaves
P24_1	Gunaseelan, 2016	*Tamarindus Indica* mature meaf	V7_2	Gunaseelan, 2004	*Coriandrum sativum L* roots
P24_2	Gunaseelan, 2016	*Tamarindus Indica* seeds	V8_1	Gunaseelan, 2007	*Daucus carota L.* leaves
P25_1	Gunaseelan, 2016	*Thespesia Populnea* mature leaf	V8_2	Gunaseelan, 2004	*Daucus carota L.* petiole
P25_2	Gunaseelan, 2016	*Thespesia Populnea* flower	V9	Zheng et al., 2013	*Glycine max L.*
S0	Nielfa et al., 2015	Sludge	V10	Buffiere et al., 2006	*Lactuca sativa L.*
S1	Hidalgo and Martín-marroquín, 2015	Animal Feed Waste Sludge	V11	Gunaseelan, 2007	*Pisum sativum L.* pods
S2	Hidalgo and Martín-marroquín, 2015	FeCl Coagulation Sludge	V12	Gunaseelan, 2007	*Rhaphanus sativus L.* Shoots
S3	Hidalgo and Martín-marroquín, 2015	Fruit Waste Sludge	V13	Qin et al., 2017	*Spinacia oleracea L.*
S4	Hidalgo and Martín-marroquín, 2015	Oily Waste Sludge	V14	Gunaseelan, 2004	*Solanum melongena* L. stalk
S5	Hidalgo and Martín-marroquín, 2015	Potato Waste	V15_1	This study	*Solanum tuberosum L.*
S6	Hidalgo and Martín-marroquín, 2015	Sewage Sludge	V15_2	Labatut et al., 2011	*Solanum tuberosum L.*
S7	Hidalgo and Martín-marroquín, 2015	Vegetable Oil Waste Sludge	W1 -11	Davidsson et al., 2007	Municipal Waste
V0	Naroznova et al., 2016	Vegetable Waste	W12:	Hansen et al., 2004	Municipal Waste
V1	Gunaseelan, 2007	*Abelmoschus esculentus L.* stalk	W13-15	Davidsson et al., 2007	Municipal Waste
V2	Gunaseelan, 2007	*Allium cepa L.* peels	W16	Pecorini et al., 2016	Municipal Waste
V3	Gunaseelan, 2004	*Beta vulgaris L.* leaves	W17	Pecorini et al., 2016	Municipal Waste
V4_1	Gunaseelan, 2007	*Brassica oleracea L.* var. capitata	W18	Nielfa et al., 2015	Municipal Waste

APPENDIX B – BIOGAS COMPOSITION DETERMINATION

The Fig.B.1 demonstrates, schematically, the procedure adopted to determine the amount of methane in the biogas.

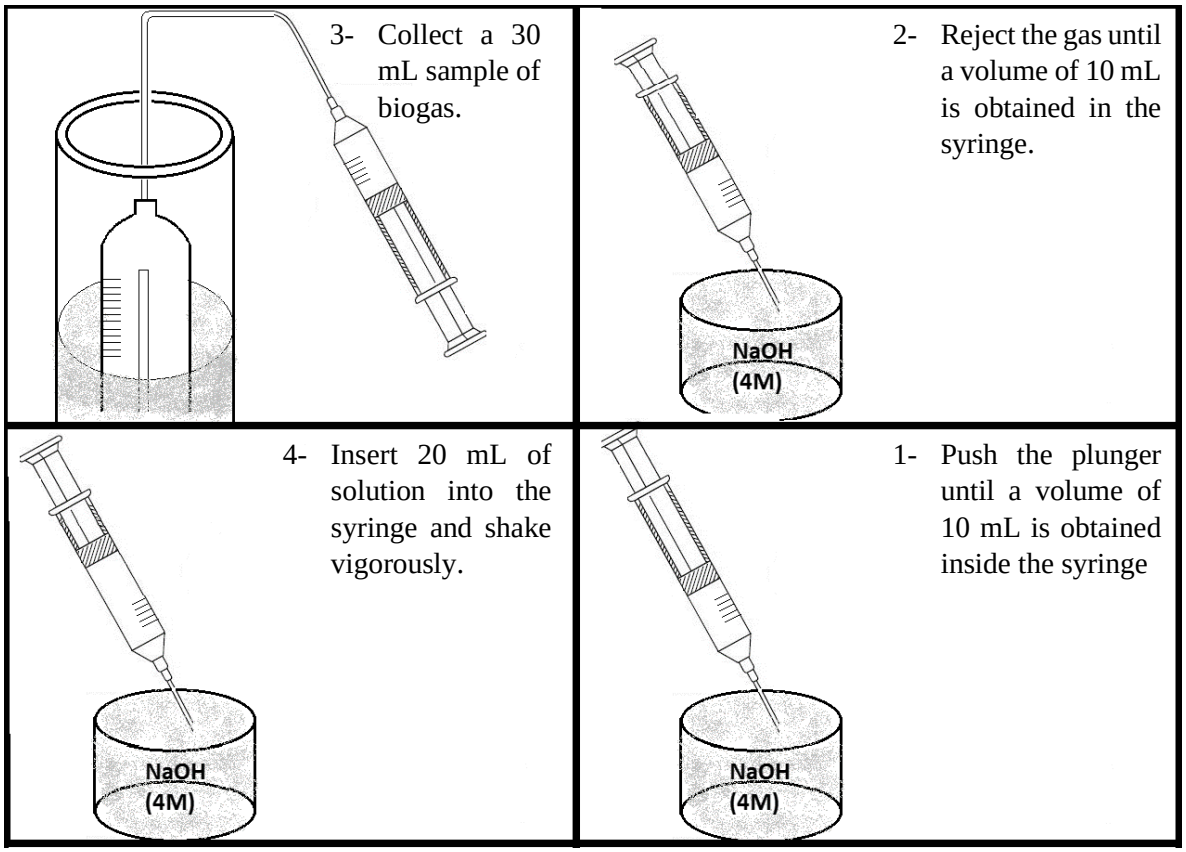

Fig.B. 1. Scheme of the determination of methane content in biogas (adapted from Abad, 2015)

APPENDIX C – CHEMICAL OXYGEN DEMAND CALIBRATION

The COD determination for liquid and solid samples required the preparation of acid and digestion solutions as well as a calibration curve.

For the liquid samples, the digestion solution was prepared with 1,1% K_2CrO7, 16,7% H2SO4, 7,4% HgSO4 and 74,6% H2O (mass %). The acid solution consists of 90,4% w/v H2SO4 and 9,6% w/v AgSO4. For the solid samples, the Acid solution was prepared diluting 9.6 g of silver sulfate in 1 L of concentrated sulfuric acid and the digestion solution consists in an aqueous solution of potassium dichromate (0.25 M).

In Table C.1 and C.2 are presented the content of calibration vials, for both liquid and solid samples, respectively. The calibration curve obtained for the two COD determinations can be observed in Fig.C.1 and Fig.C.2.

Table C. 1.Calibration solutions for COD of liquid samples.

	C_{KHP} (mg L^{-1})	V_{sample} (mL)	$V_{acid\ solution}$ (mL)	$V_{digestion\ solution}$ (mL)
0	0	1.0	1.2	2.8
1	425,5	1.0	1.2	2.8
2	638,3	1.0	1.2	2.8
3	851,1	1.0	1.2	2.8
4	1276,6	1.0	1.2	2.8
5	1489,4	1.0	1.2	2.8
6	1702,1	1.0	1.2	2.8
7	2127,7	1.0	1.2	2.8

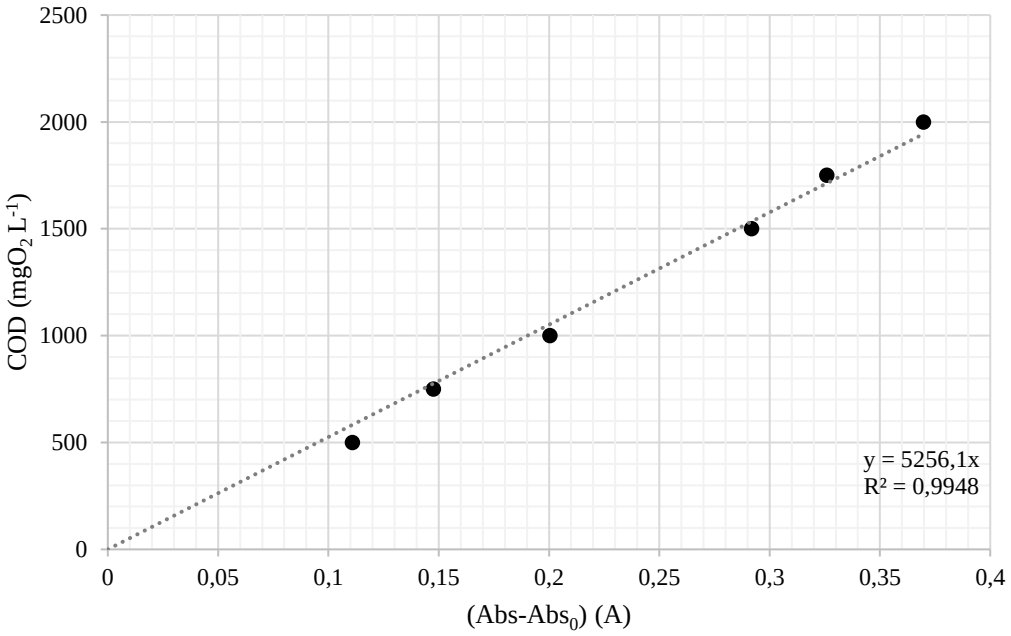

Fig.C. 1. Calibration curves for COD of liquid samples.

Table C. 2. Calibration solutions for COD of solid samples.

	m_{KHP} (mg)	$m_{distilled\ water}$ (mg)	$V_{acid\ solution}$ (mL)	$V_{digestion\ solution}$ (mL)
0	0	400.0	3.6	3.6
1	0.5	399.5	3.6	3.6
2	1.0	399.0	3.6	3.6
3	1.5	398.5	3.6	3.6
4	2.0	398.0	3.6	3.6
5	2.5	397.5	3.6	3.6
6	5.0	395.0	3.6	3.6

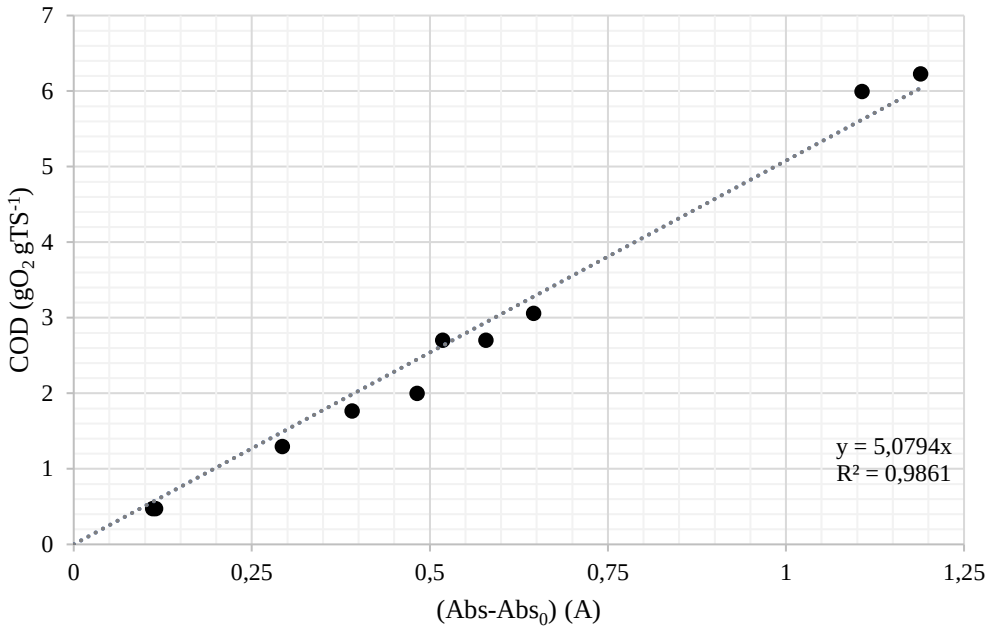

Fig.C. 2.Calibration curve for COD of solid samples.

Where Abs is the measured absorbance of the sample vials and Abs_0 is the measured absorbance of the blank vial.

APPENDIX D – CORRELATION ANALYSIS

In order to evaluate the dependency between the different variables, a correlation analysis was made. This analysis was carried using a correlation matrix, which is present in Fig. D.1

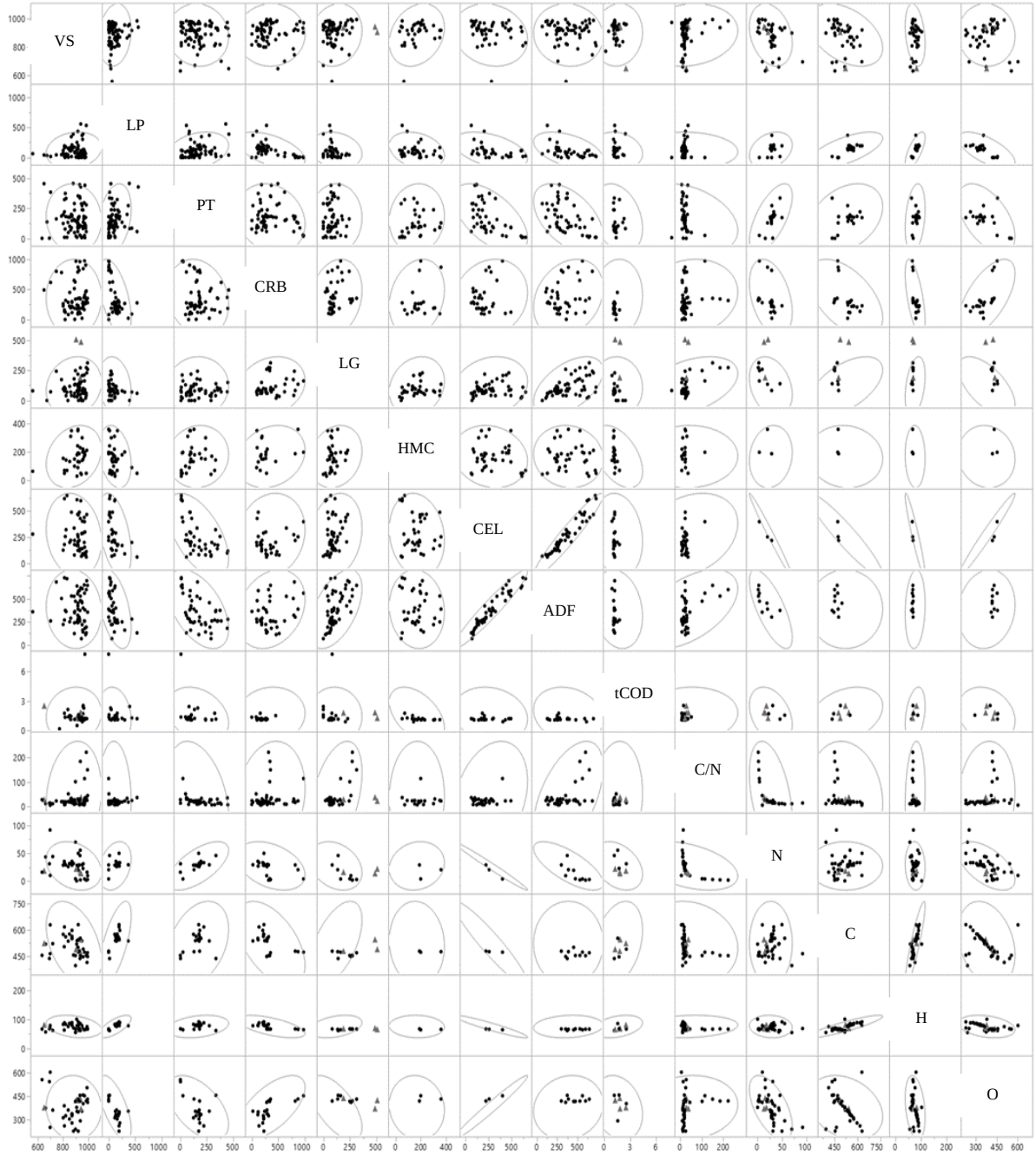

Fig.D. 1. Correlation matrix with the 14 different variables.