Carlos Krus Galvão Lucas

Licenciado em Ciências de Engenharia de Ambiente

Biogas Production from Potato Peel Waste

Dissertação para obtenção do Grau de Mestre em
Engenharia do Ambiente, perfil de Gestão e Sistemas Ambientais

Orientador: Nuno Carlos Lapa dos Santos Nunes, Professor Auxiliar da FCT/UNL
Co-orientadora: Maria da Graça Madeira Martinho, Professora Auxiliar da FCT/UNL

Júri:

Presidente: Prof. Doutora Ana Isabel Espinha da Silveira
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Ao meu pai.

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RESUMO

A digestão anaeróbia (DA) é um processo biológico com aplicação na valorização energética de resíduos orgânicos, devido à produção de biogás que contém metano.

Atualmente, em Portugal, os resíduos orgânicos são depositados na sua maioria em aterros sanitários, conduzindo à perda de matérias e à produção de compostos orgânicos que se libertam para a atmosfera. Por outro lado, a DA permite obter energia a partir desses materiais orgânicos e reduz significativamente a libertação de compostos orgânicos para a atmosfera.

Na presente tese, a DA de um resíduo de casca de batata de uma unidade industrial foi realizada num reator mesófilo do tipo UASB, à escala laboratorial, para produção de biogás. Quando submetidos a pré-tratamentos, por exemplo pré-tratamentos químicos, a degradação biológica destes resíduos pode melhorar o rendimento do biogás e do CH_4.

Esta investigação pretendeu avaliar o efeito de diferentes pré-tratamentos químicos sobre o resíduo de casca de batata, em condições de digestão mesófíla. O resíduo foi submetido a um pré-tratamento mecânico que consistiu numa trituração até uma dimensão inferior a 2 mm. Foi aplicado um pré-tratamento térmico, a 50 ºC durante 30 minutos, à pressão atmosférica, o qual foi combinado com um pré-tratamento químico com ácido sulfúrico, a pH 2 e 4, e hidróxido de sódio, a pH 10 e 12. Estes ensaios foram identificados pelos códigos A2.50.30, A4.50.30, A10.50.30 e A12.50.30, respetivamente. No ensaio de controlo, At50.30, o resíduo foi triturado e submetido a pré-tratamento térmico, porém, sem pré-tratamento químico. O ensaio A12.50.30 apresentou os valores mais elevados da CQO total e da CQO solúvel, tendo apresentado aumentos de 15.8% e 42.8%, respetivamente, relativamente ao ensaio de controlo.

Os ensaios A12.50.30 e At50.30 foram selecionados para serem replicados no digestor mesófilo do tipo UASB. Diversos parâmetros físico-químicos foram determinados no afluente e efluente de cada ensaio realizado no digestor. O biogás produzido foi também recolhido, quantificado o seu volume e analisada a sua composição relativamente a diferentes gases. Em ambos os ensaios de DA, os teores máximos de CH_4 foram superiores a 72% (v/v). O teor mais elevado de CH_4 foi obtido no ensaio A12.50.30, tendo sido de 78.3% (v/v). Os rendimentos mais elevados de CH_4 foram obtidos no ensaio A12.50.30 (209 $cm^3.g^{-1}$ $VS_{removidos}$ e 271 $cm^3.g^{-1}$ $CQOt_{removidos}$), correspondendo a um incremento de 116% e 109%, respetivamente, relativamente ao rendimento de CH_4 no ensaio de controlo.

No final deste estudo, a conclusão a que se chega é a de que o ensaio A12.50.30, de pré-tratamento alcalino, é o mais adequado para aumentar a biodisponibilidade do resíduo de casca de batata, mediante as condições estudadas.

Palavras-chave: Digestão anaeróbia; Pré-tratamento químico; Rendimento em biogás; Rendimento em metano; Resíduo de batata.

ABSTRACT

An anaerobic digestion (AD) is a biological process that can be adapted for the energetic recovery of organic wastes used as biomass supply for biogas production. The management of biowastes applied to AD is essential from an energetic and environmental point of view, in which landfills and biogas plants represent two contrasting guiding principles of sustainable management.

Currently, in Portugal, solid organic wastes are placed mostly in landfills leading to loss of these matters and to organic compounds released into the atmosphere. In this way, the AD allows obtaining energy through organic matter and reduces significantly the output of organic compounds, such as methane (CH_4), into the atmosphere.

In the present thesis, AD of the potato peel waste of an industrial unit was carried out in a mesophilic reactor of UASB type, in laboratory scale, for biogas production. When subjected to pre-treatments, for example chemical pre-treatments, the biological degradation of these wastes can improve the biogas and CH_4 yield.

This research intended to evaluate the effect of different chemical pre-treatments on potato peel waste, in conditions for mesophilic digestion. The waste was subjected to a mechanical pre-treatment that consisted in triturating until its size was inferior to 2 mm. A thermal pre-treatment was applied, at 50 ºC during 30 minutes, at atmospheric pressure, and combined with chemical pre-treatment with sulphuric acid, at pH 2 and 4, or sodium hydroxide, at pH 10 and 12. These assays were identified by codes A2.50.30, A4.50.30, A10.50.30 and A12.50.30, respectively. In the control assay, At50.30, the waste was triturated and subjected to thermal pre-treatment without undergoing chemical pre-treatment. Assay A12.50.30 presented the highest values of total COD and soluble COD, with increases of 15.8% and 42.8%, respectively, over the test assay.

Assay A12.50.30 and test assay At50.30 were selected to be replicated in the mesophilic digester of UASB type. Different physicochemical parameters were determined in influent and effluent of each assay in the anaerobic digester. The biogas produced was also collected, quantified the volume and analysed the composition in relation to different gases. In both AD assays, the maximum s CH_4 contents above 72% (v/v) were obtained. The highest CH_4 content was obtained in assay A12.50.30, which was of 78.3% (v/v). The highest CH_4 yields were obtained in assay A12.50.30 (209 $cm^3.g^{-1}$ $VS_{removed}$ and 271 $cm^3.g^{-1}$ $CODt_{removed}$,), corresponding to an increment of 116% and 109%, respectively, over the CH_4 yields in the control assay. At the end of this study, the conclusion that has been reached is that the assay A12.50.30, that had undergone alkaline pre-treatment, is best suited for enhancing the bioavailability of the potato peel waste, for AD, under the conditions studied.

Keywords: Anaerobic digestion; Biogas yield; Methane yield; Potato peel waste; Chemical pre-treatments.

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LIST OF ABBREVIATIONS AND SYMBOLS

AD - Anaerobic digestion
At - Test assay
BOD_5 - Biochemical Oxygen Demand over a period of 5 days
CHP - Combined Heat and Power
CODs - Soluble Chemical Oxygen Demand
CODt - Total Chemical Oxygen Demand
db - Dry basis
DCTB - *Departmento de Ciência e Tecnologia da Biomassa*
FCT - *Faculdade de Ciências e Tecnologia*
FS - Fixed Solids
g - Centrifugal force
GEP - Gross Electricity Production
HRT - Hidraulic retention time
LEL - Lower Explosive Limit
LNEG - *Laboratório Nacional de Energia e Geologia*
MSW - Municipal Solids Wastes
n.a. - not appropriate
n.d. - not determined
NREAP - National Renewable Energy Action Plan
OHPA - Obligate Hydrogen Producing Acetogens
OL - Organic load
PNREAP - Portuguese National Renewable Energy Action Plan
PPB - Primary Production of Biogas
Qm_{Biogas} - Daily average flow rate of biogas produced
Qm_{CH4} - Daily average flow rate of methane produced
RES - Renewable Energy Source
SRB – Suphate-reducing bacteria
TS - Total Solids
UASB - Up-flow Anaerobic Sludge Blanket
UNL - *Universidade Nova de Lisboa*
VFA - Volatile Fatty Acids
VS - Volatile Solids
WAS - Waste Activated Sludge
Wastewater Treatment Plant - WWTP
wb - Wet basis
$\eta_{Biogas/SV\ removed}$ - Biogas yield in relation to volatile solids removed
$\eta_{Biogas/Total\ COD\ Removed}$ - Biogas yield in relation to total COD removed
$\eta_{CH4/SV\ removed}$ - Methane yield related to volatile solids removed
$\eta_{CH4/Total\ COD\ Removed}$ - Methane yield in relation to total COD removed

1. INTRODUCTION

1.1 Overview

The climate changes, the imbalances in the energy supply and storage, the instability of energy prices and the energetic dependency related to hydrocarbons, and their critical declining availability, spurred an ambitious reduction of primary energy consumption and a diversification of the energy sources in Europe and worldwide (Ferreira et al., 2009). It is of paramount importance today to focus on the sustained economic use of existing limited resources and on identifying new technologies and renewable resources, for example, biomass, for future energy supply (Deublein and Steinhauser, 2008).

Biomass is the non-fossil and biodegradable organic material originating from plants, animals and micro-organisms, for which a set of activities and programs in Europe is supported to stimulate its use for energy production, for example in the Biomass Action Plan and in an EU Strategy for Biofuels (Ferreira et al., 2009). Biogas has an important role in these programs, increasingly in the centre of attention as a subsequent product of anaerobic treatment for biodegradable waste from municipal sewage, agriculture, food industry or households.

Biogas is a renewable and sustainable secondary energy source generated via biochemical conversion of biomass by a well-known process designated by anaerobic digestion (AD) (Budzianowski, 2011; Ferreira et al., 2009). The organic waste treatment applied to AD is beneficial from an energetic and environmental point of view, when comparing to these organic matters placed in landfills. Therefore, landfills and biogas plants present two contrasting points of sustainable biowaste management.

Currently, in Portugal, a great amount of solid organic wastes of urban areas are still landfilled. Thereby, the AD of the biodegradable organic matter in landfill releases biogas into the air without any benefit or use, such as methane (CH_4), a greenhouse gas (GHG) with energetic value.

The AD in biogas plants reduces the air pollution in two main ways: it takes place in a sealed tank preventing the output of CH_4 into the atmosphere, and at the same time the combustion of bio-CH_4 releases green end-product to the atmosphere. The latter is carbon–neutral carbon dioxide (CO_2), that is to say, it does not add more CO_2 or other GHG to the air (Khalid et al., 2010; Ferreira et al., 2009) because the CO_2 absorbed by plants for their growth is the same as the one released into the atmosphere when using organic matter as biomass support for energy recovery (Ferreira et al., 2009).

The spread of biogas technology emerged in the developing world in the 1970s, when high oil prices motivated research in the alternative energy sources (Monte, 2010; Bond and Templeton, 2011). It has, currently, definite advantages, even when compared to other renewable energy sources (Holm-Nielsen et al., 2009). As Bond and Templeton (2011) reported, biogas technology has the potential to reduce nearly 4% of the global anthropogenic CH_4 emissions. Another advantage mentioned is the reduction of emissions of nitrous oxide (N_2O), now regarded as the biggest manmade threat to the ozone layer with a global warming potential over 300 times higher than CO_2.

In recent years, AD has become a choice for sustainable organic waste treatment all over the world (Zupančič and Grilc, 2012). AD technology provides several environmental, agricultural

and socio-economic benefits, combining renewable energy recovery of biogas with the sustainable treatment of a huge variety of waste from municipal wastewater treatment plants, agriculture, household or industrial processes (Appels et al., 2011; Bruni et al., 2010; Monou et al., 2008; Holm-Nielsen et al., 2009; Borgström, 2011; Crespo, 2013).

Although the traditional AD technology is most exclusively applied for concomitant biogas production, it has recently been studied and applied to the supply of biological hydrogen (H_2) (see: Zhu et al., 2008; Liu et al., 2006; Ozkan et al., 2011; Tapia-Venegas et al., 2013; Penumathsa et al., 2008) collected in the first stage of AD, and thereby giving another source of green energy production in this process. Nevertheless, the energy recovery of bio-H_2 is much less productive than bio-CH_4 (Liu et al., 2006). According to Liu et al. (2006), the two-stage system for bio-H_2 production has not won an impasse as it adds to complexity and consequently, investment and operational costs are increased.

The susceptibility of the substrates for biogas conversion under AD conditions results from both their suitable chemical composition and structure (Table 1.1). Thus, various physical, chemical and enzymatic pre-treatments are required to increase the solubility of several substrates and accelerate their biodegradation rate (Khalid et al., 2011).

Table 1.1 - Potential biogas production from wastes of the food and fodder industry (Deublein and Steinhauser, 2008).

Substrate for biogas production	**Total solids (TS) [%]**	**Biogas yield [$cm^3.g^{-1}$ TS.d^{-1}]**
Potato mash, potato pulp, potato peelings	6-18 85-96	300-900 3,000-10,000
Potato pulp dried, potato shred, potato flakes	88 94-96	600-700 -
Cereal mash	6-8 83-90	900 3,000-10,000
Mash from fermentations	2-5 90-95	500-85,000 35,000-60,000
Mash from distillations	2-8 65-85	420 14,000
Mash from fruits	2-3	300-700
Oilseed residuals (pressed)	92	900-1,000
Colza/flax extraction shred	88-89	400-900
Colza/flax cake	90-91	700
Molasses	77-90	300-700
Molasse of lactose	30	700
Wheat flour	88	700
Wheat bran, wheat powder bran	87-88	500-600
Malt germ	92	600

Although not considered in this study, it is worth mentioning that preferably co-digestion of two or more kinds of substrates are used for improving yields of AD rather than one substrate alone (Parawira et al., 2004b; Khalid et al., 2011; Monou et al., 2008; Kryvoruchko et al., 2009).

Besides the better known and widely used substrates, most by-products of the food industry are not currently used in biogas plants (Kryvoruchko et al., 2009). According to Deublein and Steinhauser (2008), the biggest output of energy from renewable resources will be provided by using biowaste from the food industry to produce biogas, as for example from potato processing industries.

In the specific case of potato waste, it is of paramount importance to manage such biowaste, from food and potato processing plants for bioenergy production, as potato remains the third largest food crop in the world (Zhu et al., 2008).

1.2 Scope of Thesis

The aim of this thesis is to evaluate the energy recovery through AD from potato peel waste subjected to chemical pre-treatments combined with thermal pre-treatments. In order to achieve the objective of this study, the experiments were carried out in two separate steps:

1. Thermo-chemical pre-treatments of the waste prior to AD trials - 4 assays carried out with chemical pre-treatments, either acid (H_2SO_4) or alkali (NaOH) tested at 2 different dosages each, to evaluate the increase of the bioavailability of the waste for AD;
2. AD trials with a chemical pre-treatment selected for the assays among those tested in the previous step - based on the previous step, an AD trial with the chemical pre-treatment selected was carried out to evaluate the increase of biogas and methane production and yields.

The AD trials of the present thesis were carried out in an up-flow anaerobic sludge blanket (UASB) reactor, under mesophilic conditions (37 ± 1 ºC, 1 atm). The subsequent biogas production and composition, chemical oxygen demand (COD) and the volatile solids (VS) of the waste, among others parameters, were also examined.

To the best of the author's knowledge, no treatment to increase biogas production through the AD of potato peel using a chemical pre-treatment has been reported in literature before.

1.3 Structure of Thesis

The chapters in the present thesis will be elaborated in the following structure.

The introduction will present an overview and the objectives of the thesis. Next, a detailed and extensive literature and conceptual review related to the subject of the thesis will be elaborated. The procedures and methods used for the experiments in the scope of the thesis will then be described, the results of which will subsequently be presented and discussed. Based on the discussions and results, the aim of the thesis will be analysed and concluded, wherein, finally, further research will be suggested for the pursuit of new goals and barriers related to the sector of biogas energy recovery.

2. LITERATURE REVIEW

2.1 Anaerobic Digestion

Anaerobic Digestion (AD) is a biological process of organic matter decomposition into simpler chemical components by specific microorganisms in the absence of oxygen (O_2). The result is a liquid and a solid fraction of a digestate enriched in nutrients, which can be used as a fertilizer in agriculture (Crespo, 2013), and a mix of gases, most commonly known as biogas, a renewable energy then used to produce green electricity and heat and less frequently injected in natural gas grids or used for vehicle fuel (Monnet, 2003; Deublein and Steinhauser, 2008; Holm-Nielsen et al., 2009).

AD occurs naturally in, for example, swamps, the digestive tract of ruminants and flooded rice fields; or in an anthropogenic environment like municipal landfills and artificial environment set up specifically for AD such as biogas plants. Controlled AD combines environmental benefits regarding the waste treatment, pollution reduction, energy production and improvements in agricultural practices (Deublein and Steinhauser, 2008; Parawira et al., 2004b). Figure 2.1 sets a sustainable potato life cycle with AD, in which the potato wastes are reused and recycled energetically by two main ways: production of biogas and of biofertilisers.

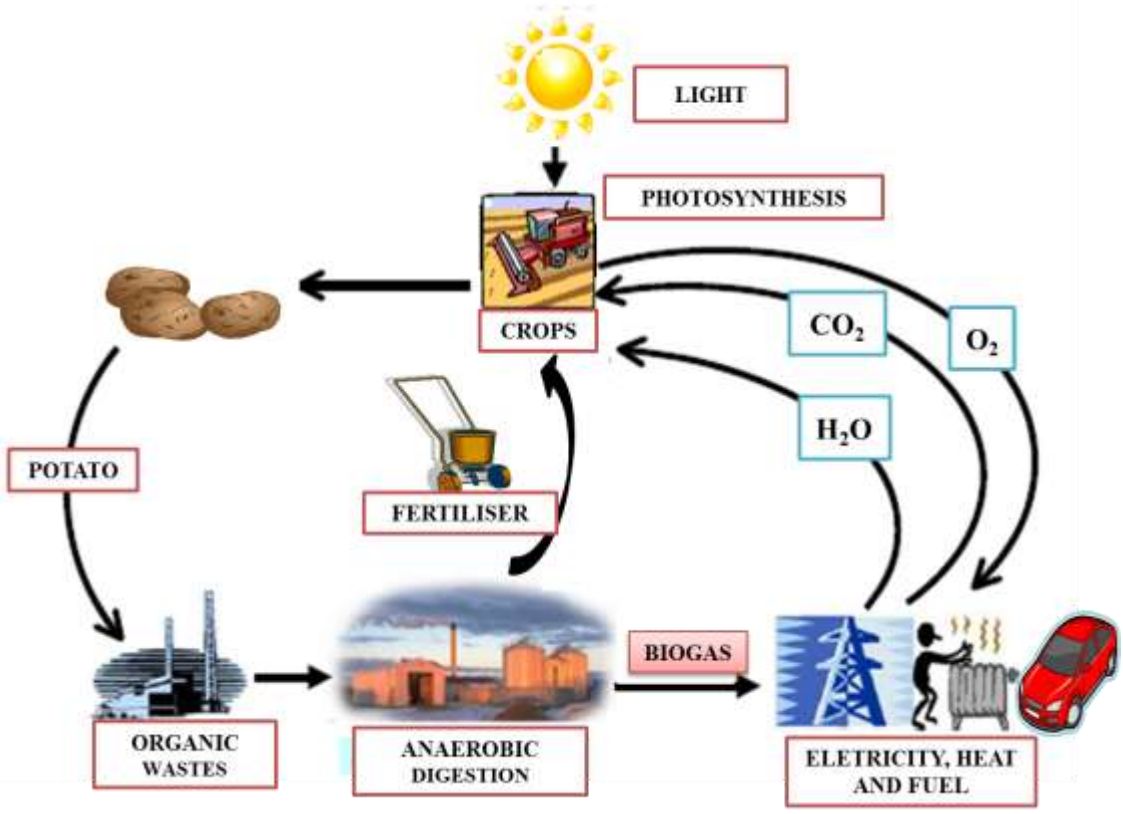

Figure 2.1 - Schematic representation of the sustainable cycle of anaerobic digestion of potato wastes from industry (adapted of Al Seadi, 2002, apud Holm-Nielsen et al., 2009).

AD requires twenty times less energy than an aerobic process. In this latter process, only low-energy compounds CO_2 and H_2O are formed, and a great deal of energy is lost to the air. In the case of AD, high-energy metabolic products are formed instead (e.g. alcohols, organic acids,

and, in the long run, methane). These are suitable for use as nutrients for microorganisms [alcohols, organic acids], or as energy carrier [biogas] (Deublein and Steinhauser, 2008). Thus, AD recycles the nutrients whilst providing clean renewable energy and fertilisers (Khalid et al., 2011; Deublein and Steinhauser, 2008).

Controlled AD occurs in biodigesters with a good record in treating a wide spectrum of waste streams such as municipal, agricultural or industrial waste operating over 20 years. Before being digested, the feedstock has to go through one or more pre-treatments, such as mechanical, thermal or chemical, to increase the biodegradability of the substrate (Deublein and Steinhauser, 2008; Khalid et al., 2011).

Furthermore, the biodigester requires specific characteristics and properties for different feedstocks differing in temperature, solid content and on the number of stages (single or multi-stage). Thus, the reactor may operate in mesophilic (around 37 ºC in the present study or/and 35 ºC in other literature) or thermophilic (55 ºC) conditions, in wet or dry digesters and in multi-stage processes - with the aim of optimizing digestion and improving control of the process by separating stages of digestion, or in batch processes; which are less expensive and less complex but also less efficient (Khalid et al., 2011; Monnet, 2003). Currently, 90% of full-scale biogas plants in Europe rely on one stage processes due to the lower cost comparing to two-stage processes (Liu et al., 2006; Bouallagui et al., 2005).

Historical evidence indicates that the AD process is one of the oldest technologies and, in recent times, European countries have come under pressure to explore the AD market for two significant reasons: higher energy prices and increasingly stringent environmental regulations. The financial aspect of AD includes high operating and capital costs, but the source of incomes coming from the sale of electricity, heat, vehicle fuel and fertilizer allows benefits (Monnet, 2003).

The management of organic waste treatment applied with AD is beneficial from an energetic and environmental point of view, comparing with landfills. Therefore, landfills and biogas plants represent two contrasting point of views of sustainable management.

Currently, in Portugal, a great amount of solid organic wastes are placed mostly in landfills following a selective collection and composting system. In this way, the AD of biodegradable biowastes releases end-products [biogas] into the air without any benefit or use, such as CH_4, a greenhouse gas GHG with energetic value. Contrastingly, the AD in biogas plants reduces air pollution preventing the output of CH_4, within biogas, into the atmosphere and promoting its combustion with energetic use.

2.2 Biochemistry of Anaerobic Digestion

The AD results in the decomposition of complex organic materials into simple compounds in a chain of groups of microorganisms, in the absence of oxygen, to produce methane and carbon dioxide as end-products under ideal conditions. The biochemistry underlying AD is complex and has been described as a sequential process, as illustrated in Figure 2.2, of four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Deublein and Steinhauser, 2008; Monnet, 2003; Kossman et al., 1997; Aldin et al., 2011; O'Flaherty et al., 2010; Khalid et al., 2011).

Due to the strong link of the phases in each stage, the nutrients availability, pH, temperature and other parameters will affect the activity and the growth rate of the different anaerobically facultative and/or obligatory microbial groups involved, such as the fermentative, acetogenic, and methanogenic bacteria (Deublien and Steinhauser, 2008; Kossman et al., 1997). Some authors describe an intermediary phase in acetogenesis: homoacetogenesis, in the presence of the homoacetogenic bacteria (Deublein and Steinhauser, 2008; CCE, 2000), although it is only responsible for 2 to 5% of the acetate production in the digester.

In the first stage, during the hydrolysis, the fermentative bacteria convert insoluble complex biopolymers into soluble monomers (e.g. carbohydrates into monosacarids). Hydrolysis of wastes with high organic content, such as long chain fatty acids (lipids), proteins or carbohydrates (sugars), may become rate limiting (Monnet, 2003; Monou et al., 2008; Kryvoruchko et al., 2009). Fermentative bacteria are more suitable in lower pH than methanogenic (6.5-7.5), thus a chemical, like hydroxide sodium (NaOH), can be added during the hydrolytic activity in order to decrease the digestion rate and to increase pH and to improve the methane yield (Bouallagui et al., 2005; Monte, 2010; Monou et al., 2008).

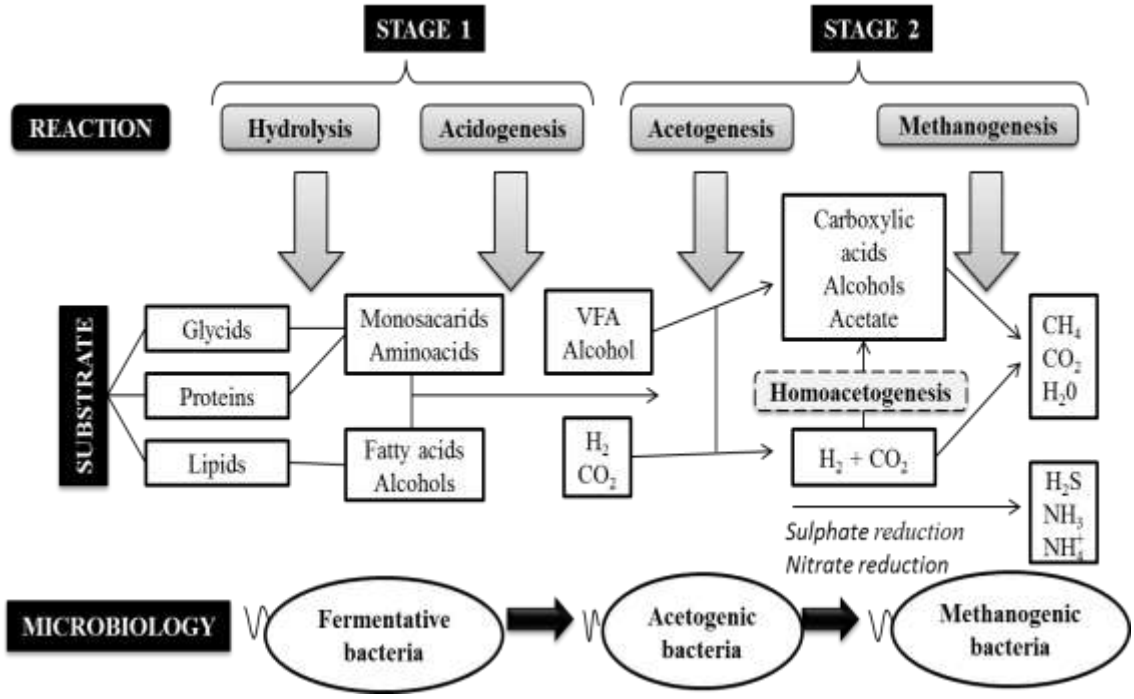

Figure 2.2 - Schematic representation of the biochemistry processes of AD (Deublein and Steinhauser, 2008; Kossman et al., 1997; Bouallagui et al., 2005; CCE, 2000; O'Flaherty et al., 2010).

In the second stage, acetogenic bacteria use volatile fatty acids (VFAs), also known as short-chain fatty/carboxylic acids, as intermediary products from the first stage to convert into carbon dioxide, hydrogen and simple organic acids, (e.g. acetic acid, butyric acid, propionic acid and ethanol) (O'Flaherty et al., 2010). The final reaction of methanogenesis occurs with methane production by methanogenic bacteria in two different paths: two acetic acid molecules generate carbon dioxide and methane, and reduction of carbon dioxide with hydrogen. The acetate reaction is the primary producer of methane because of the limited amount of hydrogen available (Deublein and Steinhauser, 2008; Monnet, 2003).

2.2.1 Hydrolysis

The hydrolysis reaction is the substrate breakdown of complex insoluble high level molecular compounds (polymers), such as proteins, carbohydrates and lipids, into simple soluble organic substances (monomers), for example, amino acids, sugars and long-chained fatty acids. The reactions in this phase can be merely biological [using hydrolytic microorganisms] or combined: bio-chemical [using extracellular enzyme], chemical (using catalytic reactions) as well as physical [using thermal energy and pressure] (Zupančič and Grilc, 2012).

This reaction is catalyzed by extracellular enzymes, such as proteases, amylases, cellulases and lipases, segregated by fermentative bacteria (Barlaz at al., 2010; O'Flaherty et al., 2010; Parawira et al., 2005; Deublein and Steinhauser, 2008; Monte, 2010), mainly obligatory anaerobic (Alves, 1998) and representing 90% of the bacteria population in the digester (Zeikus, 1980). These exoenzymes crack the complex molecules into monomers (water soluble fragments). Hitherto the bacteria had had no capacity to assimilate the organic matter in particulate form, thus providing the inflow of these compounds inside the cells (Bouallagui et al., 2005; Parawira et al., 2005).

Generally, the duration of this step is two or three days for easily biodegradable compounds (Monte, 2010), although the reaction rates vary widely depending on the type of substrate used (Aldin et al., 2011). Each molecule type has a different reaction rate to decompose into simpler compounds: the carbohydrates hydrolyse in some hours, the proteins and the lipids within a few days or months. Lignocellulose and lignin are degraded only slowly and incompletely (Khalid et al., 2011; Deublein and Steinhauser, 2008), often considered as substrates which are difficult to biodegrade and, therefore, undesirable for AD.

According to Yang et al. (2010), the hydrolysis rate is affected by several factors; the pH, the temperature, enzyme types and hydrolytic substance. For instance, the higher the temperature, the higher the solubilisation of volatile suspended solids of the organic matter and the concentrations of dissolving reducing sugars. Thus, the combination of heat and enzymes had positive effects in the recalcitrant organic matter broken into dissolving organic compounds.

The hydrolysis rate can be lower if the concentration of particulate organic matter is high, due to a surface-limiting area of cells with disruptions in the mass transference (Myint et al., 2007). According to Myint et al. (2007), a quantity of organic substrate has to be in the same ratio of the hydrolytic enzymes, provoking an effective reaction of the hydrolytic bacteria, not limiting the next step of AD: acidogenesis. However, according to Aldin et al. (2011), the benefits of particle size on solubisation and increased gas production are not exactly correlated.

The hydrolytic phase is often considered the rate-limiting step of AD (O'Flaherty et al., 2010; Vavilin et al., 2001; Ge et al., 2011; Molino et al., 2013; Kim et al., 2003; Penumathsa et al., 2008; Parawira et al., 2004a), whereby the content of scarcely biodegradable products determines the efficiency of the substrate conversion into biogas (Li et al., 2011; Deublein and Steinhauser, 2008).

2.2.2 Acidogenesis

In the this step, also known as fermentation, the acidogenic bacteria, anaerobically obligatory and facultative, proceed into the degradation of the monomers formed after the hydrolytic phase with the formation of VFAs [e.g. primarily acetate, butyrate, propionate], alcohols, ammonia,

H_2 and CO_2, and other alcohols and carboxylic acids such as lactate, ethanol, acetone or alanine (Barlaz et al., 2010; O'Flaherty et al., 2010; Deublein and Steinhauser, 2008; Liu et al., 2006).

Hereafter, the oxidation of propionate and butyrate into acetate, CO_2 and H_2 is only thermodynamically favourable at very low H_2 concentrations. Thus the high partial pressure of H_2 acts as an inhibiting factor of acetate formation and other reduced compounds (O'Flaherty et al., 2010; Liu et al., 2006; Deublein and Steinhauser, 2008), although the conditions are favourable to produce more reduced substrates such as lactate, ethanol, acetone, alanine (Liu et al., 2006).

According to Liu et al. (2006), the pH parameter is a good correlation factor of H_2, acetate and butyrate for the shifts of the fermentation system: at a higher pH of 5.2, more production of H_2 was registered and subsequently more production of acetate as almost the only final product, although at a lower pH of 4.8, less H_2 was produced and butyrate increased. Liu et al. (2006) observed an optimum pH range of 5-5.5 to increase the acetate production.

According to the aforementioned author, the CO_2 concentration can also affect the H_2 synthetic pathway. High CO_2 concentration favours the production of fumarate or succinate, which consumes electrons in the medium, and therefore decreases H_2 production. Another reason for it may be due to the removal of carbon monoxide (CO) in the system. Levin et al. (2004) reported that CO could influence bacterial metabolism away from H_2 production towards solvent [i.e. ethanol] production.

The acidogenesis is a fast reaction, thus not considered a rate-limiting phase of AD (Appels et al., 2008) and the growth rate of acidogenic bacteria is typically much higher than that of CH_4-formers (Vavilin et al., 2001).

2.2.3 Acetogenesis

In this step, the pathways of the reactions are complex and variable as the processes are tightly linked, and the production and consumption of intermediate products are rapid and in balance with acidogenesis and methanogenesis phases.

The end-products of the degradation of polymers to H_2, CO_2, formate, acetate and higher VFA by the fermentative bacteria [1 in Figure 2.3] are further oxidised to acetate, H_2, and CO_2 in a complex metabolic process known as acetogenesis, mediated by the obligatory H_2-producing acetogens (OHPAs) bacteria [2 in Figure 2.3].

OHPA, a slow growing bacteria, is dependent on hydrogenotrophic methanogens, homoacetogens, fatty acid synthesizers and sulphate-reducing bacteria [6, 3, 4 and 7, respectively, in Figure 2.3] to jointly decrease the hydrogen pressure that they increase with the oxidation of substrates, such as proprionate [2 in the Figure 2.3], H_2/formate [2 in Figure 2.3], and CO_2 [2 and 4 in Figure 2.3] to produce acetate (Borgström, 2011; O'Flaherty et al., 2010).

Thereby, OHPA, which are sensitive to high hydrogen pressure, are only capable to act in favourable thermo-dynamic conditions like in exergonic reactions, when the H_2 partial pressure is low. OHPA must therefore grow in syntrophy with hydrogenotrophic (or H_2-using) bacteria, sulphate-reducing bacteria (SRB), or homoacetogens [6, 7 and 3, respectively, in Figure 2.3), in order to facilitate interspecies H_2 transfer (acetate, formate and CH_4) and to gain energy from growth in the products of acidogenesis (O'Flaherty et al., 2010; Deublein and Steinhauser, 2008; Bruni, 2010).

In an endergonic reaction, an energy input for OHPA is necessary for acetate formation and its survival and growth. Therefore, in the absence of an energy input, OHPA must only grow at very low H_2 concentration with synthropic associations, optimum temperatures [25-45°C] and pH levels [6.3-8.5] (Bruni, 2010; O'Flaherty et al., 2010; Deublein and Steinhauser, 2008).

Propionate and butyrate are the most important intermediates in the syntrophic reactions, whereby their degradations are regarded as the rate-limiting steps of AD due to their thermodynamic restrictions (Amani et al., 2012). In a recent study, Amani et al. (2012) found optimum conditions to be 1.93 $g.L^{-1}$ propionate, 2.15 $g.L^{-1}$ butyrate, 2.50 $g.L^{-1}$ acetate, for a HRT of 22 hours, and a population ratio of 2.5 [adimensional] related to a maximum of VFA removal and biogas production rate.

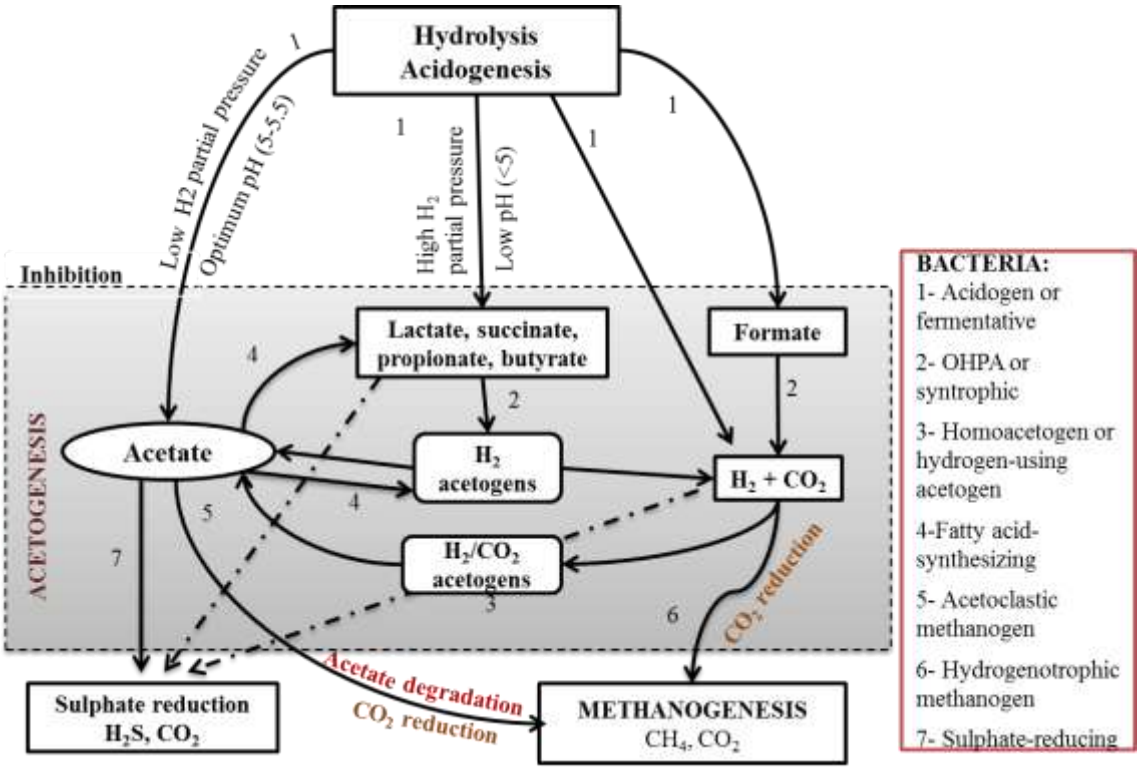

Figure 2.3 - Diagram of the biochemistry synthetic pathways processes of acetogenesis and the bacteria involved (Sötemann et al., 2004; Liu et al., 2006; Bouallagui et al., 2005; CCE, 2000; O'Flaherty et al., 2010; Kotsyurbenko et al., 2004; Deublein and Steinhauser, 2008; Zinder, 1993).

Homoacetogens are capable of both autotrophic and heterotrophic growth and responsible for generating among 2% or 5% of the acetate in this phase. The acetate formed by the homoacetogens is the sole end-product of the degradation of either H_2/CO_2 or multicarbon, therefore competing with methanogens, which produce CH_4 (Alves, 1998; O'Flaherty et al., 2010; Barlaz et al., 2010; CCE, 2000; Bruni, 2010).

The fatty acid synthesizers (4 in Figure 2.3) produce lower fatty acids by transforming the acetate and/or ethanol when the concentration of H_2 is high, thus reversing the reactions of the syntrophic bacteria. The activity of these organisms can be an indicator of reactor instability (O'Flaherty et al., 2010), as well as the presence of propionate in the digester. Propionate is the first metabolic step of acetogenesis, since their degradation generally occurs faster in normal conditions of this step. When in case of increasing concentrations of H_2, the oxidation of VFA is affected, and thereby the inhibition of the oxidation of propionate occurs (CCE, 2000).

The action of the OHPAs converts a range of fermentation intermediaries into usable methanogenic substrates and thus provides a link between the initial fermentation stages and the ultimate methanogenic phase during AD (O'Flaherty et al., 2010).

2.2.4 Methanogenesis

Methanogenesis occurs strictly in anaerobic conditions and is the last and predominant metabolic process with CH_4 production by two types of methanogen bacteria: hydrogenotrophic, also known as hydrogenophilic genera and the acetoclastic species, also known by acetotrophic [6 and 5, respectively, in Figure 2.3]. Methanogens can produce CH_4 by other substrates, such as methanol, methylamines, and formate (O'Flaherty et al., 2010).

Methanogens [archaebacter genus] are anaerobically obligatory, and therefore very sensitive to environmental changes. Under standard conditions, compounds with a low molecular weight, formed during the previous step, are decomposed by methanogens in exergonic reactions to generate CH_4 by in two ways: the hydrogenotrophic, by the reduction of CO_2 via formyl, methenyl, and methyl intermediates, in association with specific coenzymes and using H_2 as electron donor, and the acetoclastic, by the decarboxylation of acetate (Kossman et al., 1997; O'Flaherty et al., 2010; Deublein and Steinhauser, 2008; Bruni, 2010).

In opposition with the hydrogenotrophic process, the acetoclastic reaction is less exergonic and only some methanogen species are able to produce CH_4 by this pathway, whereas nearly all the well-known methanogen species are able to use H_2/CO_2 as a substrate for CH_4 production. Nevertheless, approximately 70% of the CH_4 produced during AD is via acetoclastic genera whilst 27-30% of the CH_4 results from the hydrogenophilic pathway (Bruni, 2010; O'Flaherty et al., 2010; Deublein and Steinhauser, 2008).

As Bruni (2010) states, close proximity between hydrogenotrophic methanogen and the acidogenic bacteria [6 and 1, respectively, in Figure 2.3] ensures that the partial pressure of H_2 is within the optimal range to allow a balance of H_2 formation and consumption to be exergonic. Furthermore, Kim et al. (2002) study indicates the importance of microbial consortia proximity and it was hypothesized that the non-mixing reactor configuration has closer microbial consortia proximity than others.

This phase is related to acetogenesis: if methanogenesis occurs, the acetogenesis is active and stable, if methanogenesis is disturbed, the acidogenesis leads to the acidification of the digester, and thus an inhibition of both phases occurs. The H_2S-producing bacteria affect the methanogenics, competing with methanogens for H_2 (Deublein and Steinhauser, 2008; O'Flaherty et al., 2010), whilst the specific activity of methanogenic bacteria has been found to decrease with increasing concentrations of ammonia (Khalid et al., 2011).

According to Lee and Zinder (1988), acetate was not used at high H_2 partial pressures, meanwhile the ethanol was oxidised into acetate. The acetate utilisation only occurred at the remaining H_2 partial pressures of approximately 10 Pa, indicating a threshold for H_2 utilization by methanogens.

Methanogen regeneration in acetate is, theoretically, very slow, having a growth rate of 100 h, approximately five times lower to other groups of bacteria, such as acidogens (CCE, 2000; Deublein and Steinhauser, 2008). However growth rates can be substantially longer under real conditions (Deublein and Steinhauser, 2008).

2.3 Parameters Affecting Anaerobic Digestion

Due to the different metabolic processes of the various bacteria in each stage of AD, a number of environmental factors influence the microbiology and application of AD and their understanding can provide the correct management of these factors to enhance the microbial activity and the AD efficiency and stability (O'Flaherty et al., 2010; Deublein and Steinhauser).

Thus, in order to achieve an increased overall potential performance of the reactor, the knowledge and control of the operational parameters are crucial, including temperature, pH, C:N ratio, hydraulic retention time (HRT) and TS content, alkalinity, redox potential, VS, agitation, availability of nutrients and presence of toxic components (O'Flaherty et al., 2010; Deublein and Steinhauser, 2008; Kossman et al., 1997; Monnet, 2003; Meena et al., 2011; Zhang, 2010).

Nevertheless, the parameters shall be associated with the characteristics of the biomass used as substrate for AD in order to optimise the reactions for bacteria growth (O'Flaherty et al., 2010). Optimum conditions for all microorganisms involved in the degradation can only be set in a two-stage reactor with one stage for hydrolysis/acidification and one stage for acetogenenis/methanogenesis (Deublein and Steinhauser, 2008). However the investments and the operational costs increase with the separation and management of the two phases (Liu et al., 2006). Another benefit of the two-stage system is that it prevents the presence of pathogen microorganisms in the final digester.

It is worth mentioning that the reactor design has a strong effect on the digester performance (Bouallagui et al., 2005; Meena et al., 2011).

2.3.1 Temperature

The AD process is strongly influenced by temperature and its key role is distinguished in one of three temperature ranges: psychrophilic (0-20 ºC), mesophilic (20-45 ºC) and thermophilic (45-65 ºC). The mesophilic and thermophilic ideal temperature ranges are 35-38 ºC and 50-55 ºC, respectively. Nevertheless, the optimum temperature of the digestion may vary depending on feedstock composition and type of digester (Bouallagui et al., 2005; O'Flaherty et al., 2010; Monnet, 2003). Hellström et al. (2009) mentioned a fourth temperature range for microbial activity: hyperthermophilic, between 70-95 ºC.

The main issue associated with the various operational temperature ranges and the related microbial activity remains the level of specialization of the bacteria, which will increase or decrease the efficiency of the biological process, and hence the production of biogas (Azeitona, 2012).

AD plants typically operate in mesophilic conditions, therefore operational regimes adopted for the digester are 30-40 ºC (Kim et al., 2002), or more precisely in the range of 35-37 ºC (Kim et al., 2006), since these temperatures assure favourable conditions for the microbial growth and increase the efficiency of the reactor. Although thermophilic digestion is generally more efficient than mesophilic, it is harder to control and less stable; the bacteria is more sensitive to temperature variations, that cause substantial decreases in its activity, organic loading rate (OLR) and the presence of toxic compounds; it also requires a high amount of energy, giving it a less favourable energetic balance than with mesophilic digestion (Hagelqvist, 2013; Deublein and Steinhauser, 2008; Abbasi et al., 2012).

Temperatures in the range of 40-45 °C are particularly critical for mesophilics and can affect their activity irreversibly (Deublein and Steinhauser, 2008). Changes in temperature, in the type of substrates or in the substrates concentrations may lead to a failure in biogas production. These disruptions can occur within several weeks, until the microbiological system adapts to the new conditions (Deublein and Steinhauser, 2008; Khalid et al., 2011). The acetogenesis and methanogenesis phases are observed as more sensitive to temperature than either hydrolysis or acidogenesis (O'Flaherty et al., 2010).

The sterialisation of the waste is also related with temperature; the higher the temperature, the more effective is the elimination of pathogens, viruses and seeds. Therefore thermophilic systems are more efficient (Monnet, 2003). Mesophilic processes require longer hydraulic retention times than thermophilic (Deublein and Steinhauser, 2008).

According to Kim et al. (2002), in a comparative study of mesophilic *versus* thermophilic conditions, thermophilic AD often manifested higher VFA accumulation, especially of propionate, which showed a higher performance than in mesophilic.

The temperature effect in biogas production was tested by Hellström et al. (2009): in mesophilic conditions, the "net exergy" is nearly 0.35 GWh.°C^{-1}.$y.^{-1}$ for a change in the temperature around the "the normal operating situation" of a 20-day retention time and a temperature around 35 °C.

2.3.2 pH Level

The pH level interacts differently with the specific functions of the microbial activity in AD. Polymers such as carbohydrates (e.g. potato, which presents a high content of carbohydrates), are likely to acidify and it is only through proteins degradation that pH-buffering ions are released. Therefore the pH value decreases easily and additionally carbohydrates degradation raises H_2 partial pressure easily, whilst reduced acidic intermediaries (products) are formed, such as, acetic, latic and propionic acids formed in acidogenesis (Deublein and Steinhauser, 2008; Monnet, 2003).

According to literature by Khalid et al. (2011), the processes of AD can be inhibited at different pH levels: whilst hydrolysis and acidogenesis occurs preferably at pH 5.5-6.5, respectively, methanogenesis occurs efficiently at pH 6.5-8.5 (Meena et al., 2011), with a corresponding optimum pH range of 7.0-8.0. Moreover, some authors reported a specific optimum pH of around 7 (Khalid et al., 2011; Meena et al., 2011). Although other preferred pH ranges can be found for each microbial group and process of AD, depending on other factors, such as the substrate (Korres and Nizami, 2013). For example Monnet (2003) stated an optimum pH range for methanogenesis of 6.6-7.0, and Deublein and Steinhauser (2008) stated an optimum pH range of 6.7-7.5.

Liu et al. (2006), found pH to be a key factor in a direct correlation between H_2, acetate and butyrate selecting the pathway of fermentation. Whereas pH 5.0-5.5 is the optimum range for hydrogen production and acetate was the only end-product to be found, at a pH below 4.8 less H_2 was released and butyrate started to concentrate.

Mata-Alvarez et al. (2000) stated that the constants for the hydrolysis of lipids, proteins and carbohydrates are pH-dependent.

Methanogens are more sensitive to this parameter and live best under neutral to slightly alkaline conditions, acetoclastic methanogens, for example, decreases sharply below pH 6.6. In a one-

stage digester, a high VFA concentration from acidogenesis result in inhibition of methanogenesis (Mata-Alvarez et al., 2000; Bouallagui et al., 2005) and process failure, thus requiring from the beginning a higher-than-normal pH value of the reactor maintained at conditions more suitable for methanogens to avoid an excessive growth of the acidogens (Monou et al., 2008; Meena et al., 2011; Kossman et al., 1997; O'Flaherty et al., 2010). The optimum level for volatile fatty acids degradation is in the pH range of 6.5 to 7.2 (Hagelqvist, 2013).

2.3.3 Alkalinity

The alkalinity parameter is the measure of the buffering effect of the AD substrate, or the ability of a solution to neutralise the acids to prevent pH variation, and meet its resistance to rapid changes of pH. It is generally expressed as $mg.CaCO_3.L^{-1}$ (Korres and Nizami, 2013; CCE, 2000).

According to Franco et al. (2007) and Flor (2006), the alkalinity parameter can be measured in two ways, which are useful control parameters for AD: the partial alkalinity in presence of bicarbonate concentration [titration until pH 5.8] and the intermediate alkalinity in presence of the concentration of VFA; however, in absence of VFA it was proved that AD can run at pH 6 (Flor, 2006). The partial alkalinity plus the intermediate alkalinity is the equivalent to the total alkalinity [titratation until pH 4.3 (Flor, 2006)], the latter at 1500 $mg.CaCO_3.L^{-1}$ is advisable for an appropriate performance of AD processes (Franco et al., 2007, Korres and Nizami, 2013). However, the ratio of intermediate alkalinity to total alkalinity must be controlled and regulated at lower than 300-400 $mg.CaCO_3.L^{-1}$ (Franco et al., 2007; Korres and Nizami, 2013). In many assays, phosphates are also used as a source for alkalinity (Santos, 2013), whilst the use of calcium salts is not recommended since can cause incrustrations in the reactor, diminishing it performance (Flor, 2006).

Contrastingly, according to Korres and Nizami (2013), in AD processes, the reaction of ammonia with dissolved CO_2 and carbonate ions to form ammonium bicarbonate can influence the alkalinity, when the ammonia is present in high proportions from the degradation of substrates containing high levels of proteins and amino acids in its composition.

2.3.4 Redox Potential

Redox potential is a necessary monitoring parameter at low values in a digester. For example, for methanogens activity this factor should be lower than -250 mV and in an optimum of -300 to -330 mV. Additionally the redox potential can also rise to 0 mVs in the fermenter. In order to keep a low redox potential, few oxidizing agents should be present like no oxygen, sulfates, nitrates, or nitrites (Korres and Nizami, 2013; Deublein and Steinhauser, 2008).

2.3.5 Nutrients and C:N ratio

The availability of nutrients for AD is crucial to increase the rate of the biochemical reactions. These are generally distinguished by macronutrients and micronutrients and associated with its need for cells. Thus the availability of these in the digester is extremely important for an overall

achievement of the processes involved in the AD. The macronutrients considered are mainly carbon (C), nitrogen (N), phosphorus (P) and sulphur (S) (Monte, 2010; Carrilho, 2012; Azeitona, 2012; Deublein and Steinhauser, 2008; Zupančič and Grilc, 2012). Other nutrients can be referred, such as hydrogen (H_2) (Kossman et al., 1997), which is required for methane production by hydrogenotrophic methanogens.

The carbon to nitrogen (C:N) ratio represents the relationship of carbon and nitrogen in the organic matter and is a measure of the nutrient balance required by the microorganisms for assimilation into their cell structures (Kossman et al., 1997; Monnet, 2003; Korres and Nizami, 2013). The degradation of carbon is 25-30 times faster than that of nitrogen and the optimum C:N ratio will affect the biogas yield as also varies on the nature of substrate (Korres and Nizami, 2013; Kossman et al., 1997) (Table 2.1). The optimum carbon:nitrogen:phosphorus (C:N:P) ratio for high methane yield is reported to be 100:3:1 (O'Flaherty et al., 2010), whereas the ideal C:N:P:S is approximately 100:10:1:1 (Zupančič and Grilc, 2012). According to Deublein and Steinhauser (2008), C:N:P:S of 333-167:7-4:2:1 is sufficient for methane formation.

Table 2.1 - Optimum C:N ratio ranges for AD of organic matter mentioned in literature.

Optimum C:N ratio	**Literature**
20-30:1	Monnet (2003) Zupančič and Grilc (2012) Khalid et al. (2011) Herout et al. (2011)
16-25:1	Deublein and Steinhauser (2008)
15-25:1	Korres and Nizami, 2013 (2013)
25-32:1	Bouallagui et al. (2005)

A low C:N ratio, [e.g. 3:1 (Zupančič and Grilc, 2012)], in the substrates can lead to increased ammonia production and the inhibition of methane production (Deublein and Steinhauser, 2008), whilst a high C:N ratio due to lack of nitrogen present will lead to a rapid consumption of nitrogen and to nutrient deficiency. A low buffering capacity will result in a more sensitive process, and thus a reduced protein formation and a decline in the energy and structural metabolism of the anaerobes (Deublein and Steinhauser, 2008; Korres and Nizami, 2013; O'Flaherty et al., 2010).

A digester must never operate at a low C:N ratio, or an ammonia (NH_3) concentration and pH values exceeding 8.5 (Monnet, 2003) may result, inhibiting methanogenesis at NH_3 concentrations over 3000 $mg.L^{-1}$ and even at pH values over 7.4 (Zupančič and Grilc, 2012; Korres and Nizami, 2013; Deublein and Steinhauser, 2008). In these situations, a balanced mixture is required and the co-digestion of substrates for the improvement of nutrition and C:N ratio is advisable (Deublein and Steinhauser, 2008; Khalid et al., 2011).

In order to grow, bacteria need a supply of certain mineral nutrients, known as micronutrients, with concentrations under $1x10^{-4}$ $mol.L^{-1}$. Although study of the effects of micronutrients on AD is a promising field of research, difficulties due to trace element limitation can arise (Carrilho, 2012; Kossman et al., 1997; O'Flaherty et al., 2010).

Additionally, the production of biomass requires a suitable supply of potassium (K), calcium (Ca), magnesium (Mg) and a number of trace elements usually present in sufficient amounts in most agricultural waste that are treated in anaerobic digesters, such as iron (Fe), manganese

(Mn), molybdenum (Mb), zinc (Zn), cobalt (Co), selenium (Se), tungsten (W) and nickel (Ni) for an efficient AD (O'Flaherty et al., 2010; Kossman et al., 1997). The Fe, Ni and Co are essential nutrients for a high acetate conversion into methane by acetoclastic bacteria (Alves, 1998).

2.3.6 Presence of Toxic Components

The presence of toxic or inhibiting components capable of causing a disruption in the chemical reactions of microorganisms can be generated during the AD process, such as VFA, or carried in the start-up feeding mix (Carrilho, 2012; Crespo, 2013; CCE, 2000; Azeitona, 2012). The inhibition mainly depends on the concentration of the inhibitors, the composition of the substrate, and the adaptation of the bacteria to the inhibitor substances (Deublein and Steinhauser, 2008).

The term *toxicity* is defined by the concentration of a compound at which is toxic or inhibitory to the bacteria, thus varying with the specific substance (Azeitona, 2012). The tolerance of the bacteria depends on the concentration and ion specie in the medium, as follows in Table 2.2 according to literature found. However sometimes contradictory reports have been published, that is explained, in some cases, with the microorganisms adaptation to an adverse environment (Deublein and Steinhauser, 2008).

Table 2.2 - Optimal, moderate and highly inhibitory concentrations for AD of several substances.

Ion	Optimal concentration [mg.L^{-1}]	Moderate inhibitory concentration [mg.L^{-1}]	Highly inhibitory concentration [mg.L^{-1}]
Na^+	100-200 [3]	3,500-5,500 [1, 3]	8,000-16,000 [1, 3]
K^+	200-400 [3]	2,500-4,500 [1, 3]	12,000 [1, 3]
Ca^{2+}	100-200 [3]	2,500-4,500 [1, 3]	8,000 [1, 3]
Mg^{2+}	75-150 [3]	1,000-1,500 [1, 3]	3,000 [1, 3]
NH_4^+	50-1000 [5]	1,500-3,500 [1]	3,000 [1]
S^{2-}	50 - 1000	200 [1,2]	200 [1]
Cu^{2+}	-	10-250 [2] 40 [3]	250-600 [4] 170 [3]
Cr^{6+}	0.005 – 50 [4]	110 [3] 200-2,000 [2]	420 [3]
Cr^{3+}	-	130 [3]	200 [5]; 260 [3]
Ni^{2+}	0.005 – 0.5 [4]	100-1,000 [2]	30-1,000 [1, 4]
Zn^{2+}	-	350-1,000 [2] 3-400 [4] 400 [3]	250-600 [4]

[1]Crespo, 2013; [2]Kossman et al., 1997; [3]Zupančič and Grilc, 2012; [4]Deublein and Steinhauser, 2008; [5]Santos, 2011.

Additionally, the level for an inhibition considered is also variable. For example, according to Zupančič and Grilc (2012), a concentration of heavy metals is considered inhibitory at the first

concentration shown to diminish biogas production and a toxic concentration at the concentration in which the biogas production was diminished by 70%.

The presence of the molecular O_2 in dissolved form can also have an inhibiting effect, as the AD requires strict anaerobic conditions, that is, the absence of O_2 in a corresponding redox potential under -200 mV, however it can be present with limited amounts in combined forms of nitrate [NO_3^-] and sulphite [SO_3^{2-}] (Monte, 2010).

2.3.6.1 Volatile Fatty Acids

Volatile fatty acids (VFA) are necessary for microbial growth in small concentrations and naturally present in the substrate and by-products of acidogenesis then decomposed during methane formation. However, at high concentrations, these inhibit the AD (Carrilho, 2012; Deublein and Steinhauser, 2008; Zupančič and Grilc, 2012). VFA can have an inhibitory effect on methanogenic archaea on concentrations over 10,000 mg.L^{-1} (Zupančič and Grilc, 2012). Especially the undissociated organic acids have an inhibiting effect for these bacteria due to their capacity to penetrate into the cells, and denaturate the cell proteins (Deublein and Steinhauser, 2008).

Propionate and butyrate are the most important intermediates for the syntrophic reactions to form acetate, whereas their degradation is regarded as the rate-limiting stage of AD due to thermodynamic restrictions (Amani et al., 2012). The inhibition of AD system by propionic acid occurs even at a concentration of 5 mg.L^{-1}, and in the case of butyric acid, or valeric acid, can be as low as a concentration of 50 mg L^{-1} of undissociated fatty acid without adaptation. At a pH lower than 7, the inhibiting threshold for AD is 1,000 mg.L^{-1} for the acetic acid (Deublein and Steinhauser, 2008). The optimum level for volatile fatty acids degradation is in the pH range of 6.5 to 7.2 (Hagelqvist, 2013).

In a recent study focused on the VFA degradation in an UASB reactor inoculated with enriched cultures, Amani et al. (2012) reported optimum conditions for specific VFA under a HRT of 22 hours and a population ratio of 2.5. Thereby, Amani et al. (2012) referred that optimum concentration for propionate, butyrate and acetate are 1.93 g.L^{-1}, 2.15 g.L^{-1} and 2.50 g.L^{-1}, respectively, in terms of a maximum of VFA removal and biogas production rate.

The concentration of VFA generally occurs with the AD of food wastes with high biodegradability leading to their fast degradation rate in acidogenesis. Thus, their concentration is an operational parameter of great interest to control in the AD process (Carrilho, 2012).

2.3.6.2 Ammonia and Ammonium

Ammonia (NH_3) is a sub-product of the anaerobic biological decomposition of nitrogenous (N) matter, mainly present in the form of protein and urea and primarily inhibits the CH_4-producing bacteria (Korres and Nizami, 2013; Deublein and Steinhauser, 2008).

Ammonia (NH_3) forms ammonium ions (NH_4^+) with an extent dependent on the pH value, whereas inhibition by NH_4^+ increases with lowering pH value at a constant concentration, thus the ratio NH_4^+:NH_3 found to be 99:1 at pH = 7 and 70:30 at pH = 9. Although NH_4^+ represents an ideal form of N for microbial cell growth, at pH levels below 7.4 NH_4^+ concentrations starts to be toxic for the mesophilic methanogenic bacteria over 3,000 mg.L^{-1}, whereas thermophilic

methanogenic bacteria are generally more sensitive since the inhibition occurs at 2,200 mg.L^{-1} of NH_4^+ concentration (Zupančič and Grilc, 2012).

Deublein and Steinhauser (2008) stated an inhibiting effect to NH_4^+-N concentrations above 1,500 mg.L^{-1} and toxicity levels above 30,000 mg.L^{-1}, whilst Kossman et al. (1997) stated an inhibition to occur at a NH_4^+-N concentration starting at nearly 1,700 mg.L^{-1}. Nevertheless, NH_4^+ inhibition of microorganisms varies with the type of substrate applied. A study of NH_4^+ inhibition in thermophilic digestion have shown an inhibiting concentration to be over 4,900 mg.L^{-1} while using non-fat waste milk as substrate (Korres and Nizami, 2013; Zupančič and Grilc, 2012). The inhibition of the AD by N in the form of NH_3 starts at a concentration over 80 mg.L^{-1} (Deublein and Steinhauser, 2008).

These compounds are two principal forms in the equilibrium of inorganic NH_3-N in aqueous solution and the predominating form depends mainly on the process, temperature and pH. The latter rising and the equilibrium steadily shifts towards to release free NH_3 as follows (Korres and Nizami, 2013; Azeitona, 2012) [Eq. 2.1]:

$$NH_3 \overset{\rightarrow}{\leftarrow} NH_4^+ + OH^- \qquad \text{(Eq. 2.1)}$$

Besides the pH-N factor, the balance of N species, such as NH_4^+ and the inhibiting NH_3, is temperature-dependent: as the temperature increases, the N reaction moves towards the NH_3 formation and inhibits AD (Deublein and Steinhauser, 2008).

As Korres and Nizami (2013) stated, the methanogens are the least tolerant and most likely to decrease their growth at certain NH_3 concentrations, the molecules of which diffuse into the cells causing proton imbalance and/or potassium deficiency. However, there is contradictory information in the literature about the sensitivity of the acetoclastic and hydrogenotrophic bacteria in relation to NH_3 accumulation. According to Kossman et al. (1997), using slurry as a substrate, after enough time has passed, the methanogens are capable of adapting to adverse concentrations of NH_4^+-N in the range of 5,000-7,000 mg.L^{-1} if the NH_3 level does not exceed 200-300 mg.L^{-1} of concentration.

Korres and Nizami (2013) stated that some literature about methanogens behaviour have shown the inhibitory effect as being stronger for the acetoclastic bacteria than for the hydrogenotrophic, the latter being less CH_4 productive, whilst other authors stated a relatively high resistance of acetoclastic bacteria to high levels of total NH_3-N in comparison to H_2-utilising bacteria.

Nasir et al. (2012) evaluated how different operational strategies could minimise the inhibition process of ammonia accumulation during the anaerobic digestion of Source-Sorted Organic Fraction of Municipal Solids Wastes (SS-OF-MSW) in a thermophilic CSTR. At an OLR of 11.4 kg VS.m^{-3}.d^{-1} and a 15 days HRT, a stable performance was observed when SS-OF-MSW was diluted with fresh water and when process water was re-circulated after ammonia stripping.

2.3.6.3 Sulphides

The effluents and wastes with a high content of sulphates (SO_4^{2-}) and sulphites (SO_3^{2-}) are reduced by sulphate-reducing bacteria into sulphides (S^{2-}). Thus, S^{2-} result in hydrogen sulphide (H_2S) in the form of gas, at pH levels under 6.5 (Carrilho, 2012; Azeitona, 2012). Whilst this gas is toxic to a wide group of bacteria up to concentration levels of 100 mg.L^{-1} and potentially corrosive, and can precipitate essential metals, such as Fe, Ni e Co, thus reducing their

availability in the medium (Alves, 1998; Chen et al., 2008). Additionally these microorganisms are capable of adapting to S^{2-} and surviving to concentrations of 600 mg $Na_2S.L^{-1}$ and up to 1,000 mg $H_2S.L^{-1}$ (Deublein and Steinhauser, 2008).

There are two inhibition stages of suphates-reduction: i) associated with the competition of the sulphate-reducing bacteria, related with organic and inorganic substrate also required by other bacteria involved in AD, causing a decrease in CH_4 production; ii) inhibition through S^{2-} toxicity over many groups of bacteria (Azeitona, 2012; Chen et al., 2008). The temperature also plays a key role in the toxicity of sulphides: the temperature arising is followed by higher toxicity of H_2S (Deublein and Steinhauser, 2008).

The sulphate-reducing bacteria are dominant due to their low energetic necessities and non symbiotic relation, thus growing faster in the presence of high SO_4^{2-} concentrations and decreasing the CH_4 production (Deublein and Steinhauser, 2008).

As H_2S can corrode engine components it is common to control its presence in the outlet flow from the digester. The contact of biogas with ferrous salts in a closed filter is a common method to achieve this and additionally a small amount of air can be injected into the digester headspace in order to facilitate biochemical H_2S oxidation (Bond and Templeton, 2011). As Monnet (2003) stated, other common methods for H_2S removal are: iron chloride dosing to digester slurry, activated carbon, water scrubbing and NaOH scrubbing, or commonly known as bioscrubber (Deublein and Steinhauser, 2008).

2.3.6.4 Heavy Metals

Although heavy metals as trace elements have stimulating effects on AD in low concentrations, higher concentrations can have toxic effects (Zupančič and Grilc, 2012; Deublein and Steinhauser, 2008). In particular lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn), nickel (Ni) and chromium (Cr) can cause disturbances in the AD process. The content of some heavy metals in the biomass rises during fermentation, but only slightly, others can be found in high concentrations in some substrates; for example in pig slurry, zinc is especially present, originating from pig fodder which contains zinc additive (Deublein and Steinhauser, 2008; Zupančič and Grilc, 2012).

2.4 Operational Parameters

2.4.1 Hydraulic and Solid Retention Time

The residence time of the liquid and solid fractions, that is, the Hydraulic Retention Time (HRT) and the Solid Retention Time (SRT), respectively, must be considered for the AD process (Crespo, 2013; CCE, 2000).

The Hydraulic Retention Time (HRT) is the average residence time (days) of the substrate flow in which a certain unit volume of the substrate passes through the reactor (Zupančič and Grilc, 2012; Carrilho, 2012; Crespo, 2013; Korres and Nizami, 2013; Monnet, 2003). Hence this parameter is of paramount importance for the reactor designing and operation of the anaerobic digester, involving higher or less investment costs underlying the reactor volume (Crespo, 2013; Carrilho, 2012). HRT is described by the division of the digester volume and the daily influent flow rate (Eq. 2.2):

$$\tau_H = \frac{V}{Q} \quad \text{(Eq.2.2)}$$

τ_H - Hydraulic retention time (d)
V - Reactor volume (m^3)
Q - Flow rate ($m^3.d^{-1}$)

The effective retention time may vary largely for each substrate constituent, depending, for example, on the vessel geometry and the mixing system (Kossman et al., 1997). For mesophilic digesters, the usual values for HRT are between 20-40 days, or 15-30 days according to Monnet (2003), whereas in thermophilic digesters the HRT values are lower in order to achieve an equal treatment efficiency of around 10-20 days (Zupančič and Grilc, 2012), or about 12-14 days, according to Monnet (2003). The design of the reactor is also an important factor for HRT efficiency; vertical digesters require a slightly higher HRT than horizontal digesters (Korres and Nizami, 2013).

SRT occurs within the digester capacity, through physical and mechanical means, to retain the biomass obtained through the process by longer periods than those observed for hydraulic flow, preventing the inset of dead zones or of preferential pathways in the digester (Crespo, 2013). Thus, SRT expression is as follows (Eq. 2.3):

$$\tau_S = \frac{V \times S_{vs}}{S_0} \quad \text{(Eq.2.3)}$$

τ_S- Solids retention time (d)
V - Reactor volume (m^3)
S_{vs} - Concentration of volatile solids inside the reactor (kg $VS.m^{-3}$)
S_0 - Loading rate of volatile solids (kg $VS.d^{-1}$)

Therefore, the relation HRT < SRT is desirable and shall, when possible, be evaluated for the AD effluent liquid systems. When the substrates present high concentration of solids, the HRT = SRT condition is practically always met, since there is no water flow (Crespo, 2013). Pre-treatments of the substrates are desirable to increase the specific surface of material for the cells involved in the AD system. However, by increasing the specific surface of the organic matter the main result will be an increase of the biochemical processes in the AD. Thus the increase of the specific surface for the cells shall be disregarded for substrates already met in fine particles, which start decomposing after a short time (Deublein and Steinhauser, 2008).

2.4.2 Organic Loading Rate

The Organic Loading Rate (OLR) determines the feasible amount of VS to be added into the digester, i.e. the quantity of biomass fed per unit volume of the digester per unit time (Korres and Nizami, 2013, 2013), generally expressed by Total Solids (TS), Volatile Solids (VS), or Chemical Oxygen Demand (COD) (Carrilho, 2012). The OLR is described by the following expression (Eq. 2.4):

$$L = \frac{Q \times S}{V} \quad \text{(Eq. 2.4)}$$

L - Organic loading rate ($kg.m^{-3}.d^{-1}$)
Q - Influent flow rate ($m^3.d^{-1}$)
S - Concentration of organic matter ($kg.m^{-3}$)
V – Volume of the digester (m^3)

Thus, OLR is a measure of the biological conversion capacity of the AD process of paramount importance for a suitable start-up of the feeding system. When OLR deviates from standard

conditions, there is a decrease in the degradation of the organic matter, and therefore low biogas production due to the concentration of inhibiting components (Monnet, 2003). However, OLR is a particularly important operational parameter for continuous systems, in order to prevent an overloading failure of the AD system, as occurred in many plants (Monnet, 2003). For the stability of the AD system, the specific OLR of VS should be less than 9 kg $VS.m^{-3}.d^{-1}$ (Mata-Alvarez et al., 2000; Carrilho, 2012).
Generally, OLR is expressed along with COD (kg $COD.m^{-3}.d^{-1}$) or VS per cubic meter (kg $VS.m^{-3}.d^{-1}$), of reactor, as applied in the current study and usually linked with retention time for any particular feedstock and anaerobic digester volume (Monnet, 2003).

2.4.3 Solids Content

One of the main interests in the AD of organic wastes lies in the opportunity given to fully digest the substrates with a high solid content. Thus, the AD allows the full consumption of all the residual solid organic matter available, greatly increasing its energy recovery (Azeitona, 2012; Crespo, 2013).

Additionally, the fact that the AD can operate with a low water content allows the reduction of the digester volume, and therefore the land use and the investment costs involved. However, the decrease of water use in AD can lead to its imbalance and hence its inhibition, as the biochemical reactions depends on water for their growth (Crespo, 2013).

A characterisation of the organic wastes in terms of their solids and moisture content is necessary in order to assure the balance of all the anaerobic processes in the digester. The digester has to operate with not more than 15% solids content, and 85% moisture content (or above), these value variations depending primarily on the type of waste, its seasonality and the collection period. If the moisture content of the waste is below 85%, a prior adjustment must be applied to the biological process (Crespo, 2013). It has been reported that the highest CH_4 production rates occur at 60–80% of moisture (Khalid et al., 2011).

2.4.4 Agitation

The mixing of the reactor is an important operational parameter to improve and increase the contact between the microorganisms and the substrate, thus contributing to reduce HRT and to improve the performance of the reactions in the digester (Carrilho, 2012; Monte, 2010; Crespo, 2013). The agitation provides the homogeneity of the mixture in the reactor as well as the temperature, and prevents the formation of scum and inactive zones (Azeitona, 2012; CCE, 2000).

A smooth agitation can result in the homogeneous concentration of nutrients and metabolism products for each individual microorganism. However in order to destroy a layer of H_2 around the microorganism, a significant agitation must be employed (Deublein and Steinhauser, 2008), although in this case it is also possible to destroy microbial cells and to reduce the oxidation rate of VFA, causing instability in the whole process (Azeitona, 2012).

The usual means to promote agitation of the fluid inside the digester are the recirculation and injection of part of the biogas into the digester, the mechanical agitation which implies higher

costs, the injection of the influent and the removal of effluent in the digester, or a combination all the above (Azeitona, 2012; Carrilho, 2012).

2.5 Pre-treatments of Substrates

There are several reasons to pre-treat substrates and co-substrates before the AD in the reactor (Deublein and Steinhauser, 2008):

i. The concentration of TS within the substrate is too high. For wet degradation a concentration of 10-12% TS is ideal, where the fraction of organic TS should be just as high.
ii. The substrate must be sanitized due to legal restrictions
iii. Fibrous materials, like green cuttings and straw must be comminuted in order to avoid process disturbances in the bioreactor.

Generally, in order to prepare the substrate before processing in the digester, the front-end treatment can be (Deublein and Steinhauser, 2008): adjustment of the water content; removal of the disturbing material; grinding, for example, to a diameter of less than 2 mm as in the present study; hygienisation, for 1 hour at 70 ºC; disintegration, to quicken the substrate biodegradability, thus increasing the yields of biogas production and efficiency of the AD process. This final treatment referred is the main focus of this study.

A large number of potentially suitable pre-treatment methods have been developed to solve problems, such as variation in the biological CH_4 production potential for various feedstock, despite the cost increase in the overall economics of 'second generation' digestion processes. Thus, a pre-treatment must follow a set of operations and procedures that provides optimum cost-effective parameters accompanied with minimum energy consumption (Korres and Nizami, 2013). Thereby is possible to reduce costs with operational parameters such as HRT. In the case of sludge, the pre-treatment enhances its digestion and the rate and quantity of biogas generated, reducing the retention time required between 15-25 days to approximately 7 days (Subramani and Ponkumar, 2012; Ferrer et al., 2008).

Pre-treatments are of particular importance in the case of biomass, the components of which vary in its accessibility for AD (Korres and Nizami, 2013), such as, for example, lignocellulose- a barely biodegradable substrate (Bruni, 2010). Table 2.3 summarises most of the pre-treatments found in literature.

Table 2.3 - Several disintegration methods by type of pre-treatment (Deublein and Steinhauser, 2008; Zhang, 2010; Borgström, 2011; Tyagi and Lo, 2011).

Thermal	**Mechanical**	**Chemical**	**Biological**
Pyrolysis	High pressure homogenizer	Acid or base treatment	Enzymatic lysis
Thermal cell disruption	Grinding, agitator ball mill	Peroxidation	Aerobic process
Baffle jet equipment	Chopping, cutting, mincing	Ozone-oxidation	
Lyzing centrifuge	Ultrasonic (also referred as an acoustic pre-treatment)	Detergents, salts	
Microwave irradiation	Extrusion		

Lignocellulose, present in wood and straw, is a complex and rigid matrix of plant cells resistant to enzymatic attack because of the tight association between lignin, cellulose and hemicelluloses (Bruni et al., 2010). These are therefore not degradable in AD processes without special pretreatment, such as thermal and/or chemical disintegration (Deublein and Steinhauser, 2008; Borgström, 2011; Bruni et al., 2010; Uellendahl et al., 2013).

Currently, straw substrate is often burned in fields without any energy use, instead of subjected to AD. The AD of straw, under thermophilic conditions, provided 60-65% of CH_4 content in the biogas, according to Deublein and Steinhauser (2008). In the future, energy-efficient AD of straw is seen as a suitable idea for developing countries instead of burning and releasing harmful gases, as it implies a substantial contribution to the power supply if fermented in a biogas plant with the use of pre-treatment to quicken its biodegradation (Deublein and Steinhauser, 2008).

Borgström (2011) tested different pre-treatment methods applied to many types of non-easily biodegradable substrates, such as straw, chicken feathers, maize silage and manure. The same author tested four pre-treatments such as extrusion, steam explosion, lime treatment and dewatering, to study if any of these could be an economically beneficial alternative for a reference plant and how the substrates used were affected by pre-treatment.

The low biogas yield and slow digestion process of straw is due to its high content of hemicelluloses (30-40%), cellulose (20-30%) and lignin (10-20%) (Borgström, 2011). Therefore, it is necessary pre-treatments to quicken their biodegradability and the AD rate, either by thermal, chemical, mechanical and biological pre-treatment, or the combination of any of these methods as in Sötemann et al.'s (2004) study.

Other methods of organic matter disintegration are applied, such as physical and acoustic, including various methods, such as freezing/thawing and extrusion (Korres and Nizami, 2013; Deublein and Steinhauser, 2008) and ultrasonic (Ferrer et al., 2008; Castrillón et al., 2011). Steam pre-treatment is often severally studied and referred as a method in combination with thermal and mechanical pre-treatment (Azeitona, 2012; Carapinha, 2012; Bruni et al., 2010; Borgström, 2011).

Generally, the efficiency of the pre-treatments applied in several studies is evaluated through the analysis of many chemical parameters, such as the increase of soluble COD (CODs), the raise in VS concentrations and even the increase in biogas production (Azeitona, 2012).

2.5.1 Mechanical Pre-treatments

Mechanical pre-treatments play an important role in size reduction to increase the accessible surface area of the organic matter. These facilitate the solubilisation of the organic compounds due to the reduction of the particulate size in the liquid phase (Mata-Alvarez et al., 2000; Bruni, 2010; Zhang, 2010).

Mata-Alvarez et al. (2000) reported two effects in their study: to a feedstock with high fibre content and low biodegradability, their comminution improves biogas production, and the size reduction can lead to an increased of AD rate. The comminution method can be by grinding, also known as milling, cutting, mincing or other similar processes.

The methods most commonly used in mechanical pre-treatments are cutting, ultrasonication, extrusion, grinding of various types and high pressure homogenization (Borgström, 2011;

Appels et al., 2008; Zhang, 2010; Deublein and Steinhauser, 2008; Meena et al., 2011). Although Deublein and Steinhauser (2008) precisely distinguished the ultrasonic method as part of acoustic pre-treatment, this is aggregated and referred in this section.

In an extensive study, Borgström (2011) tested many types of mechanical pre-treatments combined with temperature and high pressure applied to barely biodegradable substrates. Whilst the steam explosion of chicken waste feathers at up to 240 ºC, combined with a high pressure of up to 33.5 bar, improved the availability of the substrate, the extrusion technique of mechanically crushing the straw during a steady pressure rise and a temperature up to a maximum of 2 bar and 160-180 °C, increased the CH_4 yield of straw and the AD rate. Finally, based in scenarios and calculations, the extrusion proved to be economically profitable, whilst steam explosion proved the contrary.

Studies of hydrotreatments with use of steam applied to potato-peel waste have been reported to improve their yields of CH_4 production, as with Azeitona (2012) and Carapinha (2012). Their studies achieved optimum improvements by autoclaving the ground waste at 122 ºC for 35 min, in thermophilic conditions, and at 122 ºC for 55 min, in mesophilic conditions, respectively.

Although some researchers tested ultrasound pre-treatments (Castrillón et al., 2012) and proved it to be effective for several substrates, even with cell disintegrations of 100%, its main drawbacks are its power consumption and cost. The ultrasound probes also require replacement every 1.5-2 years, thus reflecting the unsustainability of this method.

In general, many authors recommend mechanical pre-treatments due to low operational cost and improvements in the AD of the feedstock (Bruni, 2010; Mata-Alvarez et al., 2000). In the present study, the organic matter was first submitted to a mechanical pre-treatment of comminution in a blade mill reducing the particle size to less than 2 mm.

2.5.2 Thermal Pre-treatments

Although reported by several authors as profitable for CH_4 production yields and AD efficiency processes, the successful disintegration and biogas yields obtained through thermal pre-treatments are variable on the feedstock applied. Therefore, contradictory information was found in the literature regarding temperature range for thermal pre-treatment, thus varying for the substrate.

According to Zhang (2010), thermal pre-treatment has been studied using a wide range of temperatures ranging from 60-270 ºC, although the optimum temperature is in the range of 160-180 ºC, at treatment times of 30 min to 60 min and an associated pressure to these temperatures in the range of 600-2,500 kPa. Additionally, as the author reported, although the carbohydrates and the lipids of the sludge are easily degradable, thermal pre-treatment can improve the proteins accessibility for AD, this latter protected from the enzymatic hydrolysis by the cell wall. As a result of the heat applied during thermal treatment, the chemical bonds of the cell wall and membrane are destroyed.

According to Deublein and Steinhauser (2008) and Zupančič and Grilc (2012), the yield of biogas is 30% higher if the feedstock is thermally disintegrated at an optimum temperature range of 135-220 ºC before it enters the digester; at pressures above 10 bar, and with a residence time of 2:37 h. Nonetheless, according to Borgström (2011), the steam explosion of chicken waste feathers up to 240 ºC combining with high pressure up to 33.5 bar improved the

availability of the substrate. Skiadas et al. (2005) stated that it has been proved that the thermal pre-treatment of sludge at a temperature range of 100-275 ºC significantly increases the disintegration and solubilisation of sludge solids and thus improves the AD.

Ferrer et al. (2008), Bordeleau and Droste (2011), Monte (2010) and Stuckey and McCarty (1984) stated a temperature range of 60-270 ºC for thermal pre-treatment of the substrate, whilst the optimum temperatures are between 60-180 ºC, since refractory compounds are formed over 200 ºC. As Liu et al. (2012) stated, many studies reported optimal temperatures for thermal pre-treatment of waste activated sludge (WAS) range between 160 ºC to 180 ºC.

In general, thermal pre-treatment of waste activated sludge can considerably increase CH_4 production for mesophilic AD and to a lesser extent for thermophilic AD (Zhang, 2010). Additionally, as Deublein and Steinhauser (2008) and Zupančič and Grilc (2012) reported, this pre-treatment only runs economically with heat regeneration and is suitable only for cellular material, like, for example, raw sewage sludge.

Several studies have been carried out by many authors with the objective of improving the bio-CH_4 production yields (Nielsen et al., 2004; Ferrer et al., 2008; Liu et al., 2012; Azeitona, 2012; Carapinha, 2012; Santos, 2013; Skiadas et al., 2005; Phothilangka et al., 2008; Borgström, 2011).

Nielsen et al. (2004) suggest thermal pre-treatment at low temperatures (< 100 ºC) as a bio-digestion pre-step to improve the biological activity of some hydrolytic bacteria. In the present study, although combined with chemical pre-treatment, thermal pre-treatment was applied at 50 ± 1 ºC during 30 min to the potato peel waste.

As Santos (2013) reported, thermal pre-treatments applied to potato-peel waste, in mesophilic and thermophilic conditions, improved their yields of CH_4 production, thus achieving optimum improvements due to the substrate being subjected to a steady thermostatic water bath at 70 ºC during 3 hours.

2.5.3 Chemical Pre-treatments

Chemical pre-treatments of various methods, including the application of acids, alkalis, or oxizing agents, promote the organic matter disintegration and reduction to soluble forms (Korres and Nizami, 2013) and, therefore, increase the AD rating processes and the yield of CH_4 production. Table 2.4 shows a set up of improvements in the biogas yields with some chemical pre-treatments of the substrates before AD.

Acids and alkaline hydrolysis (Tyagi and Lo, 2011; Zhang, 2010) are usually followed by thermo-chemical procedure (Tyagi and Lo, 2011), whilst oxidation (Hellström, 2009) is not. However, acid and/or alkaline solutions can be used as catalysts for other disintegration methods, such as steam pre-treatment (Bruni, 2010), with noticeable biodegradation improvements. For example, Bruni (2010) studied H_2SO_4 as the catalyst for steam treatment, at 155 ºC and the CH_4 yield was increased by 67%.

However, acid-alkaline pre-treatments are usually achieved by adding costly chemicals and have low energy efficiency, not only for the pre-treatment itself but also for the neutralisation after the pre-treatment (Zupančič and Grilc, 2012; Appels et al., 2008). Therefore, the biodegradation of the feedstock must be highly achieved and optimised to be profitable.

Table 2.4 - Biogas yield and efficiency as a function of chemical pre-treatments of different substrates.

Pre-treatment	Reagent	Substrate	Biogas yield	[1] Biogas efficiency	Other parameters	Literature
Alkaline	NaOH	Sludge	349 $cm^3.g^{-1}$ $CODt_{removed}$.	30-34%	41% of $VS_{removed}$	Lin et al. (1999)
		WAS	n.d	n.d.	53.2% VS rem. eff.	Jang and Ahn (2013)
		Sludge	381 $cm^3.g^{-1}$ VS	33.0%	7.4 $cm^3.g^{-1}$ $VS_{removed}$; 34.5% d.r	Li et al. (2013)
		Sludge	650 $cm^3.g^{-1}$ VSS	1.54%	13,659 cm^3 $COD_t.g^{-1}$; 38.3% d.r.	Li et al. (2012)
		WAS	19-45% VS rem.;	106-287% biogas; 133% VS;	39-47% CODt rem.; 123% CODt yield	Lin et al. (1997)
		Dairy WAS	862 $cm^3.g^{-1}$ VS add.	51.0%	25% VS rem.	Rani et al. (2012)
		Corn stover	420.6 $cm^3.g^{-1}$ VS; 233.0 cm^3 $CH_4.g^{-1}$ VS	20.2%	21.1 $cm^3.g^{-1}$ VS; 63.6% VS rem.	Zheng et al. (2010)
		Corn stover	372.4 $cm^3.g^{-1}$ VS	37.0%	n.a.	Zhu et al. (2010)
	$Ca(OH)_2$	Manure biofibers	234-239 cm^3 $CH_4.g^{-1}$ VS	66% CH_4	n.a.	Bruni et al. (2010)
		Pig manure	409.47 $cm^3.g^{-1}$ VS	78%; 60% CH_4	n.a.	Rafique et al. (2010)
Acid	H_2SO_4	Potato-starch	470 cm^3 $CH_4.g^{-1}$ VS	81%	n.a.	Fang et al. (2011)
	H_3PO_4	Sludge	n.r.	n.r.	80.0% CODr rem.eff.	Boulenger et al. (1999)
Oxidant	PAA	WAS	n.d.	21%	25 g $PAA.kg^{-1}$ DS	Appels et al. (2011)

[1] Biogas efficiency: increment of biogas production over the control assay.
WAS: waste activated sludge; rem.: removed/removal; eff.: efficiency; n.d.: not determined; n.a.: not appropriate; add.: added; d.r.: degradation rate.

Furthermore, chemical neutralisation (Monou et al., 2008) leads to increased salt concentrations (Charles et al., 2013), although increasing the saponification of the cell components and also other chemicals. These chemicals can be NH_3, then assuming the form of ammonium hydroxide (NH_4OH) in solution (Mata-Alvarez et al., 2000), and quicklime (CaO), often used to increase the pH (Deublein and Steinhauser, 2008) instead of NaOH. Alternatively, the neutralisation of pH value can be decreased using sulphuric (H_2SO_4) or hydrochloric acid (HCl) (Deublein and Steinhauser, 2008), or by flushing with CO_2 (Borgström, 2011).

Typically, the acid or alkaline hydrolysis is combined with heat, pressure or both (Zupančič and Grilc, 2012; Mata-Alvarez et al., 2000). In the present study, the chemical pre-treatment of the potato peel waste with acid (H_2SO_4) and alkali (NaOH) solutions is combined with thermal pre-treatment in a steady water bath at 50 ºC during 30 min, in addition to the mechanical pre-treatment previously referred.

Generally, the acidification processes uses reagents, such as H_2SO_4 (Charles et al., 2013; Fang et al., 2011) or HCl (Charles et al., 2013), among others as with H_3PO_4 (Boulenger et al., 1999), although also associated as catalysts combined with other methods. The use of H_2SO_4 releases NH_4 to the reactor in concentrations worrying for an inhibition to may occur, hence must be controlled as Fang et al. (2008) reported, besides of the promising results of 81% of biogas achieved.

The alkaline disintegration mainly uses NaOH (Charles et al., 2013; Lin et al., 1999; Jang and Ahn, 2013; Li et al., 2013; Li et al., 2012; Lin et al., 1997; Rani et al., 2012; Zheng et al., 2010), among the most investigated and promising pre-treatment method to improve the biodegradability and biogas production (Zheng et al., 2010; Bruni, 2010; Meena et al., 2011). On the other hand, soda, potassium hydroxide (KOH) or CaO (Bruni et al., 2010; Rafique et al., 2010) are the alkaline reagents amongst the least applied (Zupančič and Grilc, 2012). Nearly 55% of the TS can be decomposed, especially when using NaOH (Deublein and Steinhauser, 2008). Thermo-chemical pre-treatment of chicken manure with NaOH and H_2SO_4 at 100 °C increased both the methane yield and biodegradability (Meena et al., 2011).

Although alkaline hydrolysis with NaOH has been successfully applied to transform scarcely biodegradable substrates into readily biodegradable, such as straw or hardwood, treatment with NaOH is of great concern due to the lack of quality in the final digestate often used as a fertiliser, since sodium ions are responsible for soil erosion (Bruni, 2010).

According to Zupančič and Grilc (2012), the most real threat in case of AD inhibition is with sodium, that is, when NaOH is excessively used in neutralization of the initial or final mixture, thus reporting that sodium concentrations of 3,000 $mg.L^{-1}$ can cause inhibition. Nonetheless the anaerobic digester can operate up to concentrations as high as 16,000 $mg.L^{-1}$ of sodium, as close to saline concentration of seawater (Zupančič and Grilc, 2012).

Thereby, pre-treatment with CaO represents to be a low-cost and efficient alternative to NaOH. In the studies of Bruni et al. (2010) and Bruni (2010), the CH_4 yield enhancement was up to 66% by using CaO to raise the AD of biofibers from digested manure. Borgström (2011) tested lime pre-treatment on chicken waste feathers with higher CH_4 yields after 5 days and a production rate 5 times higher in the next 34 days. The slaked lime [$Ca(OH)_2$] added was decomposed in CaO and water, at 512 °C, pre-hydrolysing the organic matter.

Finally, the most frequent research about oxidative desintegration is ozonation and peroxidation methods (Zhang, 2010; Tyagi and Lo, 2011). The ozonation is the addition of ozone dosages to partial oxidation and hydrolysis of the substrate, thus transforming a wide range of barely degradable compounds into significantly biodegradable ones (Tyagi and Lo, 2011). Generally, is a method described by a sequential decomposition of reactions, such as, floc disintegration, solubilisation and the subsequent oxidation of the released organics into carbon dioxide, and the latter known by mineralization (Tyagi and Lo, 2011).

The peroxidation method uses the activation of hydrogen peroxide (H_2O_2) by iron salts (Fe^{2+}) to disintegrate the extracellular polymeric substances and break the cell walls, thus releasing intracellular material and increasing the concentration of soluble COD (Tyagi and Lo, 2011). Other oxidants, such as peracetic acid (PAA), have been used to enhance the AD of waste activated sludge (WAS). Appels et al. (2011) obtained a maximum increase in biogas production of 21% with PAA pre-treatment, although the inhibitions of high VFA concentrations occurred.

Besides, Charles et al. (2013) studied the effect of electrochemical pre-treatment by electrolysis, decreasing or raising the pH value and controlling the most favourable pH medium to enhance the hydrolysis rate. In this study, a 12 V two-chamber electrolysis using an anion exchange membrane dropped sludge pH from 6.9 to 2.5 in the anode chamber and increased to 10.1 in the cathode chamber within 15 h, without chemical addition. As a result, the WAS was solubilised by 31.1% in the anode chamber and 34.0% in the cathode chamber. Furthermore, the authors compared the results with the conventional acid (HCl) and alkaline (NaOH) pre-treatment. Although these methods, either chemical or electrochemical, had only little effect on the final CH_4 production, the electrolysis of both the anode and cathode chambers improved the methane production.

2.5.4 Biological and Enzymatic Pre-treatments

Biological pre-treatment are mainly based on either microbial or enzymatic activity used for pre-degradation of barely biodegradable substrates (e.g. lignocelluloses) as a cost-effective method, although it requires a specific environment to operate (Korres and Nizami, 2013).

The enzymatic hydrolysis of holocellulose requires the action of different groups of cellulases and hemicellulases or oxidative enzymes (Bruni, 2010), whilst pre-hydrolysis of specific microbial inoculum is a suitable method for pre-degradation of the substrate in optimum conditions for their operation, such as, the aerobic pre-treatment of organic waste (see: Subramani and Ponkumar, 2012; Bruni et al., 2010). Both enzymatic and microbial methods can be used for the organic matter lysis, as in the study of Kassuwi et al. (2012), in which submission of CBR-11 bacterial culture and lipase enzyme was used to pre-treat fish solid waste in co-digestion of the vegetable fraction, such as potato waste. According to Kassuwi et al. (2012), the results demonstrated that optimal mixture of CBR-11 pre-treated FSW with potato waste enhanced methane yield by 135% compared to control assay.

2.6 Anaerobic Digester - UASB

The anaerobic digesters, or bioreactor, are sealed manufactured displacements of various forms and designs where the specific inocula carry out the AD of the substrate in artificial conditions. All the digesters have an inlet for the substrate and an outlet for the biogas and the digestate (Carrilho, 2012).

The Upflow Anaerobic Sludge Blanket (UASB) is, among the Expanded Granular Sludge-bed (EGSB) and the Internal Circuit (IC), the most used industrial full-scale plants, particularly of industrial waste water. (Deublein and Steinhauser, 2008). This reactor is suitable for treating liquid effluent with high levels of organic acids at high organic rates in multi-stage systems (Monnet, 2003), such as wastewater from the potato processing industries (Deublein and Steinhauser, 2008).

Fang et al. (2011) carried out a comparative study between UASB and EGSB, while treating potato-juice for biogas production. The UASB reactor (mesophilic) with a 1.2 L working volume and a H:D ratio of 4:4 was tested at HRT from 10 to 4 days. The UASB reactor *could tolerate higher VFA concentration than the EGSB, however it had lower methane yield than EGSB under stable operation*, according to Fang et al. (2011).

2.7 Biogas

The standard composition of biogas varies in a range of 50-70% of CH_4 and 30-50% of CO_2, as well as 1-5% of traces of other gases, including O_2, H_2S, CO and H_2 (Bond and Templeton, 2011; Appels et al., 2011; Borgström, 2011; Kossman et al., 1997).

Biogas is only flammable when it has a content of CH_4 of above 45% (Deublein and Steinhauser, 2008) and it possesses a calorific value of approximately 21.48 $MJ.Nm^{-3}$, 41% less efficient than natural gas, which has a calorific value of 36.14 $MJ.Nm^{-3}$ (Monnet, 2003). Nevertheless, Kossman et al. (1997) stated a calorific value of biogas of 6 $kWh.m^{-3}$, corresponding to nearly half a litre of diesel oil. Hence, the calorific value of the biogas grows with the optimisation of the AD processes which increase the concentration of bio-CH_4 in the biogas.

Biogas is a secondary renewable energy with a final energy unit (gas with calorific power), and an energy carrier for different final processes of energy production (heating and electricity). Murphy et al. (2004) estimated an asset value of 1 m^3 of biogas producing Combined Heat and Power (CHP). Thus, 1 m^3 of biogas each for electricity and thermal energy (heating), respectively, with a yield of 35% and 40%, respectively, would generate each 2.04 kWh and 2.33 kWh and 0.143 $€.Nm^{-3}$ and 0.047 $€.Nm^{-3}$ respectively, with a final profit of 0.19 $€.Nm^{-3}$ in a CHP plant. In this study, transport proved to be the best option to apply biogas for energy consumption, based on taxes and other causes.

Biogas is a clean fuel, as its burns is environmentally friendly without releasing soot or particles to the air and not interfering in the carbon chain (Arthur et al., 2011). Nevertheless, CH_4 in the biogas is a gas of global warming potential over 300 times more than CO_2 (Bond and Templeton, 2011) retained for energy purposes instead of releasing into the air in great amounts, like in the case of landfills. Furthermore, CH_4 and H_2, potential biogas fuels, are reported as cleaner than fossil fuel, thus reducing fossil fuel dependency by an alternative renewable energy source in exchange (Khalid et al., 2011).

Several economic analyses points to the sustainability of biogas (Akbulut, 2012; Börjesson and Ahlgren, 2012; Ericsson et al., 2009; Gebrezgabher et al., 2010; Murphy et al., 2004; Uellendahl et al., 2013; Arthur et al., 2011), not only in energetic-environmental terms, but also in economic terms: for example, bio-CH_4 and/or bio-H_2 production to sell as final or carrier energy or to be directly used by the production site, and the sale of the final effluent of the biogas plant for agriculture (as a good and cheap organic fertiliser). Additionally, the production of biogas can have indirect economic advantages, such as economic incomes and increased employment opportunities in agricultural areas.

Finally, advantages of using biogas technology, in an optimum cost-efficient operating AD system, can positively impact the environment and the society, as summarised by Bond and Templeton (2011):

i. Improved sanitation: reduced pathogens; reduced disease transmission;

ii. Low cost energy source: cooking, lighting;

iii. Low cost fertiliser: improved crop yields;

iv. Improved living conditions of existing plants;

v. Improved air quality;

vi. Reduced greenhouse gas emissions;

vii. Reduced nitrous oxide emissions;

viii. Less demand for fossil fuels;

The main possible disadvantages of biogas production are associated with the optimisation of the AD processes and operations [e.g., high HRT increase operational costs and the inefficiency of biogas production and the quality of the biogas, depending on the substrate]. The high investment costs are possible disadvantages, although in some cases these are recovered relatively fast. For example, in a recent study, Akbulut (2012) found that the implantation of a biogas plant with dairy cows and stall as feedstock for biogas production, had shown a payback time under 3 or 4 years. Although, according to Carrilho (2012), a decentralised biogas plant proved to be unprofitable in a long term of 20 years, some important economical aspects were not taken into account in that study, such as: the payback indexed in students fees and state economical support, the projection was carried out in terms of electric tariffs instead of a green energy tariff; the initial investment cost with the biogas facility was set up piece by piece instead on the whole set pieces to one construction company which would be cheaper.

Other disadvantages can be the low suitability of the implantation of biogas plants in hot or cold regions and their confined operational lifetime, usually of no more than 20 years (Bond and Templeton, 2011). The hygienisation of sludge from mesophilic AD is sometimes less reliable than thermophilic processes (Bond and Templeton, 2011).

2.7.1 International and National Context

To estimate the feasibility of biogas production in a national context, a simplified analysis of the trends in the national and European Union (EU) market of renewable and non-renewable energy sources (RES and non-RES) in the last years can be undertaken, as well as the potential power installed for biogas generation per sector. It must be considered that the performance of the RES sectors in the EU and/or in national ground is variable with the goals and trend opportunities set out by each country in their National Renewable Energy Action Plans (NREAP) (EurObserv'ER, 2013), associated with the European countries commitment to the mitigation of GHG. In a final approach, the feasibility or the potential of biogas energy use is also analysed in the framework of natural gas and transportation sector.

According to data from EurObserv'ER (2013), in 2012, the Primary Production of Biogas (PPB) in Portugal from biogas plants [i.e. decentralized or centralized digesters, multiproduct plants, solid waste methanization plants] is a small amount [0.7 ktoe], while landfills assume the major source of PPB with an amount of 54.0 ktoe and Wastewater Treatment Plants (WWTP), or sewage sludge, as referred to in the EurObserv'ER reports, is of 1.7 ktoe (Table 2.5) with a steady presence in the market share, as will be mentioned later.

Table 2.5 - PPB (ktoe) in the EU in 2012 (EurObserv'ER, 2013).

Country	Biogas Plants [ktoe]	WWTP [ktoe]	Landfills [ktoe]	Total [ktoe]
Germany	5,920.3	372.1	123.8	6,416.2
Portugal	0.7	1.7	54.0	56.4
Total EU	7,988.6	1,185.1	2,841.8	12,015.5

These values must be reversed, as the landfilling of organic matter for PPB is not adequate when it is considered its effects, such as air and soil pollution with CH_4 and soil leaching, respectively. Furthermore, it do not follow the Directive 1999/31/EC on the landfill of waste.

However the reason for the phenomenon of the decreasing and lack of biogas plants in Portugal is unknown by the present author, but it is possibly due to: low tariffs purchase of electricity produced through biogas; highly isolated and distant farming and livestock units in national territory; high initial investment cost in small and medium sized biogas units, compared to the low purchase tariff value of electric energy through biogas; non-existence of network distribution of biogas and vehicles that run on biogas; high initial investment cost with biogas treatment units, in case of opting for it injection in natural gas grid; very low public incentives and enforcement for biogas production.

Germany is the best example amongst the European countries of government support and involvement in the biogas sector, with successful policies and incentives in this specific bioenergy market. It is the country in the highest position of PPB in the EU, accounting 6,416.2 ktoe in 2012, more than a half of the total EU PPB.

In 2003, the biogas sector in Portugal showed a 1MW of potential power installed, reaching 20.0 MW in the overall market of 2009 (Carrilho, 2012). From 2008 to 2009, the PPB from biogas plants enjoyed a steady growth in the Portuguese sector, inducing to a dominant presence in the market sector. However, in 2010, a sudden fall of PPB from biogas plants can be observed (Figure 2.4), whilst the energetic recovery of biogas from landfills increased significantly and was followed by a scarce PPB in municipal WWTP. This trend must be reversed in the model of, for example, Germany, with a dynamic implementation of a feed-in tariff combining a number of premiums, such as the low fair of €0.02/kWh in 2009 (EurObserv'ER, 2010), thus the source of landfills not receiving any incentives, among other examples reported by EurObserv'ER (2010; 2011; 2013).

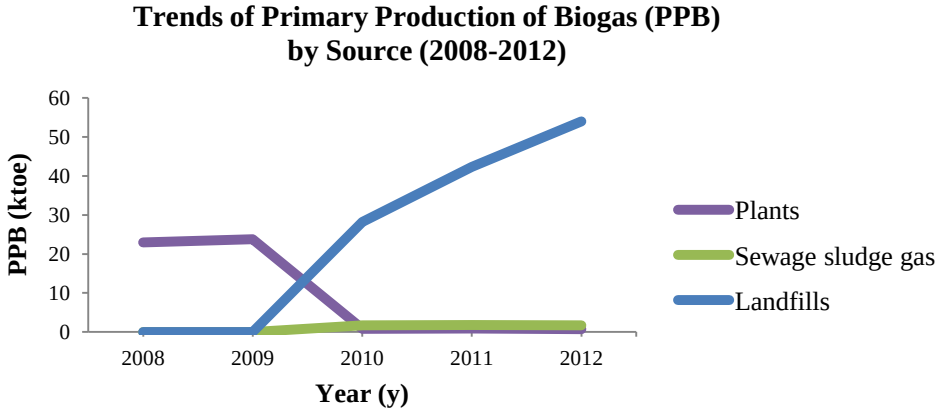

Figure 2.4 - Trend of PPB in Portugal by source in the sector (EurObserv'ER, 2010; EurObserv'ER, 2011; EurObserv'ER, 2013).

The main appplication of biogas energy in the EU is for electricity and heat generation, combined or otherwise (EurObserv'ER, 2013). Although the high Gross Electricity Production (GEP) from RES in the Portuguese market (37%), it does not come from the biogas sector. In the REN (2012) annual report of national GEP, 63% is stated as coming from non RES, whilst 37% of national energy comes from RES; 20% from wind, 11% from hydro and 6% from other renewable energies including biogas. However, in Portugal, 38.3 GWh and 164.3 GWh of GEP

is either exclusively for the generation of electricity or for Combined Heat and Power (CHP) plants, respectively (Table 2.6).

Table 2.6 - Gross Electricity Production (GEP) from biogas in Portugal and EU countries (EurObserv'ER, 2013).

Country	CHP Plants [GWh]	Electricity only plants [GWh]	Total electricity [GWh]	Total RES [1] [%]
Germany	21,332.0	5,917.0	27,239.0	24.0
Portugal	10.0	199.0	209.0	37.0 [2]
Total EU	30,002.1	16,250.9	46,253.0	23.4

[1]Total RES: Total Renewable Energy Sources for electricity production.
[2] EurObserv'ER (2013) reports a contradictory value of 35.6% in opposition with the 37.0% of REN (2012).

Contrary to 2011 (REN, 2012), in Portugal, 2012 has shown a 9% increase of GEP coming from non RES. This negative trend is explained by unfavourable hydrological conditions throughout the year, which meant that wind energy was in higher demand, and in fact reached its best achievement of GEP ever. Thus, two negative dependencies for the biogas sector to grow are noted in the market of non RES and RES. While the non RES have shown growth, the dependency in the RES is clearly of hydro and wind energies. However, there is a high potential for the growth of biogas power in the RES market because of its advantages of not depending on weather conditions (like in the case of hydraulic power), the currently scarce competition with wind and hydro power, and the relative lack of its implementation.

Despite the positive trend of increasing GEP in the biogas sector from 2008 to 2012 (Figure 2.5), this is accompanied by an improper depositing of organic wastes in landfills for PPB, instead of their management and submission into biogas plants or anaerobic digesters of any kind as previously observed.

Furthermore, Portugal is one of the countries with the highest hydro potential in the EU and great amounts of state investments and policies in the RES are veered towards a 54% reduction in hydro potential use to 33% by 2020, that is to say, turning the potential into real utilisation, and thus, increasing hydro use (INAG, 2014). This is laid out in the governmental commitment with the Portuguese National Renewable Energy Action Plan (PNREAP), in order to achieve the targets of the European Directive 2009/28/EC. Therefore there is a strategy towards major investment in hydro power and a lack diversification of bioenergy investment that affects, namely, the biogas energy sector.

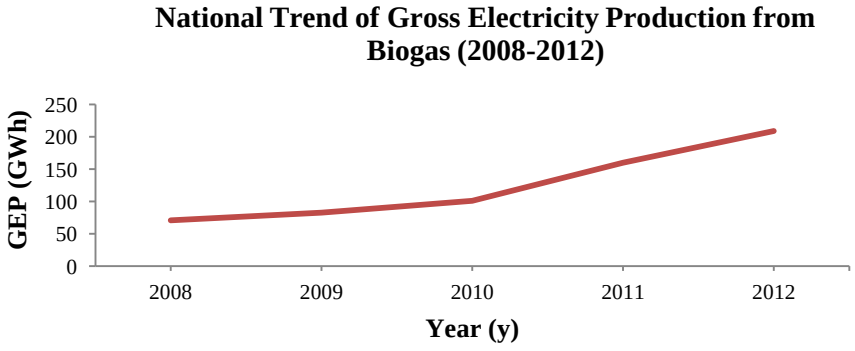

Figure 2.5 - Trend of Gross Electricity Production (GES) in the biogas sector (EurObserv'ER, 2010; EurObserv'ER, 2011; EurObserv'ER, 2013).

Although solar and wind energy are more efficient RES than biogas in generating electricity because of losses in biogas energy conversion, a well-guided biogas production has greater positive ecological impacts and is more environmentally friendly than hydro or wind sources, considering the advantages of nutrient recycling; the prevention of soil leaching in landfills; the incomes from the sale of enriched organic fertilisers; the harmless and clean sanitation through a biological treatment/management of organic wastes; and the final output of a green energy without nearby ecological disruptions related to its production or a relatively high land use.

Biogas also has a huge growth potential in the private, or decentralized, market. According to Carrilho (2012), in 2003, a study estimated the potential installed power share in the biomass sources for biogas production on national ground of 12.2% from the agro-food sector, followed by 23.6%, 30.9% and 33.3%, for agro-husbandry, Municipal Solids Wastes (MSW) and WWTP, respectively (Table 2.7).

Table 2.7 - Potential market share of energy production in the organic waste market from which biogas is obtained on national ground in 2003 (Carrilho, 2012).

Sector	Potential Power [MW]	Share [%]
Agro-husbandry	15	12.2
Agro-food	29	23.6
MSW	38	30.9
WWTP	41	33.3

Thus, in 2003, biomass supply for energy production was highest for WWTP, most likely due to the already incorporated facilities with relatively low investment and operational costs for their start-up, and the use of public money. However, to the best of the author's knowledge, it is believed that the potential of the diversification of the biogas sector into well-supported decentralised sources, such as agro-food or energy crops, as is currently the case in Germany, was not taken into account. It is clear that the private sector required, and continues to require, enforced governmental impulses and policies to help with the high investment and other initial costs implicated in the installation of biogas plants.

According to Ferreira et al. (2012), there is a huge biogas potential still to explore in Portuguese ground, only recognized in 2007, however the installed power in 2012 is only about 10% of the potential electrical power [229 MW]. This author stated "it is desirable to strengthen the national and regional biogas market".

Finally, the natural gas grid and transports are other potential beneficiaries of biogas energy and the most efficient ways of upgrading biogas into usable energy in the sector (Holm-Nielsen et al., 2009).

In 2012, the natural gas demand was of 50.2 TWh in natural gas networks, without any introduction of bio-CH_4 purified biogas, (REN, 2012; Moreira, 2011), despite the European measures established to encourage the introduction of bio-CH_4 in natural gas networks, as laid out in the European Directive 2003/55/EC (Moreira, 2011). It has, however, already been introduced in other EU countries, particularly in Sweden, the United Kingdom, the Netherlands, Austria and Germany (Moreira, 2011), and is the third recovery technique of biogas emerging in the EU, after electricity and heat (EurObserv'ER, 2013). From 1992 until the end of November 2013, Germany was also in the leading role for this technique with a bio-CH_4 injection of 80,390 Nm^3 into the natural gas network by 130 biogas plants among the total 152 biogas plants in Europe with this process (EurObserv'ER, 2013; Moreira, 2011).

The upgrade and utilisation of biogas as a vehicle fuel in transports is slowly emerging as a technique in the EU. A remarkable example of a country that upgrades biogas and uses it for vehicle fuel is Sweden, according to Holm-Nielsen et al. (2009). The advantage that lies in the use of bio-CH_4 in transports instead of its injection into the natural gas grip, is the direct use of it, whilst if the injection of the biogas produced in isolated facilities, such as in farming areas, requires long distances of transport to the natural gas grids.

Nevertheless, Moreira (2011) reported the first biogas plant with bio-CH_4 injection into the natural gas grid of Portugal to be installed in the Sermonde landfill site in Portugal in the 2011 International Gas Union Research Conference. The referred landfill is projected to handle the cleaning and purification of biogas in an Organic Waste Recovery Waste Plant (OWRP) serving an area of 384 km^2 and a population of 462,681 inhabitants. The OWRP has the capacity to treat 20,000 $t.y^{-1}$ of biomass from undifferentiated MSW by mesophilic digestion from 36-38 ºC with a HRT of 24 days. The biogas recovered from the AD of MSW is retained in a gasometer of 4,000 m^3 and cooled to 6 ºC. The plant is projected to produce 2,654,400 $m^3.y^{-1}$ of biogas recovered from 3,229 t of MSW, corresponding to a flow of 316 $m^{-3}.h^{-1}$.

However, the main constraints indicated by Moreira (2011) for this project - considered a Project of National Interest (PNI) - are: the lack of legislation to regulate and standardize the process, particularly with respect to the quality of the bio-CH_4 injection into the natural gas network; and the absence of a system of incentives and know-how for the taxes to apply.

2.8 Substrate

The main studied substrate to be seen in literature was the sludge of WWTP, probably due to the already incorporated municipal anaerobic facilities for their treatment, implying reduced investment and operational costs, and also due to the combination of public wastewater treatment and energy recovery of biowastes. However, Deublein and Steinhauser (2008) state the supply of biomass from the food industry for biogas generation as the biggest output of energy among the RES in the future.

Therefore, it is extremely important to consider the quality of the substrate used in the AD process, as, for example, lignocellulosic substrates are barely biodegradable, thus would slow down the process and increase the operational and maintenance costs. The HRT of the digester may change from polymer to polymer, as the hydrolysis of carbohydrates occurs in a few hours and of proteins and lipids within a few days (Dublein and Steinhauser, 2008).

The organic matter used as substrate typically possesses a high or a low content of polymers with higher or lower potential to boost the formation of CH_4 in the final biogas composition (Table 2.8).

Nonetheless, the high content of lipids present in the oil wastes from the industries of fried potato-processing are not easily biodegradable substrates, which is why the co-digestion with other substrates, such as potato peel rich in carbohydrates and easily biodegradable, should be suitable.

Table 2.8 - Potential CH_4 formation in biogas from different polymers, based on different wastewaters substrates (adapted from Franco et al., 2007).

Polymer	Composition	CH_4 [%]
Carbohydrate	$(C_6H_{10}O_5)_n$	50
Protein	$C_5H_7NO_2$	50
Lipid	$C_{57}H_{104}O_6$	> 75
Ethanol	C_2H_6O	75
Acetate	$C_2H_4O_2$	50
Propionate	$C_3H_6O_2$	58

2.8.1 Potato Peel

Potato is the third largest food crop in the world (Zhu et al., 2008). In Portugal, in the year of 2012, 445.6 Mt of potato were produced in Portugal out of a total of 54,456 Mt of the EU production (Eurostat, 2013).

The several studies on the feasibility for AD of by-products from potato processing (Linke, 2006; Fang et al., 2011; Parawira et al., 2004b; Kryvoruchko et al., 2009; Monou et al., 2008; Zhu et al., 2008; Azeitona, 2012; Carapinha, 2012; Santos, 2013) have shown the greater interest of the industry and agriculture sector for biogas recovery through the treatment of those wastes by AD.

According to Herout et al. (2011), the chemical composition of dry mass of plant biomass varies considerably depending on the plant species, soil and climatic conditions, the fertilisation, time and manner of harvest and means of conservation.

The composition of potato peel pulp is illustrated by Kryvoruchko et al. (2009) (Table 2.9). However there is no reference to carbohydrates in that composition by those authors. Therefore, additionally the composition of raw potato peel stated by Schieber and Saldaña (2009) is: 83.29 $g.100g^{-1}$ of water; 2.57 $g.100g^{-1}$ of protein; 0.1 $g.100g^{-1}$ of lipid; 1.61 $g.100g^{-1}$ of ash; 12.44 $g.100g^{-1}$ of carbohydrate; 2.5 $g.100g^{-1}$ of fibre (Azeitona, 2012). Kossman et al. (1997) stated a C:N ratio of 25:1 and 1.5% of N in the potato peel's composition.

Table 2.9 - Nutrients content of potato peel pulp for AD (Kryvoruchko et al., 2009).

Substrate	Crude [% TS]				N-free Extracts [%]	Nitrogen [%]	Carbon [%]	Gross Energy [Mj.kg^{-1} VS]	C:N Ratio
	Protein	Lipids	Fibre	Ash					
Potato peel pulp	16.4	1.3	7.0	8.2	66.2	3.8	45.8	19.5	12.1

Although, potato organic matter possesses a high content of carbohydrates, usually regarded as more suitable feedstock for the production of bioethanol rather than of biogas (Parawira et al., 2004a), it is less efficient to convert it into ethanol, thus not advisable.

According to Gunaseelan (2004), the CH_4 obtained from fruit and vegetable wastes is in the range of 180-732 $cm^3.g^{-1}$ VS and 190-400 $cm^3.g^{-1}$ VS respectively, indicating fruit wastes as having a higher potential for biogas production. However, Parawira et al. (2004b) has shown that the co-digestion of solid potato waste with sugar beet leaves improved the accumulated CH_4 production and the CH_4 yields by 31% to 62%, respectively, when compared with the AD of potato waste alone.

3. MATERIAL AND METHODS

The following experiment was carried out from October 2013 to March 2014 in the laboratories of the Departamento de Ciências e Tecnologia da Biomassa (DCTB) of the Faculdade de Ciências e Tecnologia (FCT) of the Universidade Nova de Lisboa (UNL).

Two trials of AD were carried out in a bench-scale (2.75 L) UASB mesophilic digester at 36.7 ± 1.1 ºC to study the efficiency of a chemical pre-treatment applied to a sample of potato peel waste obtained from an industry of potato crisp.

The experiment was carried out in seven separate steps as follows (Figure 3.1):

1. Sampling of potato peel waste;
2. Physicochemical characterisation of the potato peel waste;
3. Trials carried out with potato peel subjected to different thermo-chemical pre-treatments and neutralisation;
4. Physicochemical characterisation of the pre-treated samples;
5. Selection of either acid or alkaline thermo-chemical pre-treatment and beginning of AD trials in the bench-scale UASB;
6. Physicochemical characterisation of the influent and effluent of the bench-scale digester in each AD trial;
7. Measurement and analysis of the composition of the biogas produced over the course of each AD assay.

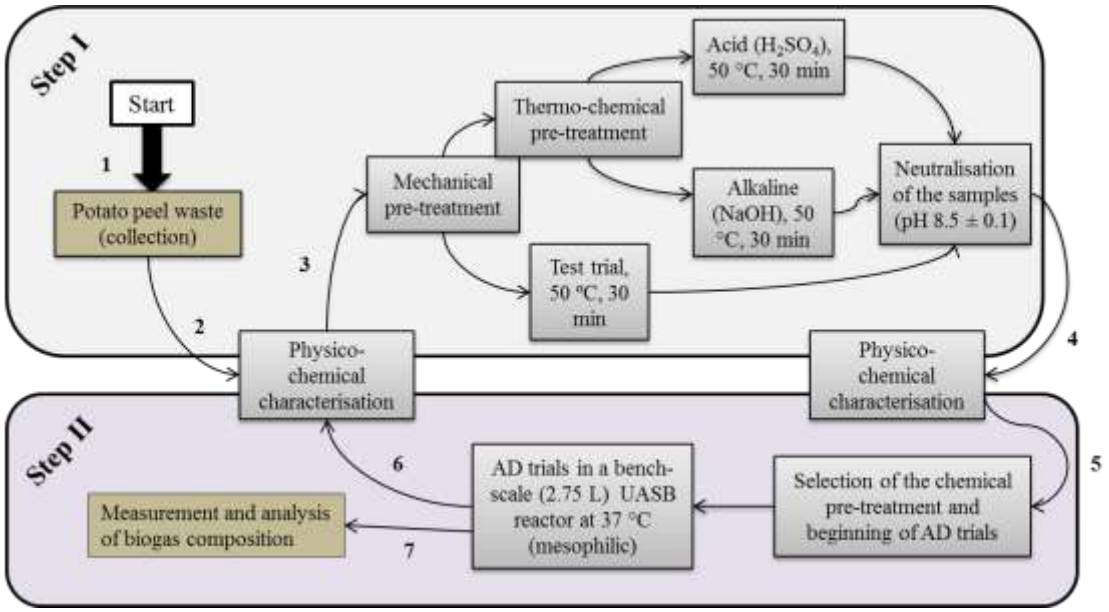

Figure 3.1 - Diagram of the sequence of procedures developed in the laboratory.

The following tasks were carried out over the course of the experiment:

1. Sampling of potato peel waste:
   a. Visit to the plant of potato crisp located in the Central region of Portugal for sampling the potato peel waste;
   b. Roughly 4 kg of waste was packed in a polyethylene bag and transported in a cooler box at a temperature of 4 °C;

c. The potato peel waste was split in polyethylene bags holding around 230 g each. These bags were frozen at a temperature of -18 °C, until usage in each of the AD trials.

2. The physicochemical characterisation of the potato peel waste comprised the following parameters:

a. Moisture content; Solids content: Total Solids (TS), Fixed Solids (FS) and Volatile Solids (VS);
b. Total chemical oxygen demand (COD);
c. Total phosphorus and total nitrogen content;
d. Biochemical oxygen demand (BOD);
e. Carbon, hydrogen, nitrogen, sulphur and oxygen content.

3. Trials carried out with potato peel subjected to mechanical and thermo-chemical pre-treatments and neutralisation (pH 8.5):

a. The potato peel waste was ground and submitted to four different thermo-chemical pre-treatments;
b. 200g of ground potato peel waste were weighed; 600 mL of deionised water were added; in 4 different assays the pH was adjusted with sulphuric acid (H_2SO_4), until pH levels of 2 and 4, or sodium hydroxide (NaOH) base, untill pH levels of 10 and 12; then the assays were heated at 50 ºC during 30 min at standard atmospheric pressure with mechanical agitation; after were cooled down at room temperature; finally, each asasy was neutralised with either H_2SO_4 or NaOH until pH values of nearly 8.5 were reached;
c. A control assay was carried out in which the potato peel waste was not subjected to a chemical pre-treatment after comminution. This assay was likewise subjected to thermal treatment at 50 ºC during 30 min and neutralised with NaOH until pH values of nearly 8.5 were reached, to be used as test control for the COD analysis in the next step.

4. Physicochemical characterisation of pre-treated samples, using the following parameters:

a. Solids content: TS, FS and VS;
b. Total and soluble COD.

5. Selection of the thermo-chemical pre-treatment and beginning of the AD trials:

The results of the trials with described in item 3 above were analysed on the basis of COD and its improvements with either of the thermo-chemical pre-treatments;

a. The thermo-chemical pre-treatment with the higher result of COD was the method selected and applied for the final trial to be used in the bench-scale digester;
b. A control trial was carried out without chemical pre-treatment. Therefore 200g of potato peel waste was subjected to comminution and thermal treatment at 50 ºC during 30 min. Finally, it was neutralised with NaOH until pH values of nearly 8.5 were reached, being used as test control for the AD trial;
c. A final trial was carried out in which the potato peel waste was subjected to comminution and the pH adjusted with alkali (NaOH) until reaching pH 12. Then was heated at 50 ºC during 30 min, and cooled down. Finally the assay was neutralised with acid (H_2SO_4) until reaching pH values of nearly 8.5, being used as the final AD trial with thermo-chemical pre-treatment.

6. Physicochemical characterisation of the influent and effluent of the anaerobic digester in each AD trial, for the following parameters:
   a. Solids: TS, FS and VS;
   b. Total and soluble COD.

7. Measurement and analysis of the composition of the biogas produced over the course of each AD assay:
   a. Measurement of the headspace created inside the biogas collection cylinder, using a measuring tape. The headspace corresponded to the amount of biogas produced up until the measurement date;
   b. Characterisation of the biogas composition through a *Gas Data GFM Series* biogas analyser to determine the CH_4, CO_2, O_2, CO, H_2 and H_2S content, as well as its Lower Explosive Limit (LEL);
   c. Calculation of the total volumes of biogas and CH_4 produced at the end of each AD assay.

Each of the stages of the experiment will now be explained in detail.

3.1. Origin and Characterisation of the Potato Peel Waste

The potato peel waste used was provided by a factory located in the Central region of Portugal that produces potato crisp. After fetching the waste, it was stored at -18 ºC, in batches of roughly 230 g each, until its usage in each of the AD assays. The samples were weighed on a *Kern PRJ 8200-1M* scale (precision ± 0.1 g).

The waste is a by-product of the potato-processing in the factory which produces approximately 1.00 $Mg.h^{-1}$ of fried potato in a tank with a 4,000 L of capacity for oil. However, other by-products results in this factory, such as wastewater with a high content of starch, fried potato with oil and other fried potato rejected in the standardised process and quality plans.

Figure 3.2 - Potato peel waste in laboratory.

The characterisation of the potato peel waste was determined by the parameters of moisture, TS, FS, VS, COD, BOD, total nitrogen and total phosphorus content through the use of *CEM*

fibreglass crucibles with 100 mL capacity. The elementary composition of the potato peel waste (C, H, N, S and O) was determined in the Portuguese National Laboratory of Energy and Geology (LNEG).

3.1.1 Moisture Content

In order to determine the moisture content of the potato peel waste, the mass of the dry sample was subtracted from the mass of the wet sample. The weight of the dry sample corresponded to the material dried in a *CEM MAS 7000* microwave muffle furnace, at 105 ± 2 °C, for 120 minutes, and weighed on a *Denver Instrument Company TR 603* scale (precision ± 0,001 g).

The moisture content in wet basis (wb) was determined using the following equation 3.1:

$$m_{wb} = \frac{W_1 - (W_2 - W_0) * 1000}{W_1} \quad \text{(Eq 3.1)}$$

where,

m_{wb}: Moisture content on wet basis (g.kg^{-1} wb)
W_0: Mass of the crucible at 105 ± 2 °C (g)
W_1: Wet mass of sample (g)
W_2: Dry mass of sample and mass of crucible at 105 ± 2 °C (g)

3.1.2 Solids Content

3.1.2.1 Total solids

To determine the total solids (TS), the samples were dried in a *CEM MAS 7000* microwave muffle furnace, at 105 ± 2 °C, for 120 minutes. The drying of the samples was programmed to have three heating ramps: ramp 1 took 5 minutes to reach 50 °C, it remained here for 1 minute; ramp 2 took 8 minutes to reach 100 °C and was left there for 1 minute; ramp 3 took 2 minutes to reach 105 °C, where it was kept for 2 hours.

The waste, after drying, was cooled for 30 minutes until it reached room temperature, in a desiccator, and then weighed on a *Denver Instrument Company TR 603* scale (precision: ± 0,001 g).

The TS represent the solids that remain in the sample after the water has evaporated by drying at 105 ± 1 ºC, indicating the quantity of mineral and organic material present in the sample.

The TS content, in the wet basis (wb) was determined using equation 3.2:

$$TS_{wb} = \frac{(W_2 - W_0) * 1000}{W_1} \quad \text{(Eq 3.2)}$$

where,

TS_{wb}: Total solid content (g.kg^{-1} wb)
W_0: Mass of crucible at 105 ± 2 °C (g)
W_1: Wet mass of sample (g)
W_2: Dry mass of sample and mass of crucible 105 ± 2 °C (g)

3.1.2.2 Fixed Solids

The fixed solids (FS) are a fraction of the TS that remains in ash form after calcination at 550 ± 50 ºC, being mainly made up of an inorganic or mineral fraction.

To determine the FS after drying, the samples were incinerated at 550 ± 50 ºC in a cycle of 60 min, in a *CEM MAS 7000* microwave muffle furnace. This programme had a 45 minute ramp time to reach 550 ºC, and was maintained at this temperature for 60 minutes. They were then cooled to room temperature in a desicator, and weighed on a *Denver Instrument Company TR 603* scale (± 0.001 g precision).

The FS in the wet basis (wb) were determined following equation 3.3:

$$FS_{wb} = \frac{(W_2 - W_0) * 1000}{W_1} \quad \text{(Eq 3.3)}$$

where,

FS_{wb}: Fixed solids ($g.kg^{-1}$ wb)
W_0 : Mass of the crucible at 550 ± 50 ºC (g)
W1 : Wet mass of sample (g)
W2 : Dry mass of sample and mass at 550 ± 50 ºC of crucible (g)

3.1.2.3 Volatile Solids

The volatile solilds (VS) represent a fraction of the TS that undergo volatilization at a temperature of 550 ± 50 ºC, constituting a mainly organic fraction. The VS may be determined as the difference between TS and FS.

3.1.3 Chemical Oxygen Demand

The Chemical Oxygen Demand (COD) is the parameter that measures the organic material content present in a sample, through chemical oxidation adding potassium dichromate ($K_2Cr_2O_7$), in a solution acidified by highly concentrated (96% v/v) *Panreac* sulphuric acid (H_2SO_4) heated at 180 °C during 60 min. The organic matter content is measured by the amount of a strong oxidising agent added, the potassium dichromate, $K_2Cr_2O_7$ (1N) until the full oxidation of the organic matter present in the sample. The full oxidation is indicated by a change of colour (red) with the addition of the $K_2Cr_2O_7$ during titration. Therefore, in this assay is measured the quantity of the oxidising agent that is not reduced by the organic material present in the sample. This measurement is expressed by the O_2 consumption required to oxidise all of the organic material as the oxidation of the organic carbon into CO_2 takes place at the time of the addition of the oxidising agent.

The measurements were carried out using five replicas of the potato peel waste, with masses of 0.3464 g, 0.1425 g, 0.1270 g and 0.1560 g. The solutions with the sample were acidified using 30 mL of sulphuric acid, H_2SO_4 (*Panreac*, 96% v/v), and mercury sulphate, $HgSO_4$, was also added (II), to complex any possible chlorides present in the replicas. A blank test was carried out without the presence of the potato peel waste and under the conditions detailed above.

After the digestion of the sample and the blank test in a *Behr Labor-Technick* thermo reactor at 180°C for 60 minutes, and the cooling of the digested mixture, the excess potassium dichromate that did not react with the organic material was titrated with ammonim ferrous sulphate,

$Fe(NH_4)_2(SO_4)_2$ (0.5 N), using nearly 8 drops of ferroin ($C_{36}H_{24}FeN_6$) as a coloured indicator to detect the final point of titration of the potassium dichromate. The titration must stop when the solution is changing from a yellowy-green to a bright red.

The COD, in dry basis, was calculated following equation 3.4:

$$COD = \frac{(V_{t-blank} - V_{t-sample}) * 8000 * T}{M_s * 8000} \quad \text{(Eq. 3.4)}$$

where,

COD: Chemical oxygen demand ($g.kg^1$)
$\boldsymbol{V_{t\text{-}blank}}$: Volume of the titrant used in blank (mL)
$\boldsymbol{V_{t\text{-}sample}}$: Volume of titrant used in sample (mL)
$\boldsymbol{M_s}$: Mass of dry sample (g)
T: Titrant (N)

The titrant, *T*, was determined by the titration of 10 mL of potassium dichromate (1N) with ammoniacal ferrous sulphate (0.5M), calculated using equation 3.5:

$$T = \frac{V_d * N}{V_t} \quad \text{(Eq. 3.5)}$$

where,

T: Titrant (N)
N: Normality of the potassium dichromate (N)
$\boldsymbol{V_d}$: Volume of potassium dichromate used in the titrant (mL)
$\boldsymbol{V_t}$: Volume of titrant (ammonium ferrous sulphate) used in the titration of the titrant standard solution (mL)

The concentrations of potassium dichromate and ammonium ferrous sulphate used were higher than those usually used to calculate the COD, given the higher organic content present in the samples. It was therefore necessary to assure the presence of excess oxidising agent at the end of the oxidation using the potassium dichromate. Using this methodology meant that it was unnecessary to drastically reduce the mas sample, which may have limited the quantification of the method.

3.1.4 *Kjeldahl* Nitrogen

Total *Kjeldahl* nitrogen (TKN) refers to the joint determination of ammonium nitrogen and organic nitrogen. The method used was the digestion of 2 replicates of the sample of 2.0544 g and 1.0816 g, with 30 mL of H_2SO_4 *Panreac* (95% v/v), and a catalyst of zinc and selenium salts. A blank test was carried out under the same conditions, without the addition of sample.

Digestion occurred in 4 stages: 100 °C for 30 minutes, 180 °C for 30 minutes, 260 ºC for 30 minutes and 340 °C for 90 minutes, in a *Velp ® Scientifica* digester.

The digested samples obtained were distilled in a solution highly alkalinised by NaOH (pH > 8.2) by steam-dragging the solution in a *Kjeltec System 1002* distilling unit. Two different volumes of digested samples were distilled: 10 mL, 20 mL and 40 mL. The distilled samples obtained were collected in a boric acid indicator solution, H_3BO_3 ($0.32\ mol.L^{-1}$), with a purple colouration. In the samples containing organic and ammonium nitrogen, the colouration of the boric acid solution changed from purple to green. After the distillation, the solution was titrated

with H_2SO_4 (0.020 N), until the solution returned to a purple colour. Equations 3.6 to 3.9 describe the stages of digestion, distillation and titration of N-*Kjeldahl*:

Digestion:

$$\textbf{Organic } N + H_2SO_4 \rightarrow (NH_4)_2SO_4 \quad \text{(Eq. 3.6)}$$

Distillation:

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O \quad \text{(Eq 3.7)}$$

$$NH_3 + H_3BO_3 \rightarrow NH_4^+ + H_2BO_3^- \quad \text{(Eq. 3.8)}$$

Titration:

$$H_2BO_3^- + H^+ \rightarrow H^+ \rightarrow H_3BO_3 \quad \text{(Eq. 3.9)}$$

The N-*Kjeldahl* content in the digested samples was calculated using equation 3.10:

$$N - Kjeldahl_{digested} = \frac{(V_{t-sample} - V_{t-blank}) * 280}{V_{t-digested}} \quad \text{(Eq. 3.10)}$$

where,

***N- Kjeldahl*$_{digested}$**: Digested nitrogen (mg N.L^{-1})
$V_{t\text{-}sample}$: Volume of titrant used in the titration of sample (mL)
$V_{t\text{-}blank}$: Volume of titrant used in the titration of blank test (mL)
$V_{t\text{-}digested}$: Volume of digested sample used in titration (mL)

The mass of the N-*Kjeldahl* present in the digested samples was calculated using equation 3.11:

$$N - Kjeldahl_{in\ digested\ sample} = \frac{N-Kjeldahl_{digested} * V_{flask}}{1000} \quad \text{(Eq. 3.11)}$$

where,

N-Kjeldahl in digested sample: mass of nitrogen in digested sample (mg N)
***N-Kjeldahl*$_{digested}$** : Digested nitrogen (mg N.L^{-1})
V_{flask}: Volume of round-bottomed flask with digested sample (mL)

The concentration of N-*Kjeldahl* in the dry sample (ds) was calculated using equation 3.12:

$$N - Kjeldahl_{residue} = \frac{N-Kjeldahl_{in\ digested\ sample}}{M_s} \quad \text{(Eq. 3.12)}$$

where,

***N- Kjeldahl*$_{residuee}$**: Total mass of nitrogen in the potato peel waste (mg N.g^{-1})
N-digested: Mass of nitrogen in digested sample (mg N)
M_s: Dry sample mass (g)

The N-*Kjeldahl* parameter is often referred to as total nitrogen, since the nitrogen content in the solid organic material is mainly made up of organic and ammonium nitrogen, whilst the fractions relating to nitrates and nitrites are greatly reduced in comparison to the nitrogen fractions. This parameter will hereinafter be referred to as Total Nitrogen.

3.1.5 Total Phosphorus

To determine total phosphorus (P-total) were used the digested samples carried out to determine the N-*Kjeldahl*.

200 mL of reducing agent was prepared with 100 mL of sulphuric acid H_2SO_4 (5N), 30 mL of ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ (0.032 mol.L^{-1}), 1.056 g of ascorbic acid $C_6H_8O_6$ (176.13 M), 10 mL of potassium tartarate $K(SbO)C_4H_4O_6.0,5H_2O$ (0.0090 mol.L^{-1}) and *Milli-Q* water.

The standard phosphate solution (100 mL) was prepared with 2 mL phosphate stock solution (0.0016 mol.L^{-1}) and 100 mL of *Milli-Q* water.The standard solutions were prepared with different volumes of the standard solution (0, 5, 10, 15, 20 and 25 mL) and 8 mL of reducing agent, then filled up to 100 mL with *Milli-Q* water.

The samples were prepared with different volumes of digested samples that had previously been alkalinised with NaOH (6 N) using phenolphthalein as an indicator to attain a pH between 8.2 and 12. 8 mL of reducing agent was then added, and the volume was filled up to 100 mL using *Milli-Q* water. The blank test was carried out as aforementioned, but using the sample corresponding to the digested blank sample [i.e. without the potato peel waste].

The absorbances of the standard solutions (0, 5, 10, 15, 20, 25 µg P) and of the samples were measured in a *Shimadzu UV-120-11* spectrophotometer at a wavelength of 880 nm.

The values of the P mass in the digested samples were calculated using the *absorbance vs mass of P* (*yy;xx*) calibration curve (equation 3.13).

$$y = 0.0104x - 0.0028 \quad \text{(Eq. 3.13)}$$
$$R^2 = 0.964$$

Where,

x: Mass of P (µg P)
y: Sample absorbance at 880nm

The concentrations of P-total in the dry sample (ds) were calculated using equations 3.14 and 3.16.

$$Mass\ of\ P\ in\ digested\ sample\ = \frac{Mass\ of\ P * V_{flask}}{V_s} \quad \text{(Eq. 3.14)}$$

Where,

Mass of P in digested sample: mass of phosphorus in the digested sample (µg *P*)
Mass of P: Mass of phosphorus (µg *P*) measured using the calibration curve [see: equation 3.13]
$\boldsymbol{V_{flask}}$: Volume of round-bottomed flask containing digested sample (mL)
$\boldsymbol{V_s}$: Volume of sample (mL)

$$[P]\ = \frac{Mass\ of\ P\ in\ digested\ sample}{M_s} \quad \text{(Eq. 3.15)}$$

Where,

[***P***]: Concentration of total phosphorus (µg P.g^{-1})
Mass of P in digested sample: Mass of P in digested sample (µg *P*)
$\boldsymbol{M_s}$: Mass of dry sample (g)

3.1.6 BOD_5

The BOD_5 allow to measure the organic material present in organic waste or in wastewaters, through biological oxidation. The oxidising agent used is a set of microorganisms, hereinafter

referred to as inoculum. The temperature at which biological oxidation takes place is 20°C (incubation temperature), for a period of 5 days. The assay must be carried out in the dark to reduce the oxygen produced by the photosynthetic organisms that may be present in the sample or inoculum.

In the Respirometric Method used in the current experiment, a calculation of the oxygen consumed in successive periods of 24 hours was carried out over a total incubation time of 5 days. Therefore 3 replicates of 1 g of the potato peel waste and a blank test were carried out for 5 days incubation.

The liquid inside the respirometers was stirred smoothly and constantly to facilitate the gas exchange between the sample and the atmosphere within the respirometer.

The BOD_5 was measured in *WTW* respirometers, with *OxiTop*® pressure detectors. The pressure detectors measure the negative pressure differences produced in the headspace of the respirometers. These negative pressure differences are caused by the microbial oxidation of the organic material, when oxygen consumption takes place. The CO_2, which is given off in the metabolic activity, is neutralised with the NaOH found in the lid of the respirometers.

The BOD_5 in the dry basis (db) was calculated using equation 3.16:

$$BOD_5 = \frac{P * F_c * V_S}{M_S * 1000} \quad \text{(Eq. 3.16)}$$

where,

$\boldsymbol{BOD_5}$: Biochemical oxygen demand (mg O_2.g^{-1} db)
$\boldsymbol{P}$: Pressure indicated on the device (mbar)
$\boldsymbol{F_c}$: Conversion factor of the mass pressure of consumed oxygen (mg O_2.$mbar^{-1}$.L^{-1})
$\boldsymbol{V_s}$: Sample volume (mL)
$\boldsymbol{M_s}$: Dry sample mass (g)

3.1.7 Elementary Characterisation

As mentioned at the beginning of the chapter (see section 3.1), the potato peel waste was characterised relative to its elementary composition, namely C, H, N, S and O by LNEG. The methods used for the elementary characterisation of the waste are described in table 3.1.

Table 3.1 - Summary of methods used for the elementary characterisation of the potato peel waste (personal information from LNEG, 2014).

Parameter	Methodology
Moisture (% db)	Drying of sample at 105 ± 2 °C, in air, until a constant weight was obtained. The moisture content is calculated based on the difference between the initial weight and the final sample weight (loss of mass).
Ash (% db)	Heating of sample at 550 °C, in air, under controlled conditions.
C (% db) **H_2 (% db)** **N (% db)** **S (% db)**	Automatic analyser of C, H, N and S based on combustion of the sample in air atmosphere and quantification of CO_2, H_2O, NO_x, and SO_2
O_2 (% db)	Calculated by the difference: ***O* = 100 – *C* – *H* – *N* – *S* – *Ash***

3.2 Anaerobic Digester

The anaerobic digester used in the present thesis (Figure 3.3) was an acrylic cylinder with a height of around 60 cm and an internal diameter of about 9 cm and a height:diameter (H:D) ratio of approximately 7. In a full scale plant, Deublein and Steinhauser (2008) recommend a bioreactor to operate with a H:D ratio of approximately 1.3. The AD trials were carried out in an Upflow Anaerobic Sludge Blanket (UASB) reactor with a 2.75 L working volume, inoculated and activated in prior studies (Carapinha, 2012; Santos, 2013). Additionally, the anaerobic digester used in the present dissertation is schematised in Figure 3.4.

The heat losses to the exterior can be decreased with the sizing of the bioreactor, dependent on the surface area and of the thermo insulation (Carrilho, 2012). If the minimum surface area of a digester decreases, the heat losses decreases (i.e. less heat lost the lower the H:D). Thermal insulation is required in order to minimise the heat losses, however if the conditions on site are favourable for the required operation mode, this factor is not as critical.

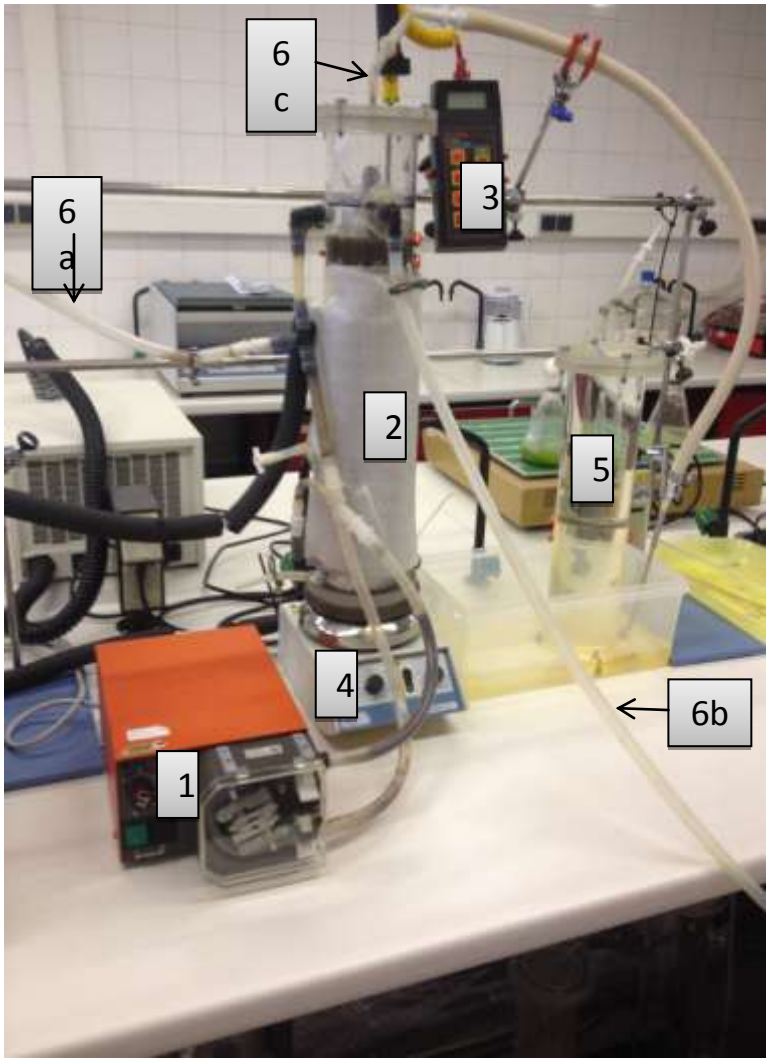

Figure 3.3 - Bench-scale UASB operated in mesophilic conditions. 1- Recirculation system of sludge and sckimmings through a peristaltic pump; 2- Heating system: pumped hot water circulating through siliconeserpentine covered with a fine styrofoam film; 3- Thermopar; 4- Stirring system of the digester content; 5- Biogas storage cylinder; 6a- Influent inlet; 6b- Effluent outlet; 6c- Biogas outlet to the storage cylinder.

The mean temperature in the present experiment was of 21.8 ± 1.4 ºC inside the laboratory and 36.7 ± 1.1 ºC inside the mesophilic digester. This temperature was maintained with a heating system of hot water circulating through a closed circuit of a silicone serpentine (see 2 in Figure 3.3 and 9 in Figure 3.4). The water flowing within the serpentine came from a hot water bath at 40 °C (see 2 in Figure 3.4). The temperature inside the digester was measured by a thermopar *Hanna instruments HI 9053* (see 3 in the Figure 3.3).

The inoculum inserted into the UASB reactor was collected from an anaerobic thermophilic digester, operating at an average temperature of 53°C (Organic Waste Treatment and Recovery Plant – ETVO of *Valorsul*). However, in the present experiments, the inoculum operated in mesophilic conditions (36.7 ± 1.1 ºC).

A magnetic low rotation stirrer in the bottom of the digester was steadly active by a magnetic plaque (see 4 in the Figure 3.3 and 6 in Figure 3.4) that aided the distribution of heat along the column, besides increasing the contact between the organic content and the cells. An inflow recirculation went from the top of the liquid to the bottom (i.e. ascendant movement; see 1 in the Figure 3.3) carried out by a peristaltic pump, operating once every 6 hours during 15 min, every day.

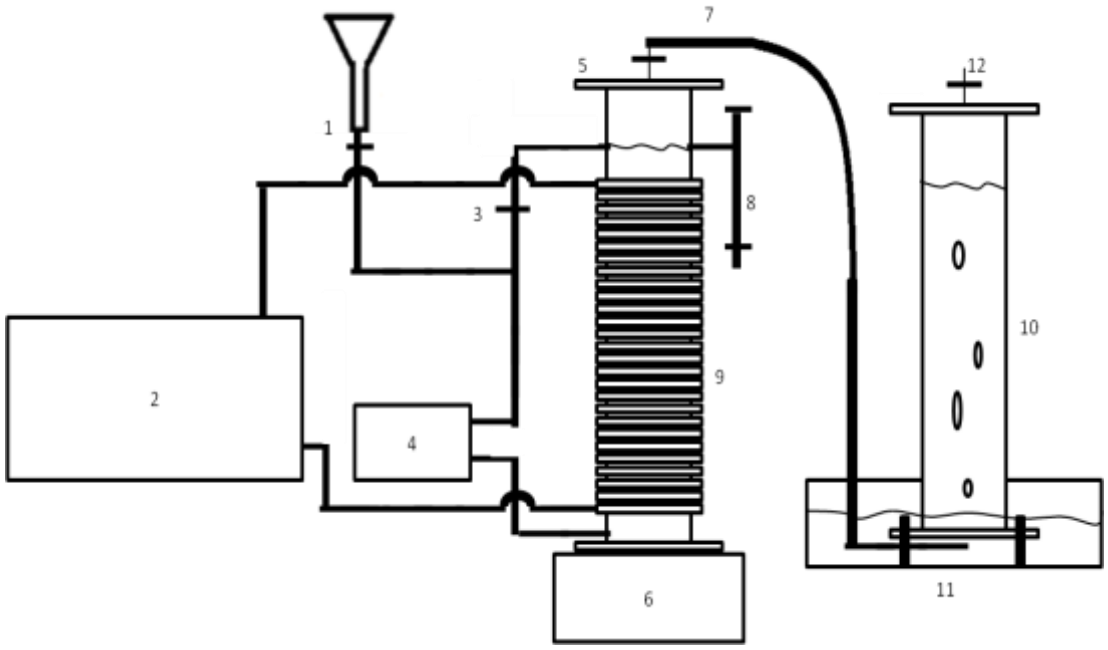

Figure 3.4 - Diagram of the bench-scale UASB. 1. Inline of influent; 2. Heated water bath with a pump for water recirculation; 3. Recirculation line of scums; 4. Peristaltic pump for scum recirculation; 5. UASB reactor; 6. Magnetic stirrer; 7. Biogas outline; 8. Outline of the effluent; 9. Silicone serpentine with hot water pumped from the hot water bath; 10. Column for biogas storage; 11. Displacement with deionised water; 12. Outlet for the collection of a biogas sample.

Before any operation with the digester, a leakage test of the reactor was carried out, as recommended by Kossman et al. (1997) in order to ensure that no oxygen could get in the reactor. Also, a feeding of the digester with 200 g wb of potato peel diluted in 600 mL deionised water was carried out for the activation of the cells before the start of the experiments.

In the present thesis, the UASB operated in a batch mode, thus the affluent used in each experiment was fully introduced at once in the reactor. Batch processes are technically simple, less expensive and more robust than other digester operating systems (Monnet, 2003).

In a full-scale, the feeding of the digester is pumped in through the bottom of the reactor as, in a small-scale, in the present thesis. Therefore, the ascendant inflow and recirculation ensures a good mixing and contact of the content with the microorganisms by the liquid stream and the development of biogas bubbles (Deublein and Steinhauser, 2008) in low HRT (Carrilho, 2012).

The biogas was collected through the biogas line (see 7 in Figure 3.4), then stored and measured in an acrylic cylinder fulfilled with deionised water (see 11 in Figure 3.4). The biogas produced was injected in the bottom of the column and moved in an ascending movement until reaching the top and press the water (see 5 in Figure 3.3 and 10 in Figure 3.4). Thus, the metering system was a water-gas pressure system operated in normal temperature and pressure conditions (NTP). To ensure that there was no biogas in the column before each AD assay, the biogas was extracted through the outlet of the column (see 12 in Figure 3.4) and the water filled the space left by the biogas.

The AD trials were carried out with the feeding of the digester in a sequential procedure. The digester inline and outline were only opened during each feeding of the digester for the AD trials.

In each assay, the new influent with the waste was poured in a polyethylene funnel and subsequently injected in the digester through the input line (see 1 in Figure 3.4). During the feeding of the digester with the influent, an equal amount of effluent was removed through the output line (see 8 in Figure 3.4), through gravitational draining, whilst the recirculation line of scums (see 3 in Figure 3.4) operated steadily. The rise of the liquid level inside the digester resulted in the attainment of the discharge level and the ejection of the effluent through gravitational draining from the output line (see 8 in Figure 3.4).

Before each feeding cycle of the digester, the biogas was collected from the storage column (see 10 in Figure 3.4). The entry (see 1 in the Figure 3.4) and exit (see 8 in Figure 3.4) lines were kept closed whilst the biogas was collected, and the recirculation line was opened (see 3 in Figure 3.4), allowing the pump for the recirculation of scums (see 3 in Figure 3.4) to take out the fluid from the upper part of the digester and inject it into the lower part.

Excluding the periods in which the digester was fed, recirculation took place automatically 4 times a day, for 15 minutes each time, with the aid of a digital timer to turn the recirculation pump on and off automatically (see 4 in Figure 3.4).

3.3. Experimental work

During the experimental work of this thesis, two steps were carried out separately, namely:

1. Analysis of the effect of 4 different chemical pre-treatments in the bioavailability of the organic matter in the potato peel waste. The bioavailability of the organic matter was assessed in terms of total COD and soluble COD present in the samples after the thermo-chemical pre-treatments;

2. AD of two assays in the digester: an assay for test control; and a final assay with a selected chemical pre-treatment based on the results obtained in the previous step.

3.3.1 Pre-treatments

In this step, several pre-treatments of potato peel waste were carried out in order to evaluate the improvements in the total COD (CODt) and soluble COD (CODs) and, thereby, the effect in the bioavailability of the organic substrate for AD in each assay. For this purpose, 4 different assays, and additionally a control test, were performed, as described in Figure 3.5.

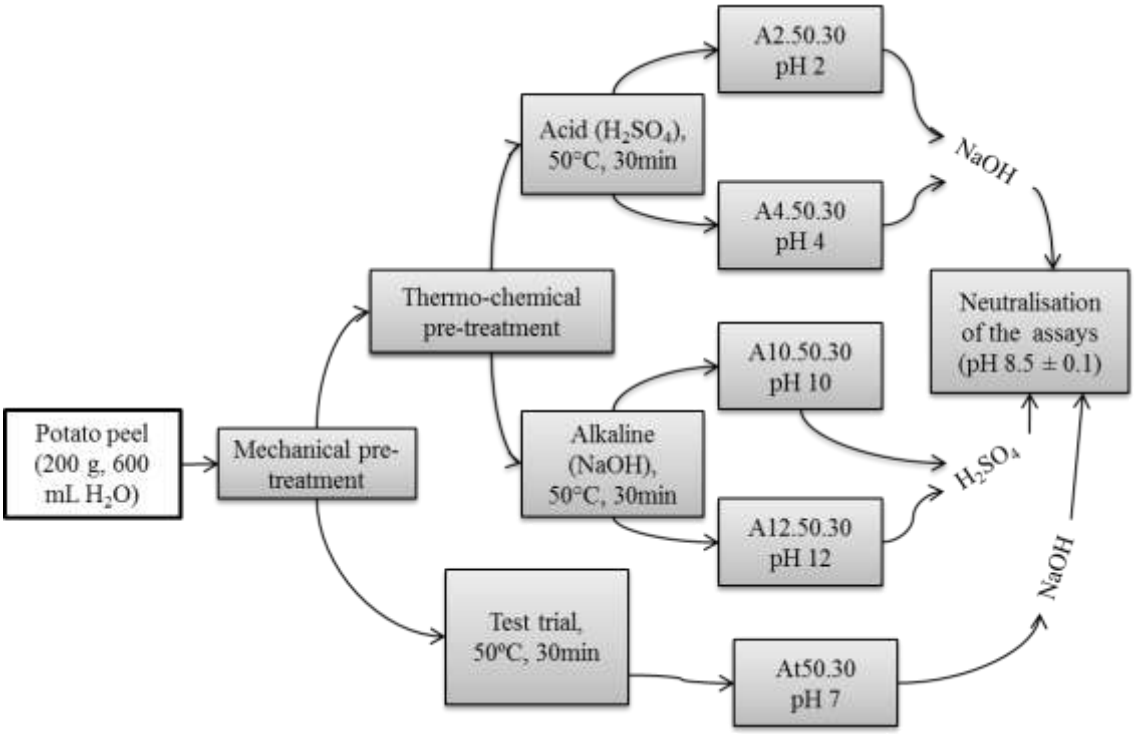

Figure 3.5 - Diagram of the chemical pre-treatments methods and the test assay.

It is worth mentioning that different volumes were used in the following experiments, although never changing the organic load (OL) used. Samples of 200 g wb weighed on a *Kern PRJ 8200 – 1M* scale (precision ± 0.1 g) were diluted in 600 mL of deionised water. Therefore, an OL of, approximately, 333 $g_{potato\ peel\ waste}.L^{-1}$ in each assay were met. However, the different volumes used were as described in Table 3.2.

The potato peel waste was primarily subjected to a mechanical pre-treatment. The mechanical treatment was carried out in all assays equally. The potato peel waste was milled in a *Fagor* blade mill for nearly 5 minutes. The milled waste was then passed through a *Retsch* sieve with a 2 mm mesh by adding 600 mL of deionised water.

Table 3.2 - Summary of the volumes used in each pre-treatment.

Assays	**Volumes used in each pre-treatment (mL)**		
	Mechanical	**Chemical**	**Thermal**
At50.30	750	-	350
A2.50.30	750	350	350
A4.50.30	750	350	350
A10.50.30	750	350	350
A12.50.30	770	770	770

The waste previously milled and sieved was subjected to 2 different chemical procedures: acid and alkaline, each carried out in 2 separated assays of different pH values (Figure 3.5). The pH values were measured in an *Orion Research, Expandable ionAnalyser EA940* electrode, previously calibrated for each assay.

Two assays in alkaline solution were carried out, whilst another two assays were carried out with acid medium. The pH values were obtained with either the addition of the acid H_2SO_4 (1N) or alkali NaOH (1N).

The alkaline pre-treatment was carried out with the addition of NaOH until pH levels of 10 were reached [assay A.10.50.30] or 12 [assay A12.50.30] (Figure 3.5). The acidification of the milled potato peel waste in solution was carried out with the addition of H_2SO_4, until reaching pH levels of 2 [assay A.2.50.30] and 4 [assay A.4.50.30] (Figure 3.5).

A thermal pre-treatment was then carried out in each assay. The acidic and alkaline mixtures were heated at 50 ºC during 30 min. Although some water was evaporated during the heating, and therefore caused a raise in the solids concentration in each assay, no water was added in compensation.

An assay for test control [At50.30] underwent the same procedures of both mechanical and thermal pre-treatment, without any chemical pre-treatment.

Finally, the neutralisation of the assays was carried out with either the addition of acid or alkali, depending on the pH present in the solution to reach a pH level of 8.5. Thus, the acidified solutions were neutralised by the addition of NaOH (1N), whilst the alkaline solutions were neutralised with the addition of H_2SO_4 (1N). The test control assay was neutralised with the addition of NaOH (1N).

The methods and procedures for each assay are detailed in Figure 3.5 and in Table 3.3.

The improvements in the bioavailability of the potato peel waste were analysed in terms of the total COD and soluble COD present in the mixtures after the thermo-chemical pre-treatments. The chemical procedure of the assay with a higher soluble COD result was then carried out for an AD assay in the digester, in addition to a control assay without chemical pre-treatment.

The COD results of the assays were compared to analyse the chemical pre-treatment procedure with greater COD improvements to be applied for the AD assay. In previous Figure 3.5, the pathways of alkaline pre-treatment at pH 12 and the test trial were the procedures followed for the subsequent AD trials in the present thesis.

3.3.2 Anaerobic Digestion Assays

Two AD assays in the UASB were carried out. The first AD assay was carried out as the test assay, as with the previous test control procedure (At50.30), with mechanical and thermal pre-treatment and no chemical pre-treatment [milling procedure as described above, heating at 50 ºC during 30 min and pH adjustment to a value close to 8.5 with the addition of NaOH] (Table 3.4).

The assay A12.50.30 was carried out with mechanical and thermo-chemical pre-treatment and was the only AD trial tested with thermo-chemical treatment. This assay was selected based on the highest soluble COD that was obtained in the previous tests (see section 3.3.1).

Table 3.3 - Summary of the methods carried out for each assay.

Assay	Chemical pre-treatment				Thermal pre-treatment		Neutralisation			
	pH		Chemical dosage (mL)				pH		Chemical dosage (mL)	
	After the mechanical pre-treatment	After pH adjustment	NaOH (1 N) added	H_2SO_4 (1 N) added	Temperature (ºC)	Time (min)	After the thermo-chemical pre-treatment	After pH adjustment	H_2SO_4 (1 N) added	NaOH (1 N) added
At50.30	6.93	-		-	50	30	7.64	8.46	-	0.3
A2.50.30	7.61	2.00	-	9.3			2.11	8.57	-	7.3
A4.50.30	6.93	4.05	-	22.30[(1)]			4.33	8.51	-	4.3
A10.50.30	7.62	10.00	1.5	-			9.07	8.50	0.3	-
A12.50.30	6.47	11.99	12.4	-			8.50	8.50	5.0	-

[(1)] The H_2SO_4 reagent initially applied was of 0.1 N, a weak acid.

Table 3.4 - Summary of the pre-treatment methods carried out for each AD assay.

Assay	Chemical pre-treatment				Thermal pre-treatment		Neutralisation			
	pH		Chemical dosage (mL)				pH		Chemical dosage (mL)	
	After the mechanical pre-treatment	After pH adjustment	NaOH (1N) added	H_2SO_4 (1N) added	Temperature (ºC)	Time (min)	After the thermo-chemical pre-treatment	After pH adjustment	H_2SO_4 (1N) added	NaOH (1N) added
At50.30	6.07	-	-	-	50	30	6.07	8.54	-	2.2
A12.50.30	6.36	12.00	12.4	-			11.31	8.56	4.7	-

The assay volumes used in both of pre-treatments and AD feeding are shown in Table 3.5.

Table 3.5 - Summary of the volumes used in each assay and pre-treatment, and the final volume for AD.

Assays	Volumes used in each assay/pre-treatment (L)			
	Mechanical	**Chemical**	**Thermal**	**Digester's feeding**
At50.30	0.760	-	0.760	0.450
A12.50.30	0.760	0.760	0.760	0.450

The pH of mixtures used in both assays were measured in an *Orion Research, Expandable ionAnalyser EA940* electrode. The pH value was adjusted to nearly 8.50, with NaOH (1N), and only after this step the digester was fed.

The next stage was to close the recirculation line of the digester and introduce the potato peel waste through the inflow using a polyethylene funnel. The gas outlet line was then closed, the effluent outlet was opened and the lid of the effluent outlet was removed. Following these procedures the recirculation pump was turned on, which would allow the influent to flow into the digester through the inlet and the effluent to flow out through the outlet at the same flow.

In each of the feedings a sample of the influent before it entered the digester and a sample of effluent as it was exiting the digester was collected. These samples were subjected to a physicochemical characterisation, as described in section 3.3.3.

3.3.3 Physicochemical Characterisation of the Mixtures Resulting From the Pre-treatments and of the Influent and Effluent of the Digester

In this section, physicochemical characterisation was carried out equally in two distinct steps:

a. Physicochemical characterisation of the assays subjected to different pre-treatments (section 3.3.1) - samples of each pre-treatment assay were analysed according to TS, FS, VS, total COD and soluble COD;
b. Physicochemical characterisation of the influent and effluent of the digester - the influent and effluent samples of the digester for each AD assay were analysed for TS, FS, VS and total COD.

3.3.3.1 Total Solids, Fixed Solids and Volatile Solids

To determine the content of solids in each sample of the pre-treatments and AD assays (sections 3.3.1 and 3.3.2, respectively), the same methods previously described in section 3.2.2 were used, but with slight differences.

As the samples had high water content, 15 mL of each sample was poured in the crucibles instead of weighing the mass of the sample. Therefore, the sample in the crucible was heated using a boiling water bath until the water content was almost evaporated.

After the water was evaporated, the samples were dried at 105 ± 2 °C for 120 minutes, in a *CEM MAS 7000* microwave muffle. The drying of the samples were programmed, as previously mentioned, with three heating ramps: ramp 1 took 5 minutes to reach 50 °C, it remained there for 1 minute; ramp 2 took 8 minutes to reach 100 °C and was left there for 1 minute; ramp 3 took 2 minutes to reach 105 °C, where it was kept for 2 hours. The samples were then cooled to room temperature in a desiccator and weighed on a *Denver Instrument Company TR 603* scale (± 0.001 g precision).

Finally, in order to determine the FS and VS, the samples were calcinated at 550 ± 50 °C for 60 minutes. The programme had a 45 minute ramp time to reach 550 ºC, as previously referred. The samples were then cooled to room temperature in a desiccator and weighed on a *Denver Instrument Company TR 603* scale (± 0.001 g precision).

Thus, in equations 3.2, 3.3 and 3.4 the mass of the samples was replaced by the sample volumes, in order to determine the TS, FS and VS in $g.L^{-1}$.

3.3.3.2 COD

In order to determine the COD, the same method previously detailed in section 3.3.2 was used. In this case, two calculations were made for each sample of the assays, as well as a blank test, after each sample was diluted to 1:20 and 1:10 and using 1 or 2 mL of the sample.

The soluble COD was also determined using the same method, with the exception of subjecting approximately 70 mL of the sample in capsules to prior centrifugation at 10,956 *g*, for a period of 10 minutes at 20 °C, in a *Sigma4K15C* refrigerated centrifuge. This procedure was based in the work of Kim et al. (2003). Only a fraction of the supernatant liquid was obtained after centrifugation. This process, in some cases, was repeated twice for each sample.

Finally, the samples were pressurised in a vacuum pump device through *Whatman 934-AH* filters. Soluble samples were then diluted in a ratio of 5:20, 10:20 and 15:20; depending on the quantity available for the determination. Therefore, the volumes were also variable from 5-15-20 mL of the soluble sample. In order to calculate the total COD and the soluble COD, in g $O_2.L^{-1}$, the sample mass was substituted in equation 3.4 by the volume of the sample.

3.3.4 Quantitative and Qualitative Analysis of Biogas

In each assay in the UASB reactor, samples of biogas were collected in a biogas storage column. The biogas was then characterised, on an almost daily basis by measuring the height of the biogas in the column, and its composition.

The height of biogas in the column was measured using a graduated metal ruler (± 0.1 cm precision).

Samples of biogas were analysed with the support of a *Gas Data GFM Series* biogas analyser. The device was first calibrated with atmospheric air. The biogas composition of the samples with a height greater than 4 cm in the column of biogas, were analysed the following gases: CH_4, CO_2, O_2, H_2S, CO, H_2 and the lower explosion limit (LEL) value. The temperature of the lab and inside of the digester were also read and registered.

The readings were carried out daily on the first few days of each anaerobic digestion assay, after which the production of biogas began to decrease, to the point that on some days there was insufficient biogas for an analysis to be performed. Thus, the frequency of analysis would wane towards the end of the assays. On the days in which the biogas level was too low to be measured, it would be left to accumulate with the biogas produced in the following days, until it reached a height preferably over 4 cm on the inside of the storage column.

Knowing the biogas accumulated and the methane content within the biogas, it was possible to calculate the average daily production of methane, following equations 3.17.

$$Vaccum_{CH_4} = \sum Vdaily_{CH_4;10d} \quad \text{(Eq. 3.17)}$$

Where,

Vaccum $_{CH4}$: Volume of methane accumulated throughout the 10 days of the assay (cm^3)
$\sum Vdaily_{CH_4}$: Accumulated methane volume in between biogas production readings (cm^3)

3.3.5 Loads, Removal Efficiencies and Production Yield

The total COD and VS loads introduced into the digester were calculated using equations 3.18 and 3.19, respectively:

$$COD_{total}load = \frac{COD_i * V_i}{V_d} \quad \text{(Eq. 3.18)}$$

$$VS\ load = \frac{VS_i * V_i}{V_d} \quad \text{(Eq. 3.19)}$$

where,

COD_{total} *load*: load of total COD applied to the digester (g O_2.L^{-1} digester)
COD_i: concentration of total COD in the influent (g O_2.L^{-1})
V_i : volume of influent introduced in digester (L)
V_d: volume of digester (L)
VS load: load of volatile solids applied to the digester (g.L^{-1} digester)
VS_i: concentration of volatile solids in the influent (g.L^{-1})

The removal efficiency of total COD and VS was calculated in accordance with equations 3.20 and 3.21, respectively:

$$Removal\ Eff.COD_{total} = \frac{COD_i - COD_e}{COD_i} \quad \text{(Eq. 3.20)}$$

$$Removal\ Eff.VS = \frac{VS_i - VS_e}{VS_i} \quad \text{(Eq. 3.21)}$$

where,

Removal. Eff. COD_{total} : removal efficiency of total COD (adimensional)
COD_i : concentration of total COD in the influent (g O_2.L^{-1})
COD_e : concentration of total COD in the effluent (g O_2.L^{-1})
Removal. Eff. VS: removal efficiency of volatile solids (adimensional)
VS_i : concentration of volatile solids in the influent (g.L^{-1})
VS_e : concentration of volatile solids in the effluent (g.L^{-1})

The biogas and methane yields were calculated based on the total COD and VS. Equations 3.22 and 3.23 were used to calculate the biogas and methane yields in relation to total COD removed. Equations 3.24 and 3.25 were used to calculate the same yields in relation to VS removed.

$$\eta_{biogas/COD_{total}\,removed} = \frac{Vt_{biogas}}{COD_{total}\,load * Removal\,Eff.\ COD_{total} * V_d} \quad \text{(Eq. 3.22)}$$

$$\eta_{CH_4/COD_{total}\,removed} = \frac{Vt_{CH_4}}{COD_{total} * Removal\,Eff.\ COD_{total} * V_d} \quad \text{(Eq. 3.23)}$$

$$\eta_{biogas/VS\,removed} = \frac{Vt_{biogas}}{VS\,load * Removal\,Eff.\ VS * V_d} \quad \text{(Eq. 3.24)}$$

$$\eta_{CH_4/VS\,removed} = \frac{Vt_{CH_4}}{VS\,load * Removal\,Eff.\ VS * V_d} \quad \text{(Eq. 3.25)}$$

Where,

$\eta_{biogas/COD_{total}\,removed}$ - Biogas yield in relation to total COD removed ($cm^3.g^{-1}$ CODt removed)
$\eta_{CH_4/COD_{total}\,removed}$ - Methane yield in relation to total COD removed ($cm^3.g^{-1}$ CODt removed)
$\eta_{biogas/VS\,removed}$ - Biogas yield in relation to volatile solids removed ($cm^3.g^{-1}$ VS removed)
$\eta_{CH_4/VS\,removed}$ - Methane yield related to volatile solids removed ($cm^3.g^{-1}$ VS removed)
Vt_{biogas} – Total volume of biogas produced in the assay during 10 days ($cm^3.d^{-1}$)
Vt_{CH_4} – Total volume of methane produced in the assay during 10 days ($cm^3.d^{-1}$)
$COD_{total}\ load$ - load of total COD applied to the digester (g O_2.L^{-1} digester)
$VS\ load$ - load of volatile solids applied to the digester ($g.L^{-1}$ digester)
$Removal\ Eff.\ COD_{total}$ - Removal efficiency of total COD (adimensional)
$Removal\ Eff.\ VS$ - Removal efficiency of volatile solids (adimensional)
V_d – Volume of digester (L)

4. RESULTS AND DISCUSSION

4.1 Characterisation of the Potato Peel Waste

The characterisation of the potato peel waste is shown in Table 4.1.

Table 4.1 - Characterisation of the potato peel waste.

Parameter	Units	Value ($\bar{x}$±σ)	Replicates (n)
TS	$g.kg^{-1}$ wb	120 ± 16	4
FS	$g.kg^{-1}$ wb	7.89 ± 0.22	3
VS	$g.kg^{-1}$ wb	114 ± 19	3
Moisture	$g.kg^{-1}$ wb	880 ± 16	4
CODt	$g\ O_2.kg^{-1}$ db	1,370 ± 168	4
BOD_5t	$g\ O_2.kg^{-1}$ db	81.7 ± 27.2	3
$BOD_5t/CODt$	adi.	0.060	-
N_{total}	$g\ N.kg^{-1}$ db	14.9 ± 2.9	2
Phosphorus	$g\ P.kg^{-1}$ db	3.95 ± 1.58	2

$\bar{x}$: mean; σ: standard deviation; n: number of replicates; wb: wet basis; db: dry basis; adi.: adimensional.

In Table 4.2, the elementary characterisation of the potato peel waste is presented as it was analysed by LNEG.

Table 4.2 - Elementary characterisation of the potato peel waste (n = 1).

Parameter	Value
Moisture (% m/m wb)	88.0
Ashes (% m/m db)	6.6
C (% m/m db)	43.9
H (% m/m db)	7.2
N (% m/m db)	0.8
S (% m/m db)	0.1
O (% m/m db)	41.4

n: number of replicates; n.r.: not reported.

As shown in Table 4.1, in the potato peel waste composition was observed a high water content of 880 $g.kg^{-1}$ wb (88.0%) of moisture and a TS content of 120 $g.kg^{-1}$ wb (12.0%). Within the TS content, 7.89 $g.kg^{-1}$ wb (6.59%) were of FS and 112 $g.kg^{-1}$ wb (93.4%) were of VS.

In relation to the potato peel composition in TS and VS, similar values were found in literature. Raynal et al. (1998), Azeitona (2012) and Santos (2013) presented 119 $g.kg^{-1}$ wb, 112 $g.kg^{-1}$ wb and 122 $g.kg^{-1}$ wb of TS, respectively, and 106 $g.kg^{-1}$ wb, 106 $g.kg^{-1}$ wb and 112 $g.kg^{-1}$ wb of VS, respectively. Kryvoruchko et al. (2009) and Carapinha (2012) obtained for potato peel pulp and potato peel, respectively, 16.4% and 6.7% of TS,in which 91.5% and 89.6% of VS, respectively. However Fang et al. (2011) obtained in potato-juice 3.3% of TS and 2.2% of VS.

According to Schieber and Saldaña (2009), the type of waste in the present thesis can be of 15.1% of VS in the set composition, although higher values were found of 22.6% of TS and

21.4% of VS by Kaparaju and Rintala (2005). Zhu et al. (2008), using a liquid substrate of potato waste, reported 10.8 $g.L^{-1}$ of TS in which 10.3 $g.L^{-1}$ were of VS.

Finally, according to Linke's (2006) study on solid wastes from a potato processing industry, a mean value of 128 $g.L^{-1}$ of TS was found in which 117 $g.L^{-1}$ of VS. These are similar values to those obtained in the present thesis.

The total COD (CODt) content of 1,370 g $O_2.kg^{-1}$ db (Table 4.1) in the present thesis is slightly lower than the CODt content of 1,730 g $O_2.kg^{-1}$ db, 1,467 g $O_2.kg^{-1}$ db and 1,565 g $O_2.kg^{-1}$ db in potato peel samples reported by Azeitona (2012), Carapinha (2012) and Santos (2013), respectively.

Raynal et al. (1998) reported a CODt of 126 g $O_2.kg^{-1}$ (wb) lower than the CODt value of 165 g $O_2.kg^{-1}$ wb in the present thesis. Fang et al. (2011) reported a CODt of 25.2 g $O_2.kg^{-1}$ wb related to a potato liquid waste.

The BOD_5t/CODt ratio in the present thesis has shown a value of 0.060. According to Deublein and Steinhauser (2008), to consider a substrate easily biodegradable, this ratio should be equal or higher than 0.500. This ratio is more suitable to test the biodegradability of substrates under aerobic conditions than of anaerobic conditions as in the present thesis; however the ratio value can be compared with the values obtained in other studies to compare its relative biodegradability.

According to Azeitona (2012), and Santos (2013), the ratio of BOD_5t/CODt was of 0.007, which differs from the value found in the present thesis. However, the ratio of BOD_5t/CODt of 0.028 found by Carapinha (2012) is closer to the value obtained in the present study.

Macro and micronutrients are necessary for the microorganisms' growth (section 2.3.5). Particularly in this study, the carbon (C), nitrogen (N), phosphorous (P) and sulphur (S) were analysed within different laboratories and methodologies (DCTB-FCT-UNL and LNEG). The S determination was important for the limited growth of sulphate-reducing bacteria.

The carbon content in the present study found by LNEG was of 43.9%, a lower C content than the 49.4% found in Azeitona's (2012) and Santos' (2013) studies and the 52.9% in Carapinha's (2012) study; the analysis of C in these studies equally performed by LNEG. However, Kryvoruchko et al. (2009) found 45.8% of C content in their potato pulp waste and 41.6% in potato pulp only.

Considering LNEG and DCTB analysis of carbon and nitrogen in the potato peel waste (43.9% of C and 1.56% of N, respectively), the C:N ratio was of 28:1; thus, in line with the optimum C:N ratio found in literature in a range of 20-30:1 (Monnet, 2003; Zupančič and Grilc, 2012; Khalid et al., 2011).

Nevertheless, Azeitona (2012), Carapinha (2012) and Santos (2013) reported a C:N of 20:1 according to LNEG analysis of the potato peel waste in their study.

The N-content (0.8%) in the present thesis determined by LNEG was lower than in the aforementioned studies (2.5%) (Azeitona, 2012; Carapinha, 2012; Santos, 2013).

However, the total nitrogen in DCTB-FCT-UNL has shown a similar value of 14.9 mg $N.g^{-1}$ db in the present thesis as with Azeitona (2012) of 15.5 mg $N.g^{-1}$ db, compared to the 5.1 mg $N.g^{-1}$ db and 6.4 mg $N.g^{-1}$ db of Carapinha (2012) and Santos (2013), respectively. According to

Linke (2006), a significant lower mean value of 2.52 mg $N.g^{-1}$ db of 3 solid potato waste samples were found.

According to Kryvoruchko et al. (2009), a different N value was found of 3.8% in potato peel pulp, although closer to the 1.8% of N found by the same author in potato pulp. The same author reported a lower C:N ratio of 12.1 (corresponding to approximately 12:1) for potato peel pulp waste, closer to the results obtained by Azeitona (2012), Carapinha (2012) and Santos (2013).

A high C:N ratio due to lack of nitrogen present leads to a rapid consumption of nitrogen and to nutrient deficiency, whilst this ratio is not close to the optimum values of 25:1 for AD, according to Deublein and Steinhauser (2008); however, the C:N in the present thesis is in the optimum range mentioned by other authors as referred above.

Different methodologies to find the content of TKN or of N can explain the differences between the values found in DCTB-FCT-UNL and LNEG laboratories or Linke (2006). Additionally, a significant change in the potato peel composition found in each study can be due to a change in the harvest of the potato [e.g. other potato specie or provider], in the agriculture practices [e.g. decrease of nutrients availability in the soil, in the case of N] or seasonal effects during the potato growth in each year.

The mean content of phosphorous determined in the present thesis in DCTB-FCT-UNL, expressed in dry basis, was of 3.95 g $P.kg^{-1}$ db. This value was similar to 3.00 g $P.kg^{-1}$ db reported by Azeitona (2012).

Thus making up a C:N:P ratio of 122:4:1 in present thesis, lower than the obtained by Azeitona (2012) of 192:5:1; nevertheless, closer to the optimum range of 100:3:1 stated by O'Flaherty et al. (2010) and lower than the optimum ratio reported by Bouallagui et al. (2003) of approximately 200:9:1; hence assesses positively the ratio found in present thesis with the literature found.

According to LNEG, the sulphur content (S) of 0.1% found in the potato peel waste of the present thesis is in line with the studies of Azeitona (2012) Carapinha (2012) and Santos (2013), in which a S content of 0.2% was measured in the same waste.

Therefore the C:N:P:S ratio in present thesis was of 439:16:4:1, relatively higher than the 333:7:2:1 optimum ratio stated by Deublein and Steinhauser (2008).

4.2 Pre-treatment Assays

The results presented in this section relate to the assays of mechanical, chemical and thermal pre-treatments to which the potato peel waste was subjected (section 3.3.1). The goal of the pre-treatment trials was to ascertain the improvements in solids content and COD [total and soluble] of the potato peel waste.

The organic load (OL) in Azeitona (2012), Carapinha (2012) and Santos (2013) study was of 400 $g_{potato\ peel\ waste}.L^{-1}$ (200 g wb potato peel waste diluted in 0.500 L of deionised water), slightly higher than the 333 $g_{potato\ peel\ waste}.L^{-1}$ (wb) OL carried out in the experiments of the present thesis.

Thereby, this fact must be assessed while comparing the results in this section with the aforementioned authors' results of the same waste; thus the samples collected of the potato peel waste for analysis in the present thesis were less concentrated than the prior studies mentioned.

The initial mean pH value in all pre-treatment assay with the potato peel waste was of 7.11 ± 0.50 (Table 3.3); this value was higher than the mean pH of 5.90 ± 0.61 in the same waste found by Azeitona (2012) in which a higher acidic medium was present.

According to Kryvoruchko et al. (2009), the pH in the potato peel pulp was of 3.9. However these authors do not state whether the potato peel pulp was mixed with water as in the present experiments or describes the medium in which the pH of that waste was measured. Nevertheless, according to Linke (2006), a similar mean value of pH of 3.87 ± 0.14 was found in potato waste samples.

However, when an acidified medium is found in the beginning of the experiments, Bouallagui et al. (2005) suggest that the correction of the pH values must be conducted with the addition of sodium hydroxide (NaOH). This process is called by neutralisation, in which the samples undergo in the digester for AD in stabilised pH at neutral levels [at pH 8.50 in the present thesis]. This author reported that in the absence of pH control the medium tends to acidify faster, then inhibiting the methanogenic bacteria.

The pH assays were neutralised after the chemical pre-treatments to an observed mean pH value of around 8.51 ± 0.05. However, according to Li et al. (2012), the initial sludge pH should be regulated at a pH lower than 8 after the alkaline pre-treatment, in which at an initial pH 8.5 the AD had not greater effect.

Nonetheless, a high NaOH added to the medium may cause changes in the osmotic potential with sodium high presence, thus conditioning the cellular growth (Charles et al., 2013). In such cases, alternative reagents for the neutralisation can be used [e.g. ammonia or quicklime] to in order to increase the pH, according to Deublein and Steinhauser (2008). According to Li et al. (2013), generally, concentrations of 3.5-5 $g.L^{-1}$ Na^{+} can moderately inhibit the activity of mesophilic methanogens whilst 8 $g.L^{-1}$ Na^{+} can lead to strong inhibition. However, it is not the case in the present thesis in which only low additions of NaOH were performed in assays during the chemical pre-treatment and neutralisation.

Figure 4.1 presents the mean content of solids [i.e. total, fixed and volatile solids] determined through samples of each pre-treatment and neutralisation assays (Table 3.3, section 3.3.1).

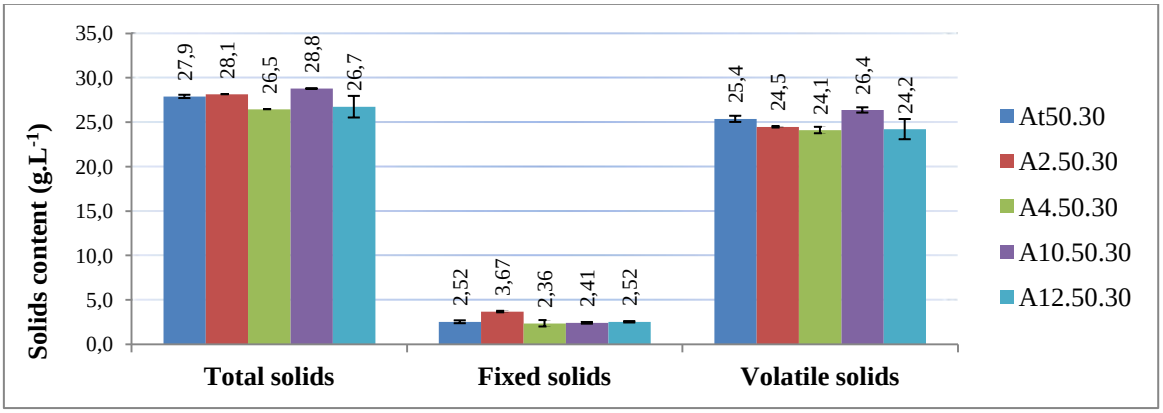

Figure 4.1 - Mean and standard deviation of the contents of total, fixed and volatile solids in each pre-treatment assay (duplicates of samples of each assay were analysed).

The highest content of TS and VS was observed in the assay A10.50.30 of 28.8 $g.L^{-1}$ and 26.2 $g.L^{-1}$ [91.7% of TS], respectively, followed by 28.1 $g.L^{-1}$ TS in assay A2.50.30 and 25.4 $g.L^{-1}$ VS [91.0% of TS] in assay At50.30, respectively. The highest reduction was achieved in assay A4.50.30 (24.1 $g.L^{-1}$) and A12.50.30 (24.2 $g.L^{-1}$), in which a solubilisation has successfully occurred.

Therefore, substantial improvement in the solids content of each assay was observed comparing with assay At.50.30; despite a negative reduction of 3.79% VS in assay A10.50.30.

In Table 4.2 are shown the total and soluble COD in the pre-treated potato peel waste.

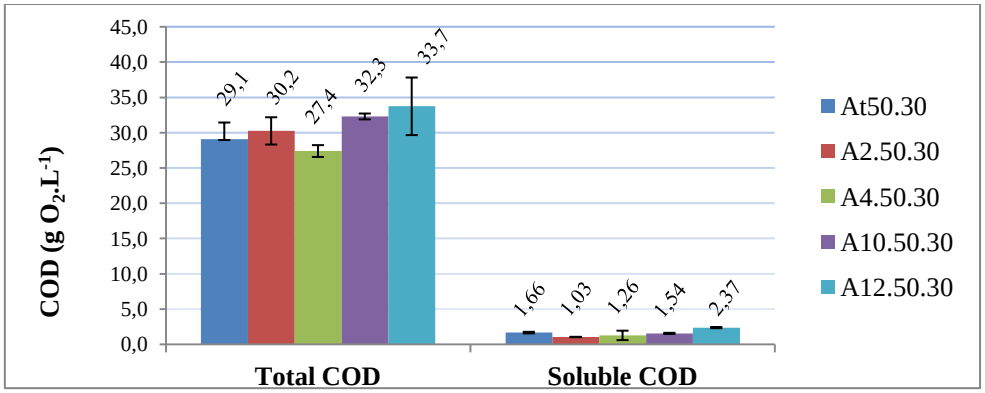

Figure 4.2 - Mean and standard deviation values in terms of total and soluble COD of pre-treatment assay samples (duplicates of samples of each assay were analysed).

A higher CODt in the assay A12.50.30 was obtained. This assay was characterised by a distinct chemical pre-treatment, with the addition of 12.4 mL of NaOH in the sample until it achieved an alkaline medium of pH 12 and then heated during 30 min at 50 ºC. Thereby, an improvement of 15.8% of CODt was obtained in assay A12.50.30 in comparison with the CODt result of the control assay At50.30. This successful result is followed by the assay A10.50.30 with 11.0% CODt improvement over the assay At50.30.

Finally, an increased CODt concentration of 3.78% in assay A2.50.30 was obtained over the assay At50.30. The assay A4.50.30 presented 5.84% less CODt than in assay At50.30.

In terms of CODs, the assay A12.50.30 was improved by 42.8% over the assay At.50.30 with no chemical pre-treatment; therefore a highly successful result in enhancing COD solubilisation.

According to Tyagi and Lo (2011), especially the alkaline pre-treatment at pH higher than 10 yields a significant reduction in microbial density and the release of CODs from sludge waste. The results of CODs in the present thesis are in line with this author report, whereby 42.8% CODs increased in assay A12.50.30 in comparison with assay At50.30; meanwhile, the assay A10.50.30 did not enhanced COD solubilisation over the test assay.

Thus, the COD results have shown greater improvements with the chemical pre-treatment used in assay A12.50.30. These improvements in the solubilisation of the organic matter for AD with the addition of NaOH are in line with the literature (Lin et al., 1997; Lin et al., 1999; Li et al., 2013; Li et al., 2012; Rani et al., 2012; Zheng et al., 2010; Zhu et al., 2010).

Rani et al. (2012) found that a pH of 12 was optimal for better solubilisation of waste activated sludge in AD; also the thermo-chemical pre-treatment was assessed at different temperatures (ranging between 50-80 ºC); 60 ºC was found to be the optimum temperature to enhance COD

solubilisation by 23%; at 50 ºC, COD solubilisation was found to improve by 17%. Furthermore, Rani et al. (2012) sate that as the treatment time was increased from 6 to 24 h, an increase in COD solubilisation was noticed. Thus the pH, temperature and time play a major role in enhancing COD solubilisation.

The greater results of the COD solubilisation test at pH 12 in the present thesis, which improved by 42.8% as mentioned above, are therefore in line with Rani et al. (2012). However, the combination with treatments of temperatures and times should be assessed in further studies, and is not the main focus of this study.

4.3 Anaerobic Digestion Assays

The following results presented in this section relate to the influent and effluent of each anaerobic digestion assays carried out in the digester. A prior mechanical, chemical and thermal pre-treatment was carried out taking into consideration the experimental conditions of the assay A12.50.30; a control assay was also performed taking into account the experimental conditions of the assay At50.30. The assay A12.50.30 was selected because it proved to be the most adequate to solubilise COD from the potato peel residue.

The goal of the following AD trials was to assess the improvements in CODt and VS removal efficiency and, especially, analyse the biogas/methane yields in assay A12.50.30 over assay At50.30.

Thereby, the AD assays results are shown in the next two sections, in terms of pysicochemical parameters and a quantitative/qualitative analysis of the biogas and, within it, methane production.

4.3.1 Pysicochemical Results

The mean pH values of the At50.30 and A12.50.30 AD influent assays were of 8.55 ± 0.01. Figure 4.3 shows the mean temperature inside the digester during each AD assay.

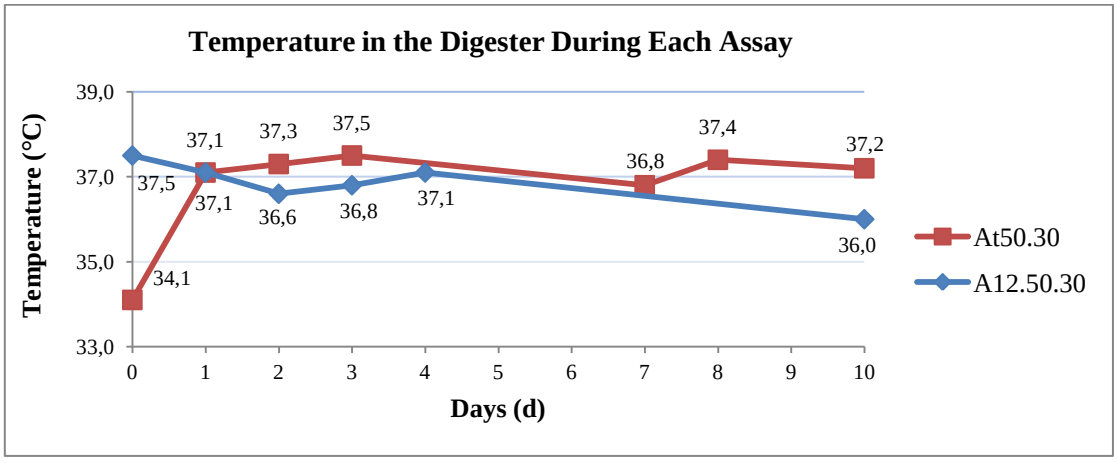

Figure 4.3 - Temperature registered by day during the assays of anaerobic digestion; for this determination, the temperatures were irregularly measured and not continuously in every days.

The temperature decreased in the first day of the test assay At50.30 due to the digester feeding of the assay being at room temperature (approximately 21 ºC), thus cooling the temperature inside the digester at the start-up. However, the assay A12.50.30 temperature was increased in a heated water bath until reaching 38 ºC and then fed to the digester.

The mean temperatures in the assays At50.30 and A12.50.30 were 36.8 ± 1.2 ºC and 36.9 ± 0.5 ºC, respectively. Thus, the goal of reaching an optimum mesophilic temperature of 37 ºC inside the digester was achieved in the AD assays At50.30 and A12.50.30.

The mean room temperature during the AD assays were of 21.6 ± 1.1 ºC and 21.9 ± 1.4 ºC in assays At50.30 and A12.50.30, respectively. Therefore the in site room temperatures were within the natural climate of Mediterranean regions whereby the AD is suitable to operate allowing lower energetic costs at steady temperature up to 37 ºC in the digester.

Figure 4.4 reports the VS, expressed in $g.L^{-1}$, determined through samples of each influent and effluent AD assay. Before the AD assays were carried out in the bench-scale UASB reactor, a sample of the mixture inside the digester have shown a VS content of 10.7 $g.L^{-1}$.

Figure 4.4 presents a higher VS content in the influent At50.30 (28.5 $g.L^{-1}$) than in the influent A12.50.30 (21.3 $g.L^{-1}$, respectively). These results are expected and in line with those obtained in the previous section (4.2); thus an increased concentration of 28.5 $g.L^{-1}$ VS in influent At50.30 and a decreased concentration of 21.3 $g.L^{-1}$ VS in influent A12.50.30 was observed. This difference in the two assays was equally observed in the pre-treatments assays (Figure 4.1); the reduction of VS in assay A12.50.30 can be explained by the solubilisation of these solids caused by the thermo-chemical pre-treatment, benefiting the AD rate of A12.50.30 to be improved.

Nevertheless, the VS content presented in assay At50.30 was similar to those obtained by Santos (2013) and Azeitona (2012) of 27.1 $g.L^{-1}$ and 27.2 $g.L^{-1}$ of VS, respectively, in the control assay within the same conditions of the At50.30 in the present thesis. Additionally, Carapinha (2012) reported lower values in the test trial of 24.8 $g.L^{-1}$ of VS.

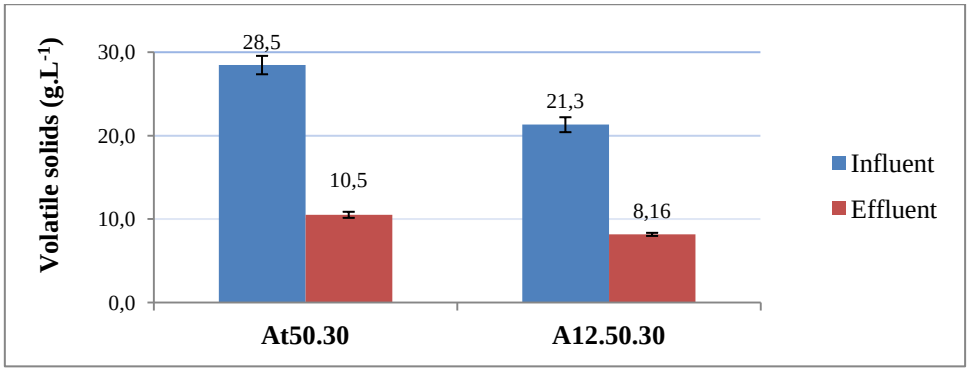

Figure 4.4 - Mean and standard deviation of volatile solids content in each influent and effluent of the AD trials (duplicates of samples of each assay were analysed).

The VS removal efficiency was of 63.1% in the case of At50.30 and higher than the 61.7% found in assay A12.50.30. According to Azeitona (2012), a mean of 75.4% VS removal efficiency was found in the test assay, higher in comparison with the test assay At50.30 in the present thesis. However, Santos (2013) reported a mean of 57.7% and 71.5% VS removal

efficiency, in a mesophilic and thermophilic test trial, respectively; Carapinha (2012) reported a mean of 55% VS removal efficiency in the test assay.

Lin et al. (1997) obtained 38% VS removal efficiency of waste activated sludge (WAS) in the test assay; thereby less efficient than an assay with the addition of NaOH in that study of 52% VS removal efficiency obtained. In other similar studies with WAS or sludge only, the assay subjected to a chemical pre-treatment with NaOH has evidenced improvements in the VS removal efficiency over the test assay (Rani et al., 2012; Zheng et al., 2010; Zhu et al., 2010; Li et al., 2013; Lin et al., 1999; Jang and Ahn, 2013).

For these reasons, the result of VS removal efficiency in A12.50.30 is expected over the test trial At50.30 in the present thesis; however not significant [a difference of merely 1.37%] in terms of efficiency, however a higher solubisation of SV is clear from 28.5 $g.L^{-1}$ (At50.30) to 21.3 $g.L^{-1}$ (A12.50.30).

However, according to Santos (2013), lower VS removal efficiencies were found in some assays compared with their test control, in mesophilic and/or thermophilic conditions, as with Azeitona (2012) and Carapinha (2012) results report.

Figure 4.5 presents the total COD results of the influent and effluent in each AD assay.

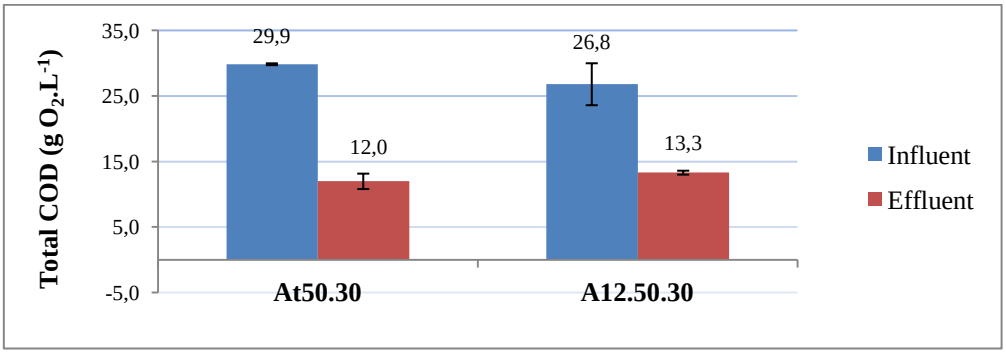

Figure 4.5 - Mean and standard deviation of the total COD in each influent and effluent sample of AD assays (duplicates of samples of each assay were analysed).

The results in Figure 4.5 demonstrate a higher CODt in assay influent At50.30 (29.9 g $O_2.L^{-1}$) over the influent A12.50.30 (26.8 g $O_2.L^{-1}$).

These results are not in line with the higher CODt concentration found in A12.50.30 in the pre-treatment assays (Figure 4.2). However, the higher CODt presence in assay At50.30 is in line with the expected higher VS content shown above (Figure 4.4), which in theory would bring back higher CODt as observed, although VS solubilisation is expected to occur in assay A12.50.30 due to the alkali pre-treatment.

Nevertheless, a standard deviation of 3.2 g $O_2.L^{-1}$ in influent A12.50.30 evidence a higher margin of error than in influent At50.30 of merely 0.3 g $O_2.L^{-1}$; also lower CODt was present in effluent At50.30 with a higher margin of error of 1.2 g $O_2.L^{-1}$ than in effluent A12.50.30.

The removal efficiency of CODt in assay A12.50.30 was of 50.4%, lower than the 59.9% that was found in assay At50.30.

Azeitona (2012) and Carapinha (2012) reported similar results of 57.7% and 50% of CODt removal efficiency in their test assays, respectively. As with Santos (2013), 46.3% and 59.8% of CODt removal efficiency was found in mesophilic and thermophilic test assays, respectively.

These values found in literature are slightly lower than in the test assay carried out in the present thesis.

The result of the CODt removal efficiency in assay A12.50.30 is higher than in all the assays carried out by Carapinha (2012) and some by Azeitona (2012) and Santos (2013) [e.g. assay E122.35 and EM37/70-1.5 in their studies, respectively] of 47.7% and 48.7%, respectively.

However it is not comparable with the 57.8% and 55.3% of CODt removal efficiency found by Azeitona (2012), although these results were obtained under thermophilic conditions. Nevertheless, Santos (2013) achieved overall higher results with a maximum of 76.5% of CODt removal efficiency in a mesophilic assay.

The VS and CODt loads in the digester are compared with each assay in Figure 4.6.

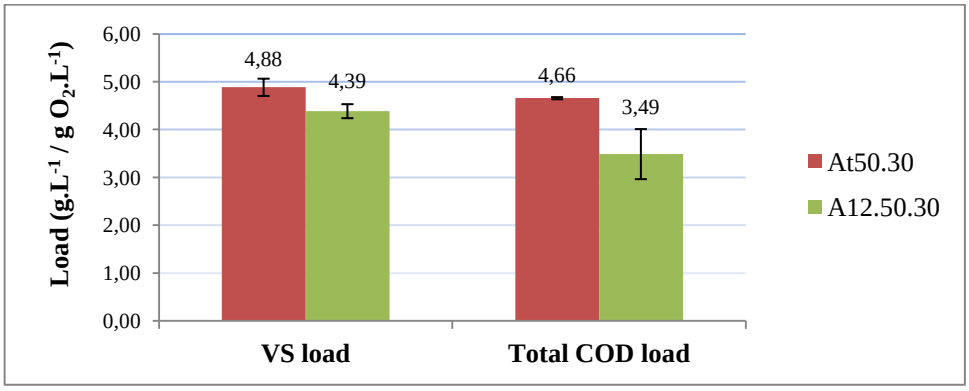

Figure 4.6 - Mean VS and CODt loads introduced in the digester in each AD assay; the load calculation was performed in terms of VS and CODt (duplicates of each assay were used).

For the calculation of these loads the At50.30 and A12.50.30 influent volumes applied in the digester were of 0.450 L for both assays, as previously referred (Table 3.6), in a digester with a working volume of 2.75 L.

Hence, at this point, the VS and COD loads are not directly comparable with the loads found by Azeitona (2012), Carapinha (2012) and Santos (2013) in which the influent volume applied in the digester in all assays was of approximately 0.550 L.

Furthermore, in Azeitona's (2012) and part of Santos' (2013) experiment, a thermophilic biodigester with a lower reacting volume of 2.1 L was used, whilst the mesophilic biodigester used in the present study was of 2.75 L.

Finally, as previously stated, the organic load (OL) in the works of Azeitona (2012), Carapinha (2012) and Santos (2013) was of 400 $g_{potato\ peel\ waste}.L^{-1}$ (200 $g_{potato\ peel\ waste}$ wb diluted in 0.500 L of deionised water), which is higher than the 333 $g_{potato\ peel\ waste}.L^{-1}$ (wb) OL carried out in the experiments of the present thesis.

These differences must therefore be considered when comparing with the aforementioned authors' results.

In the present thesis, the highest VS and CODt loads were of 4.88 $g.L^{-1}$ and 4.66 g $O_2.L^{-1}$, respectively, in assay At50.30.

4.3.2 Quantitative and Qualitative Analysis of the Biogas

The stored volumes in assays At50.30 and A12.50.30 were as shown in Figures 4.7 and 4.8, respectively.

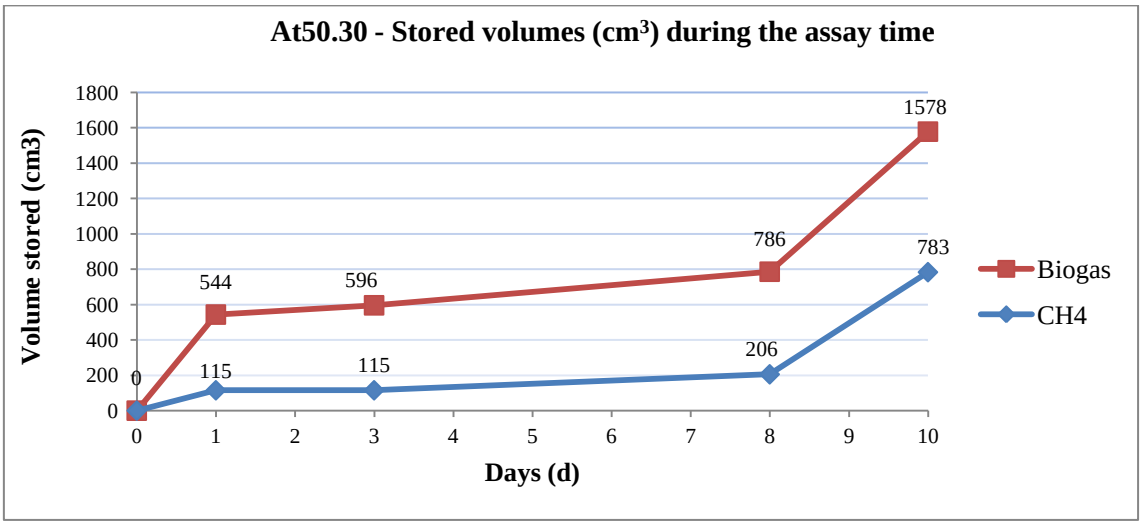

Figure 4.7 - Biogas and methane stored volumes during the anaerobic digestion assay At.50.30 over a period of 10 days.

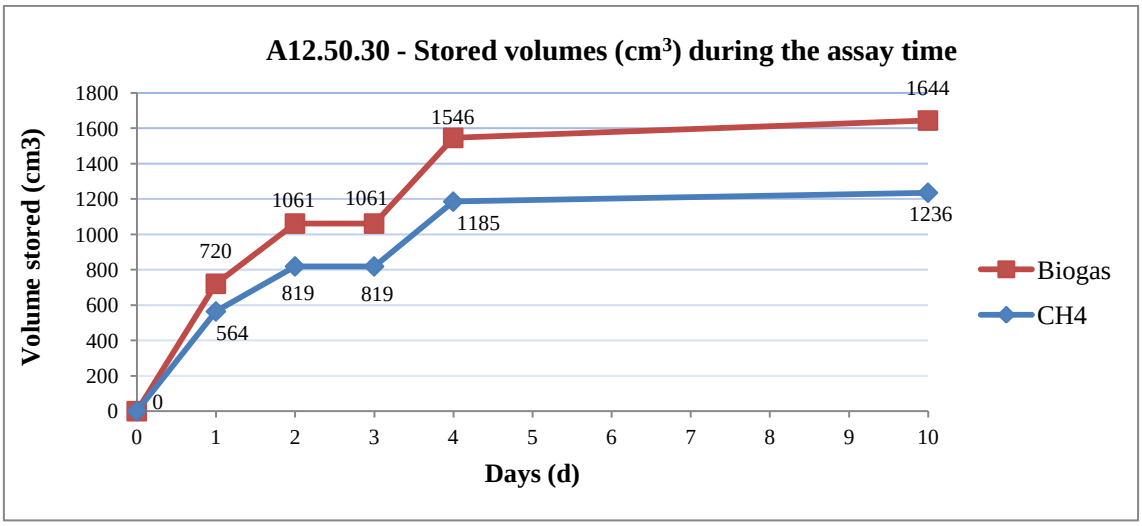

Figure 4.8 - Biogas and methane stored volumes over 10 days of the anaerobic digestion assay A12.50.30.

In assay At50.30, the final biogas volume stored was of 1578 cm^3, whilst in assay A12.50.30 it was of 1644 cm^3, meaning an improvement of 4.18% in biogas production in assay A12.50.30.

On other hand, the total CH_4 volumes stored in assay At50.30 and A12.50.30 were of 783 cm^3 and 1236 cm^3, respectively corresponding to 49.3% and 75.2% of the biogas composition, espectively; therefore in assay A12.50.30 CH_4 production improved by 57.9% in comparison with assay At50.30.

The results above proved that alkaline pre-treatment improved the accessibility of the substrate for AD.

In Azeitona's (2012) experiments, the same substrate used was subjected to thermal pre-treatment of autoclave at 122 ºC during 20, 35 and 55 minutes, to a gauge pressure of 1.2 bar, in thermophilic conditions. In this study, the highest concentration of CH_4 in the final biogas composition was of 82.0% and the assay with the highest CH_4 volume stored presented 22.8% more than the produced in the test assay.

However, Santos (2013) carried out a lower energetic cost experiments with potato peel samples subjected to thermal pre-treatment in a thermostatically-controlled hot water bath at 70 ºC, in mesophilic and thermophilic biodigesters. Additionally, according to Santos' (2013) results over mesophilic conditions, the highest concentration of CH_4 in the final biogas composition was of 81.2% and the assay with the highest CH_4 volume stored presented 13.9% more than in the test assay.

In the present thesis, a lower OL was introduced in the digester in all assays than those introduced by Azeitona (2012), Carapinha (2012) and Santos (2013) in their AD assays. Therefore, a final biogas stored volume approximately 2 to 3 times lower was obtained in both assays of the present thesis comparing with these mentioned studies. According to Linke (2006), when organic loading rate decreases biogas production increases, however referring to a continuously fed reactor.

According to Azeitona (2012) and Carapinha (2012), in their test assay the total CH_4 volumes stored were of 68.8% and 69.8%, respectively. In the same terms, Santos (2013) reported 79.3% and 69.0% in the mesophilic and thermophilic test assays, respectively. Thus, CH_4 production in assay A12.50.30 (75.2%) was higher than all test controls in similar literature to the present study, except in the mesophilic assay test reported by Santos (2013).

In the biogas, the major gases, expressed in cm^3, considered in the present thesis were methane (CH_4), carbon dioxide (CO_2) and oxygen (O_2), whilst of minor gases, expressed in ppm, were hydroxide sulphur (H_2S), carbon monoxide (CO) and hydrogen (H_2).

The results of the major gases in the biogas of assays At50.30 and A12.50.30 were as shown in Figures 4.9 and 4.10,

respectively.

A major production of CH_4 of 564 cm^3 was experienced in the first day of assay A12.50.30; representing 78.3% of the biogas composition. In assay At50.30, a similar volume of CH_4 of 578 cm^3 was only reached on day 10; constituting 72.9% of the biogas composition of that day.

The trend of CH_4 production per day is distinct when comparing assays At50.30 and A12.50.30 (Figure 4.7 and 4.8). In assay At50.30 the trend of CH_4 production per day starts low and steadily decreasing until day 8; and rapidly increasing on day 10 reaching the maximum production.

Whilst in assay A12.50.30 the trend of CH_4 production per day starts with the maximum production, it then decreases to half in the following day. The two subsequent days (day 2 to 4) of decreased production [mean volume of 183 cm^3 per day] were then followed by only residual amounts produced until the last assay day.

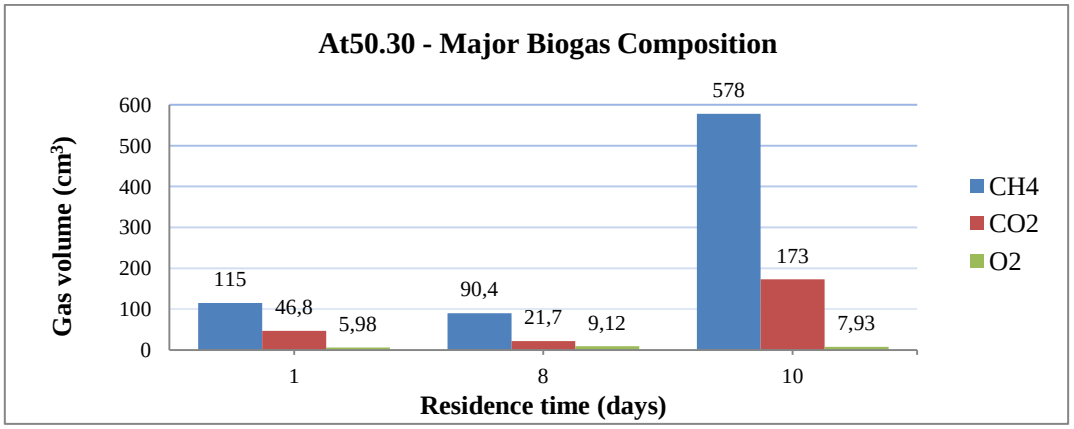

Figure 4.9 - Volume of the major gases in the biogas composition of the anaerobic digestion assay At50.30 over the 10 days of the assay.

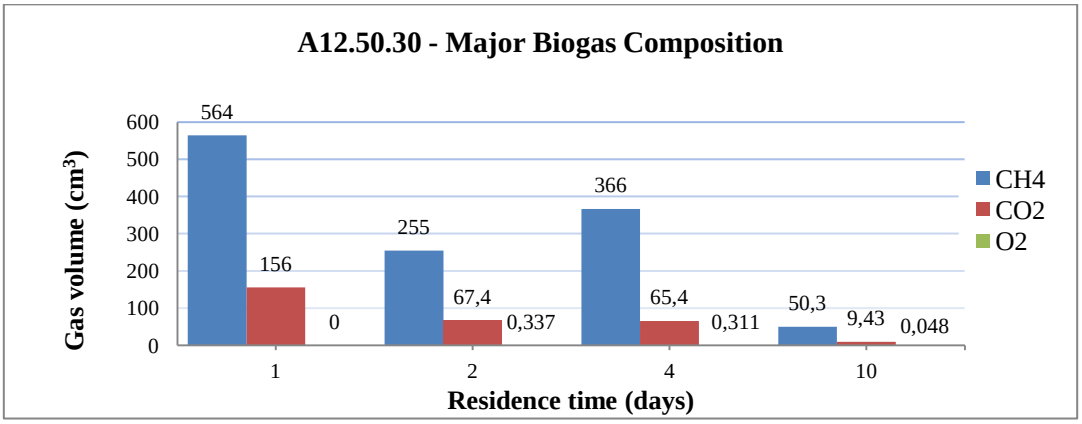

Figure 4.10 - Volume of the major gases in the biogas composition of the anaerobic digestion assay A12.50.30 over the 10 days of the assay.

This trend analysis shows a high degradation rate of the organic matter rapidly consumed from the 1st day of assay A12.50.30 with significant biogas and methane production (564 cm^3 of CH_4 production). This high consumption of organic matter was only similarly observed in the last day of the assay At50.30 (578 cm^3 of CH_4 production). Thus, these results show a methanogenic phase highly active from the 1st day of assay A12.50.30, which is only observed in the last day of the assay At50.30.

The highest CH_4 production in assay At50.30 in the present thesis was of 72.9% out of 578 cm^3 produced during 2 days. However Santos (2013) reported maximum CH_4 production in every 7th AD assay day, in which the mesophilic assay test has showed a higher value (88.1%), as well as the thermophilic test assay (81.9%) than in the present thesis.

Azeitona (2012) reported maximum CH_4 production of 87.9% in the test assay. Finally, Carapinha (2012) reported the highest maximum CH_4 production of 96% of biogas which was stored for approximately 6 days before being measured on the last day of the assay.

The highest CH_4 production in assay A12.50.30 in the present thesis is reported as 78.3% out of 564 cm^3 produced in the first day.

In assay At50.30, 15.0% of CO_2 was present in the biogas composition whilst 15.3% was found in the final biogas composition of assay A12.50.30. In relation to the concentration of O_2 in the biogas, 1.46% and 0.042% was found in assay At50.30 and A12.50.30, respectively.

Thereby, 33.6% and 6.64% of unidentified gas concentration was observed in the final biogas composition of assay At50.30 and A12.50.30, respectively. The unidentified gas is likely to be nitrogen (N), generally as a result of protein digestion, however it was not observed in greater amounts in assay A12.50.30. Thus, the biogas quality is greater in assay A12.50.30 in which a smaller amount of unidentified gas was produced.

Additionally, the O_2 concentration in assay A12.50.30 was lower than in At50.30, thus the anaerobic conditions for methanogenic activity were achieved.

The results of the minor gases in the biogas composition of assays At50.30 and A12.50.30 were as presented in Figures 4.11 and 4.12, respectively.

In assay At50.30, the maximum H_2S production of 810 ppm was registered on day 1, decreasing until its lowest concentration of 10 ppm when CH_4 production was at its highest indicating methanogenic activity. In assay A12.50.30, a minor H_2S concentration of 10 ppm was observed in the last day of the assay, whilst only steadily arising residual amounts were noticed in previous days.

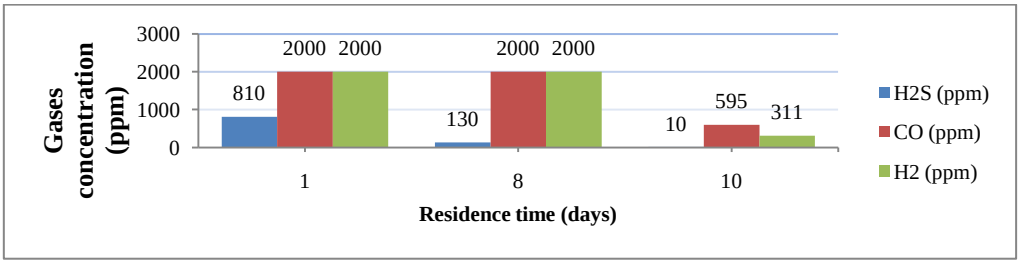

Figure 4.11 - Concentration of the minor gases in biogas of the anaerobic digestion assay At50.30 during the 10 days of the assay.

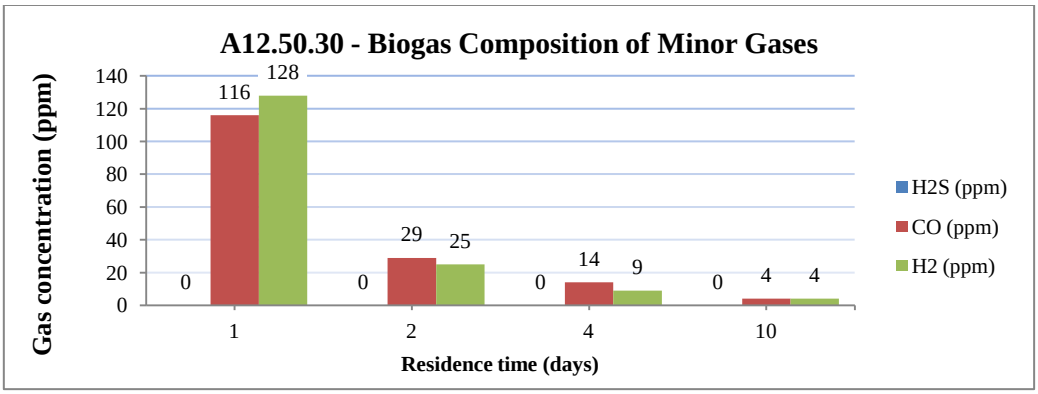

Figure 4.12 - Concentration of the minor gases in biogas of anaerobic digestion assay A12.50.30 over the 10 days of the assay.

H_2S is a corrosive gas for the equipment, thus not desirable in biogas plants (Deublein and Steinhauser, 2008). The biogas quality was for this reason superior in assay A12.50.30 to the test assay in the present thesis.

In assay At50.30, the maximum CO and H_2 concentration of 2,000 ppm each was observed during 8 days in which the gas measuring device used had no higher reading potential for this

particular gas.The high quantity of H_2 strongly indicates above all an inhibited acetogenesis phase in which the presence of this gas affects those specific bacteria, thus not progressing to the next phase of methanogenesis.

However, in assay A12.50.30, the CO and H_2 concentrations were of 128 ppm and 116 ppm, respectively, assuring improved conditions for acetogenesis to occur in the digester. Thereby, the low presence of these gases indicates an improvement in the hydrolysis/acidogenesis rate promoting acetogenic/methanogenic activity.

In Figures 4.13 and 4.14, the mean flow rate per time interval during assay At50.30 and A12.50.30 is as follows.

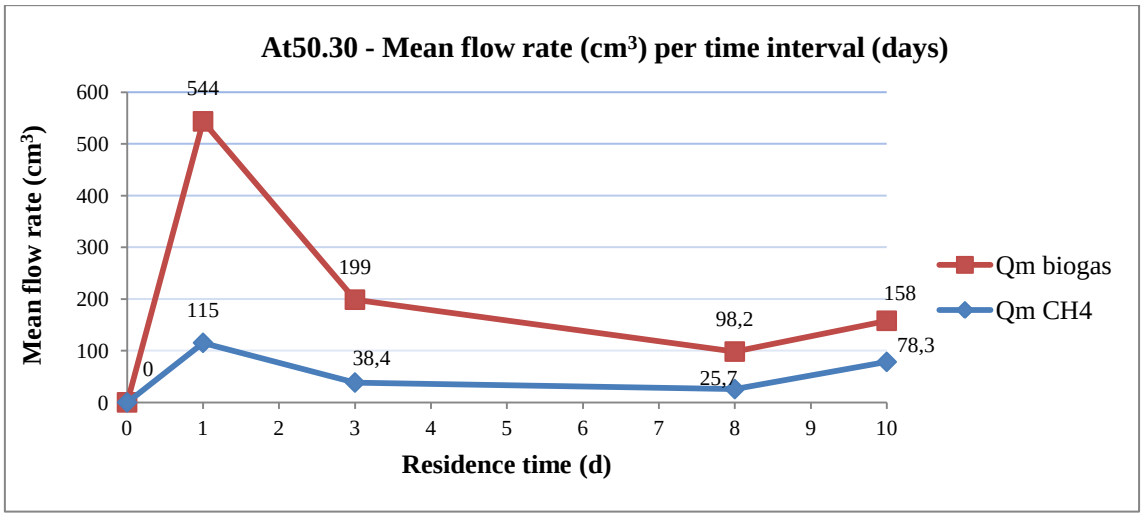

Figure 4.13 - Biogas and methane mean flow rate volumes per time interval during the 10 days of the anaerobic digestion assay At.50.30.

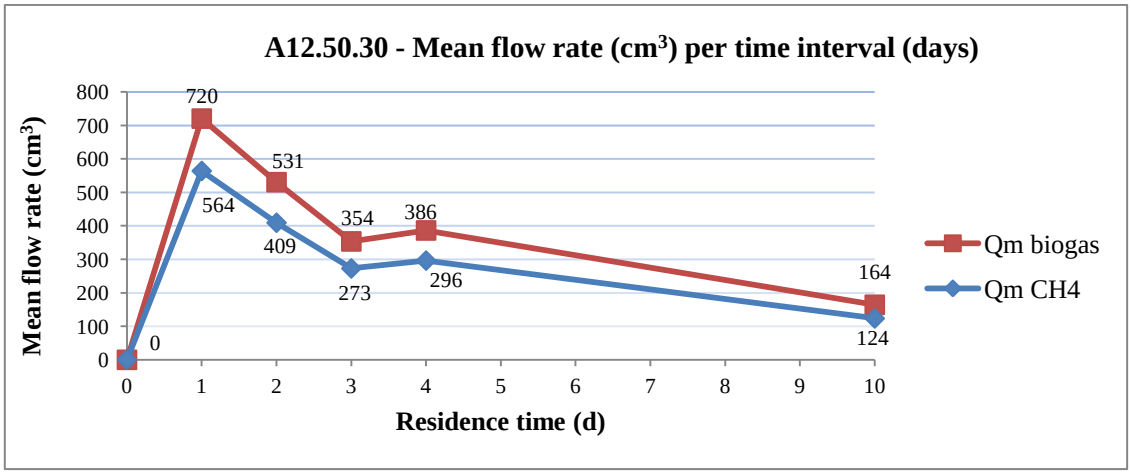

Figure 4.14 - Biogas and methane mean flow rate volumes per time interval during the 10 days of the anaerobic digestion assay A12.50.30.

The biogas and CH_4 mean flow rate (Qm) curves were distinct comparing these 2 assays.

In assay At50.30, the mean flow rate curves start with the highest biogas production whilst showing low CH_4 produced. In the following days a sudden fall in biogas was observed followed by a slower decrease of CH_4. On day 8, the flow rate curves start rising reaching 158 $cm^3.d^{-1}$ of biogas on the last day followed close by a CH_4 flow rate of 78.3 $cm^3.d^{-1}$.

In assay A12.50.30, the mean flow rate curves starts with the highest biogas production (720 $cm^3.d^{-1}$) and the highest CH_4 flow rate (564 $cm^3.d^{-1}$). Over the following days a sudden fall in the flow rate is observed in both curves of Qm biogas and Qm CH_4 (Figure 4.14). On day 3, the flow rate curves steadily increase reaching their peak of 386 $cm^3.d^{-1}$ of biogas and 179 $cm^3.d^{-1}$ of CH_4. Finally, the flow rates continue to decrease until the end of the assay indicating the end of organic matter degradation.

Comparing both assays, the highest biogas flow rate was observed in assay A12.50.30 of 720 $cm^3.d^{-1}$ against 544 $cm^3.d^{-1}$ in assay At50.30. These compared results strongly indicate a higher degradation rate in assay A12.50.30 with alkaline pre-treatment.

The biogas and CH_4 yields per VS and CODt removed in AD assay At50.30 and A12.50.30 were as presented in Figures 4.15 and 4.16, respectively.

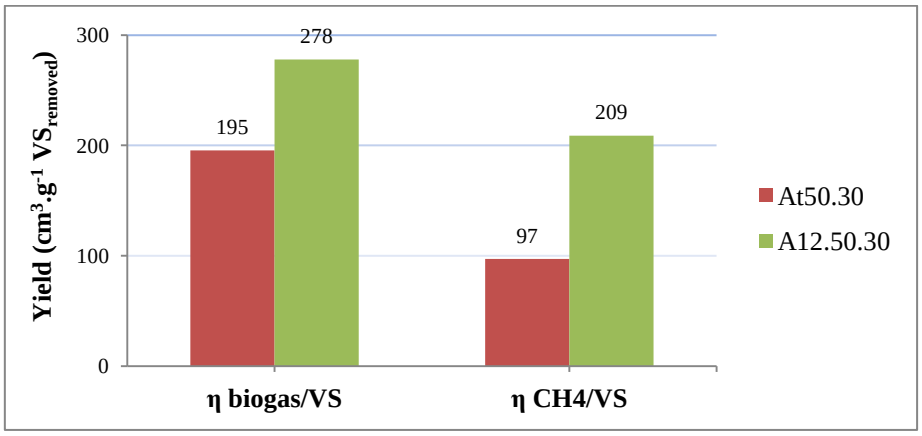

Figure 4.15 - Biogas and CH_4 yields per VS removed in AD assays At50.30 and A12.50.30.

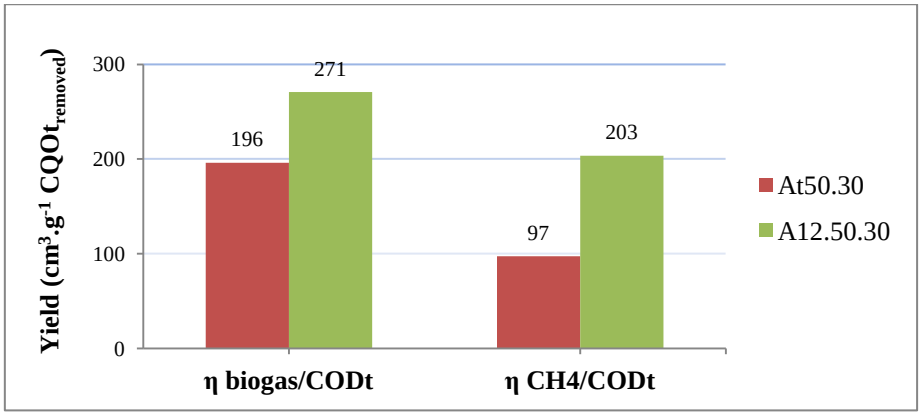

Figure 4.16 - Biogas and CH_4 yields per CODt removed in AD assays At50.30 and A12.50.30.

According to Flor (2006), the criteria to assess the reactors performance is expressed by the maximum rate that these can take, and are represented mainly by the OL introduced in the digester in terms of CODt and VS, and by the production of biogas and methane per digester volume, therefore expressed by cm^3 biogas or $CH_4.g^{-1}$ VS or CODt. These parameters along with the specific biogas and CH_4 productivity and VS and CODt removal efficiencies, define the digester performance in the process of AD, as well as the microbial activity.

In order to assess the digester performance the HRT was not considered; thus not reflecting, in a batch digester, a parameter of analysis when equal amounts of OL were applied once in both assays.

The biogas and methane yield was higher in assay A12.50.30 in all the variables observed than in assay At50.30. These results evidence a greater performance in the digester in assay A12.50.30.

Thereby, the biogas yield was of 278 $cm^3.g^{-1}$ $VS_{removed}$ in assay A12.5030 improved by 42.6% over the yield of 196 $cm^3.g^{-1}$ $VS_{removed}$ in assay At50.30. Noticeably, a CH_4 yield of 209 $cm^3.g^{-1}$ $VS_{removed}$ in assay A12.50.30 was observed over the 97 $cm^3.g^{-1}$ $VS_{removed}$ CH_4 yield in assay At50.30; which constitutes a significant 116% yield improvement in assay A12.50.30.

The biogas yield of 271 $cm^3.g^{-1}$ $CODt_{removed}$ in assay A12.50.30 improved by 38.3% over the 196 $cm^3.g^{-1}$ $CODt_{removed}$ of biogas yield obtained in assay At50.30. A distinct yield was observed in CH_4 of 203 $cm^3.g^{-1}$ $CODt_{removed}$ in assay A12.50.30 over 97 $cm^3.g^{-1}$ $CODt_{removed}$ of CH_4 yield obtained in assay At50.30; a significant 109% increase in assay A12.50.30.

4.3.2.1 Analysis of the Main Results of Anaerobic Digestion Assays

In the present section, the results of the AD assays with potato peel waste will be assessed and compared in terms of their improvements in biogas and subsequent methane production; therefore to ascertain the effect of alkaline pre-treatment in the potato peel waste (assay A12.50.30) toward no chemical pre-treatment carried out (assay At50.30).

Table 4.3 summarises the parameters of efficiency in AD assays: removal efficiency of VS and CODt; final stored volume of biogas and methane; yields of biogas and methane relating to CODt and VS removed; and maximum methane percentages obtained within each biogas composition measurement.

In relation to the removal efficiency of VS, the test assay have showed the best results of 63.1%, closely followed by assay A12.50.30 with 61.7%; a higher removal efficiency of CODt was noticed in assay At50.30 of 59.9% in comparison with 50.4% obtained in assay A12.50.30.

In terms of stored biogas volumes, assay A12.50.30 showed better results of 1644 cm^3 produced against the 1578 cm^3 produced in test assay At50.30. Therefore, the alkaline pre-treatment proved to increase biogas production by 4.18% in the current experiments.

In relation to stored methane volumes, assay A12.50.30 presented better results of 1236 cm^3 produced compared to 783 cm^3 obtained in test assay At50.30, thus proving a 57.9% increase of methane production in the current experiments when subjected to alkaline pre-treatment.

In terms of highest maximum CH_4 percentages in biogas, a superior value of 78.3% was observed in assay A12.50.30 in comparison to 72.9% in assay At50.30. Therefore, 5.4% of maximum CH_4 was observed with alkaline pre-treatment.

In Table 4.4, the biogas and CH_4 yields per VS and/or CODt removed are analysed by contrast with literature found in terms of their maximum improvement over the control assay. In this way, it is possible to establish a direct relation of the success or unsuccessful results of the alkaline pre-treatment between present thesis in face to other pre-treatments assessed in other studies regarding biogas and CH_4.

According to biogas yields associated with the VS removed, the results were greater in assay A12.50.30 - 278 $cm^3.g^{-1}$ $VS_{removed}$ over 196 $cm^3.g^{-1}$ $VS_{removed}$ obtained in assay At50.30; representing improvements of 42.6% in assay A12.50.30.

In Carapinha's (2012) study, in mesophilic AD, the maximum biogas yield improved by 35.7% over the test assay, in which the potato peel sample was thermally pre-treated in autoclave at 122 ºC during 35 minutes.

In comparison with the work of Azeitona's (2012) study, the highest yield of biogas, per VS removed, in a thermophilic digester, was improved by 42.7% over the test assay. In Santos' (2013) experiments, the maximum yield of biogas showed improvements of 32.6% and 18.1% in mesophilic and thermophilic temperatures, respectively, over the test assay.

Therefore, greater results were obtained in the present thesis than Carapinha (2012) and Santos (2013), in terms of biogas yield related to VS removed.

Noticeably, a CH_4 yield of 209 $cm^3.g^{-1}$ $VS_{removed}$ in assay A12.50.30 was observed over the 97 $cm^3.g^{-1}$ $VS_{removed}$ in assay At50.30; thus signifying 116% yield improvement in assay A12.50.30 over the test trial related to CH_4 production [per VS removed].

In Carapinha's (2012) study, in mesophilic regime, the maximum CH_4 yield per VS removed improved 33.6% over the test assay, in which the potato peel sample was thermally pre-treated in autoclave at 122 ºC during 35 minutes. Thus, greater results were obtained in the present thesis in mesophilic conditions with chemical pre-treatment.

In comparison with Azeitona (2012) the highest CH_4 yield [per VS removed], in a thermophilic biodigester, improved by 42.8% over the test trial. In Santos' (2013) experiments, the maximum CH_4 yield presented improvements of 27.0% and 27.7% in mesophilic and thermophilic temperatures, respectively, over the control assay. Therefore, significantly greater results were obtained in the present thesis than Carapinha (2012), Azeitona (2012) and Santos (2013), in terms of the CH_4 yield related to VS removed.

In terms of the biogas yield related to CODt removed, 271 $cm^3.g^{-1}$ $CODt_{removed}$ in assay A12.50.30 improved 38.3% over the biogas yield of 196 $cm^3.g^{-1}$ $CODt_{removed}$ obtained in assay At50.30.

In comparison with Azeitona's (2012) report of biogas yield per CODt removed an improvement of over 60% in all their pre-treated assays was obtained. These results were higher than observed in the present thesis; however thermophilic biodigesters are generally more efficient than mesophilics biodigesters, as previously referred previously in literature found.

Nevertheless, in thermophilic conditions the AD is harder to control and less stable; the bacteria is more sensitive to small and large temperature variations, which cause substantial decreases in their activity, organic loading rate (OLR) and the presence of toxic compounds; it also requires a high amount of energy, giving it a less favourable energetic balance than with mesophilic digestion (Hagelqvist, 2013; Deublein and Steinhauser, 2008; Abbasi et al., 2012)

In Santos' (2013) experiments, a maximum biogas yield of 386 $cm^3.g^{-1}$ $CODt_{removed}$ obtained in a mesophilic biodigester corresponded to an improvement of 22.3%, whilst 431 $cm^3.g^{-1}$ $CODt_{removed}$ obtained in a thermophilic biodigester corresponded to an improvement of 29.0% over the test assay. Therefore, greater results were obtained in the present thesis when comparing the results obtained in mesophilic conditions.

In Carapinha's (2012) study also in a mesophilic biodigester, only one assay carried out had greater improvements in CH_4 yield, of 24.6%, in which the potato peel sample was thermally pre-treated in autoclave at 122 ºC during 35 minutes. Thus, greater results were obtained in the present thesis in mesophilic conditions with chemical pre-treatment.

A distinct CH_4 yield was observed in assay A12.50.30 of 203 $cm^3.g^{-1}$ $CODt_{removed}$ over 97 $cm^3.g^{-1}$ $CODt_{removed}$ of CH_4 yield obtained in assay At50.30; thus a significant 109% CH_4 yield in assay A12.50.30 was observed related to CODt removal.

In Carapinha's (2012) study in a mesophilic biodigester, the maximum improvement in CH_4 yield was of 33.6%, in which the potato peel sample was thermally pre-treated in autoclave at 122 ºC during 35 minutes. Thus, greater results were obtained in the present thesis in mesophilic conditions with chemical pre-treatment.

Considering Azeitona's (2012) study, potato peel waste assays carried out in a thermophilic biodigester have shown CH_4 yield improvements over 60% in all their pre-treated assays was observed.

In Santos' (2013) experiments, a maximum CH_4 yield of 313 $cm^3.g^{-1}$ $CODt_{removed}$ obtained in a mesophilic digester corresponded to an improvement of 24.3%; whilst a maximum CH_4 yield of 337 $cm^3.g^{-1}$ $CODt_{removed}$ in thermophilic digester corresponded to an improvement of 37.4% over the control assay. Therefore, greater and significant results were obtained in the present thesis over the authors results.

Thereby, as observed in Table 4.4, the alkaline pre-treatment improved the efficiency of potato peel waste for biogas and CH_4 production through AD. In comparison with literature found using potato peel and/with different pre-treatments, the chemical pre-treatment in the present thesis showed greater improvements in CH_4 yield per VS removed; furthermore greater improvements on CH_4 yield per CODt removed were found than in results within mesophilic biodigesters.

Finally, in Table 4.4, it is possible to observe greater improvement in the CH_4 yield per VS removed with potato peel waste over corn stover studied by Zheng et al. (2010); therefore, it is an example of another vegetable substrate subjected to alkaline pre-treatment with lower improvement in comparison with to the potato peel substrate.

Table 4.3 - Summary of control parameters assessed of the anaerobic digestion assays.

Assay	VS removal efficiency (%)	CODt removal efficiency (%)	Volume stored of biogas (cm^3)	Volume stored of CH_4 (cm^3)	Biogas yield ($cm^3.g^{-1}$ $VS_{removed}$)	CH_4 yield ($cm^3.g^{-1}$ $VS_{removed}$)	Biogas yield ($cm^3.g^{-1}$ $CODt_{removed}$)	CH_4 yield ($cm^3.g^{-1}$ $CODt_{removed}$)	Maximum CH_4 (% v/v)
At50.30	63.1	59.9	1578	783	195	96.9	196	97.3	72.9
A12.50.30	61.7	50.4	1644	1236	278	209	271	203	78.3

Table 4.4 - Summary of maximum improved biogas and methane yield in anaerobic digestion assays over the test assay in the literature.

Literature	Substrate	Pre-treatment	Thermal Regime	Yield improvement over the test assay (%)			
				Parameters			
				η biogas/VS removed	η CH_4/VS removed	η biogas/Total COD removed	η CH_4//Total COD removed
Present thesis	Potato peel	Chemical pre-treatment with NaOH addition until pH 12; thermal pre-treatment during 30 min at 50 ºC in a hot water bath.	Mesophilic	42.6	116	38.3	109
Carapinha (2012)	Potato peel	Thermal pre-treatment of autoclave at 122 ºC 35 during 35 minutes.	Mesophilic	35.7	33.6	24.6	33.6
Santos (2013)	Potato peel	Thermal pre-treatment in a thermostatically-controlled hot water bath at 70 ºC	Mesophilic Thermophilic	32.6 18.1	27.0 27.7	22.3 29.0	24.3 37.4
Azeitona (2012)	Potato peel	Thermal pre-treatment of autoclave at 122 ºC, during 55 minutes.	Thermophilic	42.7	42.8	61.3	65.3
Zheng et al. (2010)	Corn stover	Treatment with NaOH varying the initial stover time, temperature and moisture level	Thermophilic	42.1	43.0	-	-

5. CONCLUSIONS AND FURTHER RESEARCH

5.1 Conclusions

The present thesis permitted to assess the effect of chemical pre-treatments, with acid (H_2SO_4) and/or alkali (NaOH) addition, combined with a thermal pre-treatment of a potato peel waste as a substrate for anaerobic digestion. The alkali pre-treatment was the most efficient in terms of improving the solubilisation of the waste.

The high production of biogas and, within it, methane, demonstrated that the microbial activity was not inhibited during the anaerobic digestion process of the wastes subjected to chemical pre-treatment, and even enhanced the degradation of the substrate. In terms of biogas and methane final stored volumes, it can be concluded that the alkaline pre-treatment in assay A12.50.30 was effective in enhancing the biogas and methane production with an increment of 4.18% and 57.9%, respectively.

Concerning the highest methane percentages present in the biogas composition, it can be concluded that the alkaline pre-treatment in assay A12.50.30 caused the maximum methane content of 78.3% in biogas on day 1, compared to 21.2% in the test assay of the same day, of the potato peel waste anaerobic digestion; whilst a maximum methane content of 72.9% in the biogas obtained in test assay At50.30 in the last day of the anaerobic digestion.

With regard to the biogas yield, in terms of VS removed, it can be concluded that alkaline pre-treatment was effective in enhancing the biogas yield by 42.6%. Also, concerning the methane yield relating to VS removed, it can concluded that the alkaline pre-treatment was highly effective in the methane yield, which significantly improved by 116%.

In relation to the biogas yield, in terms of the CODt removed, it can be concluded that the alkaline pre-treatment was effective as it improved production by 38.3%. Considering the methane yield relating to CODt removed, it can be concluded that the alkaline pre-treatment was highly effective in the methane yield, which significantly improved by 109%.

To conclude, it can be said that alkaline pre-treatment combined with thermal pre-treatment contributes to enhance the efficiency of potato peel waste for biogas and methane production in all variables assessed. Hence the application of a chemical pre-treatment is justified for potato peel waste, and even recommended when compared with other pre-treatments found in literature, particularly in the case of strictly mesophilic conditions.

5.2 Further Research

During the analysis of the results obtained in the laboratorial assays, some questions have been raised without answer, as others remain as good ideas for further research of AD; using the same organic waste. Below, some of the research that can be developed in the future is described, which could complement the study carried out in the present thesis.

a) Considering that one of the aims of this thesis was to study the chemical pre-treatment, at pH 12 with NaOH, it would be appropriate to study the same effect at pH 9, 10 and/or 11, for example, in order to reduce the dosage of NaOH and, therefore, reducing costs in finding an optimal pH for the alkaline pre-treatment of the potato peel waste. Additionally, it is advisable to evaluate the effect of alkaline pre-treatment in the substrate with alternative reagents, such as quicklime.

b) Concerning the chemical pre-treatment, it can be combined with thermal pre-treatment at different times and a range of temperatures, in order to find an optimal thermal pre-treatment reducing costs and enhancing the overall combined treatment of the potato peel waste. Also, it would be suitable to test combined chemical pre-treatment with steam pre-treatment at different termperatures.

c) In relation to biodigester temperatures, it is recommendable to study the alkaline pre-treatment applied in the present thesis and carry out assays of thermophilic AD to compare the different effects in the results of mesophilic AD.

d) Whereas it was studied the AD of potato peel waste alone, it is suggested the co-digestion of the waste with other by-products of the industry. Particularly with oil waste, in which presents a high biogas production potential because of the high protein content. Although oil is considered a barely biodegradable substrate, the co-digestion with potato peel is advisable in order to speculate the stimulus in biodegradation of the oil waste. Additionally, co-digestion with other types of vegetables is appropriate to assess the effect on biogas production.

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