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Utilização de geopolímeros para controlo de pH em processos anaeróbios de valorização de resíduos orgânicos

Application of geopolymers as pH buffering materials in anaerobic processes treating organic waste

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Engenharia do Ambiente, realizada sob a orientação científica da Doutora Maria Isabel Aparício Paulo Fernandes Capela, Professora Associada do Departamento de Ambiente e Ordenamento da Universidade de Aveiro e da Doutora Maria Paula da Silva Seabra, Investigadora do Instituto de Materiais de Aveiro (CICECO) da Universidade de Aveiro.

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palavras-chave

resumo

Num processo anaeróbio, o pH é um dos parâmetros que mais influencia o funcionamento dos sistemas, afetando tanto as reações bioquímicas como a atividade microbiológica, devido à sensibilidade dos microorganismos a variações de pH. Assim, é essencial o controlo do pH para que haja estabilidade de todo o processo anaeróbio, especificamente quando se tratam substratos facilmente biodegradáveis.

Este trabalho teve como principal objetivo estudar a utilização, a longo prazo, de geopolímeros para o controlo de pH em processos anaeróbios para produção de metano, tratando substratos facilmente acidificáveis. Foram usados reatores descontínuos anaeróbios com 1 L de volume de trabalho e soro de leite como substrato, e efetuados dois estudos. No primeiro estudo, e de modo a selecionar a concentração e tipo de geopolímeros que permitiam a maior produção de metano, utilizaram-se três reatores com adição de geopolímeros com diferente porosidade e em diferentes concentrações e um reator com adição de alcalinidade química (referência). O segundo estudo, com o objetivo de avaliar o comportamento e a reprodutibilidade da ação dos geopolímeros a longo prazo, foi dividido em quatro fases, com adições sucessivas de substrato. Para tal utilizou-se um reator como referência e dois reatores com as condições selecionadas no primeiro estudo do trabalho (tipo e concentração de geopolímeros).

No primeiro estudo observou-se que o reator em que se adicionou o tipo de geopolímeros mais poroso na concentração de 16 g/L foi o que produziu maior volume de metano.

Em ambos os estudos, e de um modo geral, os reatores apresentaram um comportamento semelhante em termos de evolução de pH e CQO, observando-se um aumento nos valores de CQO no período inicial (fase 1) nos reatores com adição de geopolímeros, o qual se deve à lixiviação dos seus componentes orgânicos. Após as várias adições sucessivas de substrato, a diminuição mais rápida dos valores de CQO demonstrou a capacidade de remoção de matéria orgânica e de recuperação do sistema. O rápido consumo dos ácidos orgânicos voláteis sugeriu uma boa adaptação da cultura microbiana para a produção de metano.

Os resultados obtidos confirmam que é possível controlar o pH para produção de metano em processos anaeróbios, utilizando geopolímeros à base de cinzas volantes. Assim, este trabalho pode trazer novas perspetivas para os atuais problemas relacionados quer com o controlo de pH em processos de digestão anaeróbia, quer com a deposição de cinzas volantes em aterro e aos respectivos problemas ambientais associados a este tipo de resíduos.

abstract

In an anaerobic process, pH is one of the parameters which greatly influence the performance of these systems, affecting both chemical reactions and microbial activity, due to the microorganisms sensitivity to pH variations. Hence, it is essential pH control to the entire anaerobic process stability, especially when dealing with easily biodegradable substrates.

The present work had as main objective the study of the long-term utilization of geopolymers for pH control in anaerobic processes for methane production, treating easily acidifiable substrates. It was used anaerobic batch reactors with 1 L of working volume and cheese whey as substrate, and performed two studies. In the first study, in order to select the concentration and type of geopolymers that promote a higher methane volume production, it were used three reactors with the addition of geopolymers with different porosity and concentrations and one reactor with the addition of chemical alkalinity (reference). The second study, in order to evaluate the long-term geopolymers performance and reproducibility, it was divided in four phases, with successive additions of substrate. So, it was used one reactor as reference and two reactors with the selected conditions in the first study (type and concentration of the geopolymers).

In the first study, it was observed that the reactor with the addition of 16 g/L of geopolymers with higher porosity produced the highest methane volume.

In both studies, and in general, the reactors presented a similar pH and COD performance, with an increase in COD values in the start-up period (phase 1) in the reactors with addition of geopolymers, due to the lixiviation of organic compounds from the spheres. After the various successive substrate additions, the fastest decrease in the COD values showed the capacity of organic matter removal and recovery of the system. The rapid VFA consume suggested a good adaptation of the microbial culture methane production.

The results herein obtained confirm that the fly-ash containing geopolymers allow the control of pH for methane production in anaerobic processes. In light of this, this work could bring new insights to the current problems either related with pH control in AD process, or landfill disposal of fly ash and the associated environmental problems of this type of residues.

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Abbreviations

AD	Anaerobic Digestion
C/N	Carbon to Nitrogen
GC	Gas Chromatography
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
sCOD	Soluble Chemical Oxygen Demand
tCOD	Total Chemical Oxygen Demand
DA	Degree of Acidification
FA	Fly Ash
FAN	Free Ammonia Nitrogen
GHG	Greenhouse Gasses
HRT	Hydraulic Retention Time
MK	Metakaolin
OLR	Organic Loading Rate
PEG	Polyethylene glycol
RT	Retention Time
SRT	Solids Retention Time
TAN	Total Ammonia Nitrogen
TOC	Total Organic Carbon
TSS	Total Suspended Solids
VFA	Volatile Fatty Acids
VSS	Volatile Suspended Solids
WWTP	Wastewater Treatment Plant

Chapter 1. Contextualization

Anaerobic digestion (AD) is a process carried out by microorganisms that degrade organic materials under anaerobic conditions, with the formation of biogas, a mixture of methane (CH_4) and carbon dioxide (CO_2) (Chen et al., 2008). According to Appels and colleagues, biogas is one of the most future dominant renewable energy resources, considering that it can provide a continuous power generation (Appels et al., 2011).

Currently, the global energy demand is growing rapidly, and about 88 % of this demand is insured by fossil fuels. Along with the rapid increase of the greenhouse gases (GHG) concentration in the atmosphere and energy security concerns, since most of the known conventional oil and gas reserves are concentrated in politically unstable regions (Weiland, 2010), there is a growing interest in alternative energy sources like biogas. Biogas can be used as replacement of fossil fuels in power and heat production, and it can also be used as gaseous vehicle fuel (Weiland, 2010). In addition, it can be an alternative to minimize pollution (Jha and Schmidt, 2017), considering that the industrialization processes generate large amount of effluents, with high organic content (Rajeshwari et al., 2000).

The efficiency of AD process is highly dependent on substrate characteristics, reactor configuration and various operational parameters (Montañés et al., 2014). Among the various process parameters with influence in AD, the most relevant are the concentration of volatile fatty acids (VFA), temperature, carbon to nitrogen ratio, ammonia concentration, organic loading rate, retention time and pH (Neshat et al., 2017).

The pH value is one parameter which greatly influence the digestion process (Hagos et al., 2017). It affects both chemical reactions and microbial activity (Montañés et al., 2014), due to the microorganisms sensitivity to pH variations (Braguglia et al., 2017). Hence, pH regulation is essential to the process stability and it can be accomplished by

manual or automatic methods. Currently, the most used method to control pH is the addition of chemical compounds, which may have some side effects that can lead to the process inhibition (Neshat et al., 2017). A recent approach is the use of porous biomass fly ash-containing geopolymers, which have the ability to promote pH control over time (Novais et al., 2016b). The production of this type of geopolymers contributes also to the material valorization of fly ash, thus decreasing the need of its disposal in landfill and associated environmental problems (Novais et al., 2018).

Along these lines, the dissertation main objective was the study of the long-term utilization of geopolymers for pH control in anaerobic reactors for methane production, treating easily biodegradable substrates. Therefore, the experimental work was divided in two studies: the first one was the optimization of the concentration and type of geopolymers to be used, and the second study aimed to evaluate the long-term geopolymers performance and reproducibility in anaerobic systems.

Chapter 2. State of art

2.1. Introduction

In the energy infrastructures of today the use of fossil fuels is considered the largest source of anthropogenic emissions of carbon dioxide, which is considered the main cause of global warming and climate change (Deepanraj et al., 2017). Thus, due to the finite nature of fossil fuels and their negative environmental effects, there has been a growing interest in alternative energy sources like the renewable ones (Kumanowska et al., 2017).

Examples of renewable energy resources are solar, wind, geothermal, hydropower and biofuels such as biogas, biodiesel, and bio-ethanol (Deepanraj et al., 2017). Biogas is mostly composed of carbon dioxide and methane, which is also one of the greenhouse gases but it could be captured and valued in renewable energy (André et al., 2017). This gas can be produced from a wide range of solid or liquid wastes through anaerobic digestion processes (Deepanraj et al., 2017) and is essentially used for thermal and electrical renewable energy production by combustion in combined heat and power plants (Gaida et al., 2017). Alternatively it can be upgraded to natural gas purity and be used in the production of electricity, heat and steam in household and industry, injected into the natural gas grid or used as a vehicular fuel (Ullah Khan et al., 2017).

Anaerobic digestion is a biological process in which a group of microorganisms biodegrade organic matter (substrate) in the absence of oxygen (O_2) (Montañés et al., 2014). This is not a newly emerged treatment and Alessandro Volta, who studied the relationship between organic loading and gas production, conducted the first study in 1776. The process has long been used as an energy providing method, especially in Asian countries such as China and India (Neshat et al., 2017).

The EU's renewable energy directive sets that by 2020 20% of the final energy consumption is from renewable sources (Kumanowska et al., 2017). Therefore, anaerobic

digestion is an important method to achieve this objective. The number of anaerobic digestion systems had increased rapidly in the last years especially in Europe (Lora Grando et al., 2017). This is the result of financial incentives for renewable energy facilities, governmental policies on climate change and an increasing energy need (Fagbohungbe et al., 2017). According to Ullah Khan and co-workers, it is estimated that biogas usage in the world will be doubled in the next years, growing from 14.5 gigawatts (GW) in 2012 to 29.5 GW in 2022 (Ullah Khan et al., 2017).

2.2. Anaerobic Digestion

Anaerobic digestion is a biological process under anaerobic conditions (absence of oxygen) in which a microbial consortium breaks down complex biodegradable organic matter into different end products as methane (approximately 50-80%), carbon dioxide (approximately 30-50%) (Lora Grando et al., 2017) and traces of other gases such as hydrogen and nitrogen (Kamali et al., 2016).

By contrast with other bioenergy technologies, AD can be applied to a high diversity of substrate compositions, even those with high moisture content and impurities (Appels et al., 2011; Xu et al., 2017), as long as they contain carbohydrates, proteins, fats, cellulose, and hemicelluloses as main components (Weiland, 2010). Consequentially, different types of microorganisms are involved in the degradation process (Lin et al., 2017).

The nature of the organic residue can be diversified and grouped in different categories: sewage sludge, animal manures, food industry wastes, energy crops and harvesting residues, organic fraction of municipal solid waste (Romero-Guiza et al., 2016) and wastewater sludge (Yang et al., 2016). According to Lora Grando and co-workers, the distribution of potential sources of biogas at world level are 75% W_w in agricultural crops, 17% W_w in municipal and industrial organic waste and 8% W_w in sewage wastewater treatment facilities (Lora Grando et al., 2017).

Wastewater plants involves the biological treatment of solid materials and transformation of dissolved and suspended organic matter to a large volume of sludge with high organic content and a host of pathogenic vectors (Yang et al., 2016). The sludge disposal is a potential source of soil and water pollution and, according to Neumann and colleagues, its management can represent more than 50% of the total cost of wastewater treatment (Neumann et al., 2016). Thus, wastewater sludge must be treated or stabilized

prior to environmental disposal and AD is the most widely used technology for the treatment (Yang et al., 2016).

Anaerobic digesters can be operated at different modes, namely continuous, semi-continuous or batch. In batch systems, a reactor is loaded with feed and will run until methane production stops. This type of reactors benefit from technical simplicity, low operating costs, and short digestion times (Braguglia et al., 2017). The anaerobic biological system can have one or two separated stages, where in one stage the microorganisms are kept together in a balance and with two stages there is a physical separation between the acidogenic and methanogenic phases (De Gioannis et al., 2017). Additionally, the reactors can be continuously stirred, using an intermittent stirring mode, or not be stirred at all. In an intermittent mode, the stirrer is turned on and off according to a preset time interval that can range from a few seconds of stirring per day to an almost continuous stirred mode (Lindmark et al., 2014).

In addition to methane production, AD has several advantages over other conventional techniques, such as the reduction of the produced sludge volume by 30–70% comparing with aerobic processes, design simplicity, non-sophisticated equipment requirement, cost-effectiveness in terms of low capital and operating cost, applicability in different scales and a high rate of pathogen destruction (Kamali and Khodaparast, 2015). However, the process efficiency can be influenced by a high number of factors, such as environmental conditions (e.g., pH, C:N ratio and retention time), by-products (e.g., volatile fatty acids and ammonia), physical and chemical properties of the substrate (e.g., nutrient content) (Mao et al., 2017) and reactor configuration (Montañés et al., 2014). Hence, the main difficulties of the process are the operational instability, the quality of the digested product and the substrates that can generate metabolic intermediates that are inhibitory of the microbial activity (Fagbohungbe et al., 2017).

The anaerobic decomposition process of organic matter can be divided into four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis; in **Figure 1** is presented a simplified scheme of the process. There are two main groups of microorganisms involved in the AD: Bacteria (acidogens and acetogens) and Archaea (methanogens). Bacteria decompose complex substrates into volatile fatty acids (VFA), CO_2 and H_2, while Archaea are responsible for methane production (Ren et al., 2017). These two groups differ in terms of physiology, nutritional needs, growth rates, and sensitivity to environmental conditions (Fagbohungbe et al., 2017; Jha and Schmidt, 2017). The primary cause of reactor instability occurs when the balance between these

two groups is not maintained (Chen et al., 2008) and, consequently, microorganisms can be indicators of the AD process stability or failure (P. Wang et al., 2017).
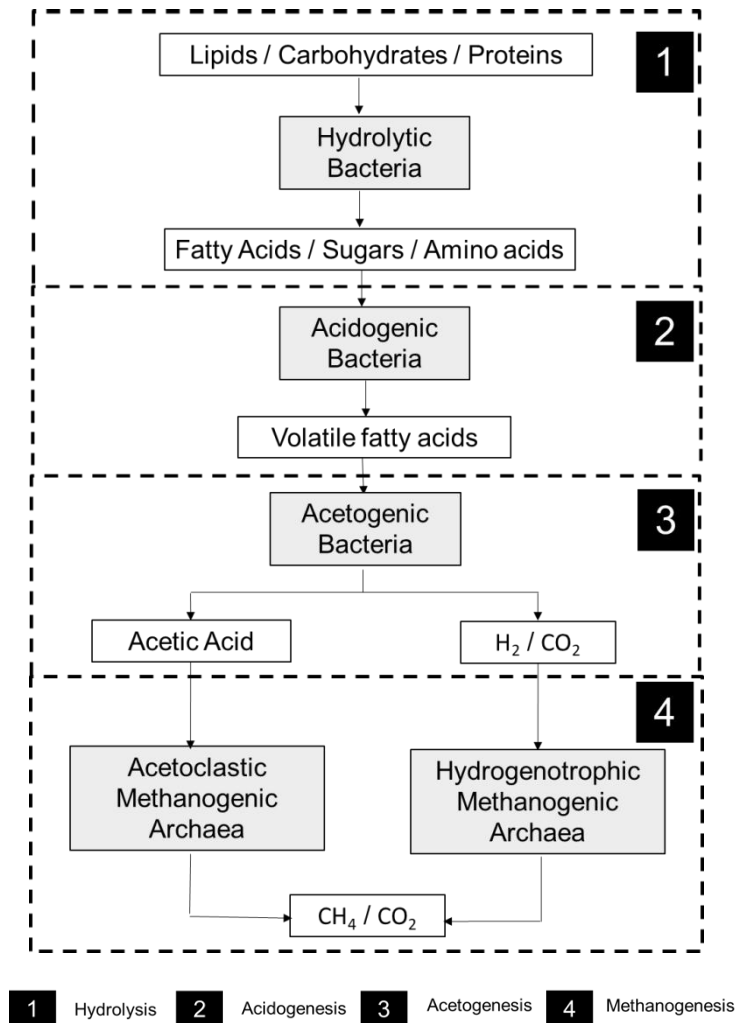
Figure 1: Schematic representation of the anaerobic digestion process, adapted from (Almeida Streitwieser, 2017; Zhang et al., 2014).

2.2.1.   Hydrolysis

Hydrolysis is the first step of the AD process, where the complex organic matter (polymers) are decomposed into smaller units (mono and oligomers) (Matheri et al., 2017). During this phase, organic matter is broken into easily dissolved monomers, including the transformation from carbohydrates, protein and fat to sugar, amino acid and long-chain fatty acid, respectively (Ren et al., 2017), by the action of a diverse community of hydrolytic bacteria (Braguglia et al., 2017) and their extracellular enzymes; as displayed

in **Figure 1**. The decomposition of proteins generally takes place faster than the transformation of carbohydrates (Adekunle and Okolie, 2015).

The organic matter composition in the substrates has a strong impact on AD performances, which shows the existence of a relationship between the quantity of methane produced and the organic matter used, not only the biodegradable fraction but also the non-biodegradable fraction (Nielfa et al., 2015). However, the non-biodegradable compounds are resistant to biological degradation (Bouallagui et al., 2005).

Hydrolysis is considered the rate limiting step for complex, hard biodegradable organic substrates, due to the formation of toxic by-products or non-desirable volatile fatty acids (Ren et al., 2017) and can be accelerated by pre-treating the substrate before digestion (Braguglia et al., 2017).

2.2.2. Acidogenesis

The second step of the AD process is acidogenesis, where sugars and other monomeric organic products obtained from hydrolysis are converted into volatile fatty acids, predominantly acetic, propionic, formic, butyric and lactic (Matheri et al., 2017), alcohols, hydrogen and carbon dioxide (Jha and Schmidt, 2017). VFA are monocarboxylic aliphatic acids, produced from a series of complex biochemistry reactions by the action of acidogenic and acetogenic bacteria (Fang et al., 2017; Lee et al., 2017).

The by-products obtained differ with the types of bacteria present as well as the environmental conditions (Braguglia et al., 2017). Carbon dioxide, hydrogen and acetic acid skip the third stage, and are converted directly by the methanogenic bacteria in the final stage to produce biogas, composed mainly by methane and carbon dioxide (Matheri et al., 2017).

This is the fastest phase in the AD process, therefore if the feedstock does not have suitable buffering capacity and/or the organic loading rate is too high, occurs a rapid accumulation of VFA. This accumulation may result in pH drop that will inhibit the methane production in the last step (Braguglia et al., 2017). Hence, this inhibition results in lower biogas output and even failure of the system (D. Wang et al., 2017).

2.2.3. Acetogenesis

As represented in **Figure 1**, in the third phase of the anaerobic digestion process, the volatile fatty acids produced in acidogenesis, are converted into acetic acid, hydrogen

(Jha and Schmidt, 2017; Ren et al., 2017) and carbon dioxide (Zhang et al., 2014). The hydrogen and carbon dioxide are reduced to acetic acid by homoacetogenic microorganisms (Braguglia et al., 2017). The by-products are used in the last step of the process by methanogens for methane production (Jha and Schmidt, 2017; Zhang et al., 2014).

The acetogenic bacteria can only survive at a very low hydrogen concentration, so if occurs an excessive production of hydrogen from the acidogenesis step, these bacteria can be inhibited (Braguglia et al., 2017).

2.2.4. Methanogenesis

The increased attention towards anaerobic digestion is due to the generation of energy (Jha and Schmidt, 2017), which happens in the last phase of the biological process, called methanogenesis. The methanogens are able to convert the products of acetogenesis (Jha and Schmidt, 2017) in to methane under strict anaerobic conditions (Braguglia et al., 2017).

Methanogens are divided into two main groups based on their substrate conversion capabilities: acetoclastic methanogens and hydrogenotrophic methanogens (Akindele and Sartaj, 2017; Montañés et al., 2014). Acetoclastic methanogens convert acetate into methane and carbon dioxide and are responsible for a fraction of about 0.7 of the methane produced. The hydrogenotrophic methanogens consume hydrogen and carbon dioxide to produce methane and are responsible for maintaining the partial pressure of H_2 at a very low level (<10 Pa), which is a needed condition for the process stability (Akindele and Sartaj, 2017; Montañés et al., 2014).

This is considered the limiting step in the production of biogas since methanogens grow slowly, resulting in a relatively small population (Montañés et al., 2014). According to Almeida Streitwieser, under mesophilic conditions (temperatures between 30ºC and 40ºC (P. Wang et al., 2017)), the methanogenesis is the slowest step and the acetoclastic methanogens are the main producers of methane (Almeida Streitwieser, 2017).

2.3. Anaerobic Digestion Process Parameters

The anaerobic digestion process occurs through the interactions of many biotic (microbial community) and abiotic (reactor parameters) factors. The understanding of

process performance and microbial community during AD is necessary to determine the optimum operating conditions (Watanabe et al., 2017). The bioreactor parameters with main influence on the process performance and stability are the concentration of VFA, temperature, carbon to nitrogen ratio, ammonia concentration, organic loading rate, retention time and pH (Neshat et al., 2017).

Considering the process instability at achieving optimum biogas production (Jin et al., 2016), precise control of these parameters is crucial, as any deviation from their optimum levels can cease the whole process (Neshat et al., 2017).

2.3.1.   Volatile Fatty Acids

VFA are short-chain fatty acids that contain from 2 to 5 carbon atoms (Jankowska et al., 2017). A high number of soluble organic acids are included in VFA, although the major components are acetic, propionic, butyric, and valeric acids (Khan et al., 2016). Among this four acids, acetic and propionic play a dominant role in biogas production (Zhang et al., 2014). Acetic acid is usually present in higher concentrations than other fatty acids. However propionic and butyric acids have a higher inhibitory effect to methanogens (Weiland, 2010) and propionic acid degradation rate is the lowest among VFA (Neshat et al., 2017). According to Montañés and co-workers, acetic acid is the least toxic fatty acid, whereas an increase in propionic acid concentration is associated with a system failure (Montañés et al., 2014).

Besides being intermediate compounds and indicators of the process state, VFA are also essential buffering agents in the AD system (Shi et al., 2017). As potentially renewable carbon sources, they have a wide range of applications, such as biological removal of nitrogen and phosphorous (Jankowska et al., 2017; Ma et al., 2016), production of biopolymers (Jankowska et al., 2017), production of biodiesel, generation of electricity through microbial fuel cells and synthesis of complex polymers (M. Zhou et al., 2017). In addition to applications of mixed VFA, individual components showed a higher application potential, for example, butyric acid can be used as a building block for pharmaceuticals. Presently, VFA and their derivatives are widely used in food, textile, pharmaceutical, leather and plastics industries (M. Zhou et al., 2017).

Currently, volatile fatty acids are mostly obtained during chemical routes from non-renewable petrochemicals; however due to intensive exploitation of oil resources and wide range of application, the VFA production from biological routes has gained more

importance (Jankowska et al., 2017). The anaerobic digestion process has been primarily applied to produce methane containing biogas. However, according to Khan and co-workers, it can also be designed to produce volatile fatty acids and biohydrogen, separately or simultaneously with biogas production (Khan et al., 2016). Hydrogen is known as an ideal, clean and renewable energy, due to only water is generated after its oxidative combustion (M. Zhou et al., 2017) and the energy density per mass is 2,5 times compared to fossil fuels (Khan et al., 2016).

Comparing with methane, the added value of volatile fatty acids is higher and their storage and transportation is easier and safer (M. Zhou et al., 2017). According to Jankowska and co-workers, the acetic acid has the highest market size, next to propionic acid, and the smallest market is for butyric and caproic acids. On the other hand, caproic and butyric acid have the highest market prices, followed by lactic acid and propionic acid; the lowest price is for acetic acid (Jankowska et al., 2017).

The operational stability of AD process is highly dependent on the accumulation of VFA (García-Sandoval et al., 2016), the products of acidogenesis and acetogenesis (M. Zhou et al., 2017). As prior mentioned, when methanogens cannot utilize hydrogen and VFA as quickly as they are produced by acidogens and acetogens, it occurs an accumulation of VFA (Shi et al., 2017). This accumulation could cause an acidification of the system (Cavinato et al., 2017), which will leads to a pH drop to a level below 6 (Neshat et al., 2017) resulting in the reduction in biogas yields, and even in the failure of the digester (Montañés et al., 2014).

According to Neshat and co-workers, in a stable anaerobic digester, the concentration of VFA is about 50–250 mg/L, while a concentration of about 1500–2000 mg/L can possibly inhibit the methane formation (Neshat et al., 2017). Hence, VFA have been widely used as sensitive and reliable indicators to control and optimize the process, considering that they reflect metabolic imbalance when operating parameters suddenly change or inhibition occur (Jin et al., 2016). The best strategy for stopping the acidification of the system due to the increase of VFA concentration is to cease the feeding to the system and let the acetoclastic microorganisms grow and increase the pH level by consuming the VFA (Neshat et al., 2017).

The amount of organic content being hydrolyzed is the primary factor that is directly responsible for the quantity of VFA produced (Khan et al., 2016). The most common way to produce these intermediate products is throughout the degradation of protein and polysaccharide, since they are the dominant macromolecular organic matters (Zhang et al., 2017). To increase the production of VFA, some strategies can be used, such as

improving hydrolysis rate to produce more soluble substrates for further fermentation, promoting acidogenesis and removing the inhibiting factors (M. Zhou et al., 2017).

There are several methods for monitoring the VFA content in a liquid sample, such as titration method, gas chromatography (GC), high performance liquid chromatography and mid-infrared spectroscopy. However, as described by Jin and co-workers, these methods are time consuming, inaccurate, expensive and typically tested manually (Jin et al., 2017). According to Lee and co-workers, CG analysis has a simple procedure, small sample requirement, and relatively low detection limit (Lee et al., 2017).

2.3.2. Temperature

Temperature, one of the most essential parameters in AD process (Nielsen et al., 2017), has a direct influence on the thermodynamic equilibrium of the biochemical reactions of the process and also controls the activities, growth rate and diversity of the microorganisms (Khan et al., 2016). Considering that the growth of microorganisms and the activity of the enzymes is only effective at certain temperatures, this parameter has an important influence on the hydrogen partial pressure of the system, and hence the metabolic pathway of the bacteria could be affected (Liu and Lv, 2016). Temperature not only influences the activity of enzymes and co-enzymes, but also the methane production and digestate (effluent) quality (Zhang et al., 2014).

The metabolic activity of microorganisms is only possible in certain temperature ranges and, in addition, a maximum activity is obtained within this interval for pure species. However, AD is carried out by a complex mixed population that have specific temperature ranges (Fernández-Rodríguez et al., 2013). In hydrolysis and acidogenesis phases, temperature variations can affect hydrolytic bacteria, which are responsible for the degradation of complex materials. Also, acidogenic bacteria cannot tolerate high temperature changes and are active in a specific temperature range; deviation from optimum temperature can cause acidification due to the accumulation of VFA (Neshat et al., 2017). In addition, acetogenesis and methanogenesis can only be performed by certain specialized microorganisms (acetogenic and methanogenic), which are very sensitive towards temperature change (Rajeshwari et al., 2000). Any temperature fluctuation can seriously affect the whole process (Neshat et al., 2017). As an example, methanogenic bacteria cannot tolerate temperature fluctuations over 1°C/day (Neshat et al., 2017).

Methane is formed over a wide range of temperatures, from low to high temperatures, though not over 65 °C. The different temperature ranges for methane formation can be defined by the microbial activity (Matheri et al., 2017), and are presented in **Table 1**.

Table 1: Conditions for anaerobic microorganisms growth, adapted from (P. Wang et al., 2017; Zhang et al., 2014).

Conditions	Temperatures ($^{\circ}C$)
Psychrophilic	10 - 30
Mesophilic	30 - 40
Thermophilic	50 - 60

An increase in process temperature causes a higher microbiological activity and metabolism, hence, the substrate consumption rate is higher (Fernández-Rodríguez et al., 2013; Liu and Lv, 2016), which will result in an increment in AD performance (Zhang et al., 2014). Accordingly, under thermophilic conditions, AD has a shortened retention time (Watanabe et al., 2017) and higher metabolic and specific growth rates (Zhang et al., 2014).

With higher temperatures, the reaction and gas yields are faster (Matheri et al., 2017), therefore thermophilic conditions have higher biogas production (Zhang et al., 2014). It is important to keep a constant temperature during the digestion process, as temperature changes or fluctuations will affect the biogas production negatively (Weiland, 2010). Nonetheless, according with Neshat and co-workers, higher temperatures generate higher biogas production, but not higher methane yields. Considering that biogas mainly consists of methane and carbon dioxide, higher biogas production with lower methane content means increased amount of CO_2. This corresponds to lower heating value of the produced biogas and the need for further purification processes (Neshat et al., 2017).

Thermophilic process has some disadvantages comparing with mesophilic conditions. The process is less stable (Matheri et al., 2017; Watanabe et al., 2017), more sensitive to environmental changes (Mao et al., 2015; Zhang et al., 2014), needs more energy to maintain the constant temperature of the reactor, is harder to control (Hagos et al., 2017), needs larger investments (Mao et al., 2015) and has a higher risk of ammonia (Wang et al., 2014; Weiland, 2010) and volatile fatty acids inhibition (Neshat et al., 2017; P. Wang et al., 2017).

Mesophilic AD is more widely used, compared with thermophilic digestion (Kamali and Khodaparast, 2015). The main advantages are suitable operating performance, stability, less sensitivity to inhibitors (Neshat et al., 2017), less energy requirements

(Kamali and Khodaparast, 2015) and higher richness in bacteria (Mao et al., 2015). A very diverse microbial population is expected under mesophilic temperatures, which could be beneficial to degrade various types of substrates (Kim et al., 2017). The mesophilic bacteria cannot survive in the thermophilic range of temperature, whereas thermophilic bacteria can survive in mesophilic range of temperature, but their growth rate is slower (Hagos et al., 2017).

2.3.3. Carbon to Nitrogen Ratio

The carbon to nitrogen ratio is an important indicator for controlling biological treatment systems (Wang et al., 2012). In anaerobic digestion processes, it represents the relationship between the amount of carbon and nitrogen in the organic materials (Matheri et al., 2017), furthermore is one of the main parameters that critically affects the whole process (Hagos et al., 2017; Piatek et al., 2016). An optimum C/N ratio is needed to keep a favorable nutrient balance for anaerobic bacteria's growth, as well as for maintain a stable environment (Piatek et al., 2016; Zhang et al., 2014).

An appropriate balance between carbon and nitrogen is required for effective digestion, and the ideal C/N ratio for anaerobic digestion depends on the feedstock and inoculum, (Zhang et al., 2014). Most studies considered an optimum C/N ratio in the range of 20/1 to 30/1 (Matheri et al., 2017; Shi et al., 2017), with a ratio of 25/1 being the most commonly used (Yan et al., 2015). The optimum C/N ratio is responsible for the regulation of nutrient balance to the methanogens within the reactor (Hassan et al., 2017), accordingly deviations from the optimum value affects the biogas production (Neshat et al., 2017).

When the C/N ratio value is higher than the optimum range, it induces a low protein solubilization rate and leads to low total ammonia nitrogen and free ammonia concentrations within a system (Mao et al., 2015), then resulting in higher nitrogen consume rate by the methanogens (Hassan et al., 2017; Matheri et al., 2017). The lack of nitrogen leads the process to lower methane production yield and could even cause the failure of the entire process (Neshat et al., 2017). According to Miqueleto and co-workers, with the increase of the C/N ratio, the daily growth of the microorganisms' population decreased. This suggests that the deficiency of an essential nutrient such as nitrogen can limit cellular growth (Miqueleto et al., 2010).

In the other hand, lower C/N ratios limit the microbial growth due to carbon shortage (Neshat et al., 2017), which also leads to ammonia accumulation (Wang et al., 2012). This

may result in higher total ammonia nitrogen release and high VFA accumulation in the digester, which are inhibitor factors for AD performance (Kamali and Khodaparast, 2015; Yan et al., 2015).

In a study conducted by Piatek and colleagues, tests with lower C/N ratios led to ammonia accumulation and increased the pH values, which were toxic conditions to methanogenic archaea. On the other hand, under high C/N ratios, the nitrogen was consumed rapidly by methanogens and the pH value was lower than the optimum demand to anaerobic digestion systems (Piatek et al., 2016). According to Wang and co-workers, C/N ratio may also interact with temperature and that interaction will result in different concentrations of ammonia, as well as inhibitory effects. Hence, when temperature increased, an increase was required in the feed C/N ratio, in order to reduce the risk of ammonia inhibition (Wang et al., 2014). Thus, it can be concluded that an adjustment of the carbon to nitrogen ratio is needed for a stable anaerobic digestion in a long-term operation (Zhang et al., 2014).

2.3.4. Ammonia

Ammonia is formed during the biodegradation process of protein or other nitrogen-rich organic substrates (Zhang et al., 2014). It is an essential nutrient for the growth of microorganisms involved in anaerobic digestion yet, as previously described, it can also be toxic when present at high concentrations (Jha and Schmidt, 2017; Zhang et al., 2014). The excess of ammonia leads to an increase of pH, a lowering of biogas production, the occurrence of inhibitory effects, and eventually, it may led to process failure (Shi et al., 2017; Zhang et al., 2014).

Ammonia exists in two forms, as ionized ammonia or ammonium (NH_4^+) and as unionized ammonia or free ammonia nitrogen (NH_3) (FAN). The combination of these two forms of ammonia is expressed as total ammonia nitrogen (TAN) (Akindele and Sartaj, 2017; Rajagopal et al., 2013). FAN has been suggested to be the main cause of inhibition of methanogenic microflora since it is freely membrane-permeable (Chen et al., 2008; Rajagopal et al., 2013).

Free ammonia concentration primarily depends on TAN, pH and temperature. In addition, ionic strength can also be considered as a significant parameter, especially for concentrated solutions (Rajagopal et al., 2013). Ammonia toxicity increases with increasing temperature, and washout of microbial population can occur (Weiland, 2010). At thermophilic temperatures, the FAN concentration is expected to be six times higher than under mesophilic conditions at the same pH (Rajagopal et al., 2013). The indicated

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conditions will led to severe inhibition, accumulation of VFA (being acetate the main type (Shi et al., 2017)), and eventual process failure (Yirong et al., 2017). Consequently, working under mesophilic conditions was proposed as a solution to prevent the ammonia inhibition in the digestion process (Neshat et al., 2017).

Among the different types of anaerobic microorganisms, the methanogens are the least tolerant and the most likely to cease growth due to ammonia inhibition (Chen et al., 2008). These microorganisms can be affected in two ways: ammonium ion may inhibit the methane producing enzymes directly or hydrophobic ammonia molecule may diffuse passively into bacterial cells, causing proton imbalance or potassium deficiency. A fraction of NH_3 that enters into the cells causes a pH change due to its conversion into ammonium, while absorbing protons in the process (Rajagopal et al., 2013).

Nonetheless, ammonia has also advantages for the digestion process, as it can enhance the buffer capacity of the AD (Zhang et al., 2014), acting as a pH neutralizer against VFA accumulation and maintaining the pH at optimum levels and being a valuable nitrogen source for methanogenic archaea (Neshat et al., 2017). As formerly explained, different ammonia concentrations cause different effects on the process, and **Table 2** summarizes the concentrations at which ammonia is beneficial, inhibitory or toxic to AD.

Table 2: Effects of ammonia levels on anaerobic digestion process (Rajagopal et al., 2013).

Ammonia Concentration (mg NH_4-N/L)	Effect on AD process
50 - 200	Beneficial
200 - 1000	No antagonistic effect
1500 - 3000	Inhibition (especially at higher pH values)
> 3000	Complete inhibition or toxic at any pH

As it can be observed in **Table 2**, between 50 and 200 mg NH_4-N/L ammonia has beneficial effects, while at higher concentrations (from 1500 mg NH_4-N/L) it occurs the inhibition of methanogenesis. At high pH values, the unionized form prevails and this form is more inhibitory than the ammonium ion. In consequence, an increase in pH results in increased toxicity (Rajagopal et al., 2013).

2.3.5.   Organic Loading Rate

The organic loading rate (OLR) is a critical operational parameter which represents the biological treatment capacity of the anaerobic digestion system (Sun et al., 2017), in other words, represents the amount of organic material that is fed to a digester (El Achkar et al., 2017). OLR is expressed as chemical oxygen demand or volatile solids fed to the system daily per m^3 of the digester volume (Matheri et al., 2017) and depends on the technology and type of wastes fed to the digester (Dhar et al., 2016).

This is an important factor for viability of the microorganisms and their optimum activity (Neshat et al., 2017). According to Sun and co-workers, microbial analysis revealed that the increase on the OLR influenced significantly the structure and behavior of microbial consortia (Sun et al., 2017). As an example, loading a high amount of organic material into the system at once can lead to a shock, resulting on higher activity of acidogenic bacteria when compared to methanogens. Thus, as the organic loading increases, the risk of inhibition due to excessive VFA production also increases (Neshat et al., 2017; Nghiem et al., 2017).

The biogas production is significantly affected by this parameter (Sun et al., 2017). With increasing OLR, the biogas yield increases to an extent, but the equilibrium and productivity of the digestion process can also be disturbed (Mao et al., 2015). Thus, an appropriate increase in OLR favored the biogas production, while excess OLR increase restrained it and may cause system failure (Sun et al., 2017). If the feeding capacity of the system is exceed by the OLR, the gas production decreases due to accumulation of fatty acids in the digester sludge (Matheri et al., 2017).

As previously described, a high organic load enables the microorganism's growth and high biogas production, but it also puts pressure on the microorganisms and can lead to process collapse due to acid accumulation, during the acidogenesis stage (El Achkar et al., 2017). Thus, digesters are usually operated at low organic loading rate (P. Wang et al., 2017). The main advantage of higher loading rates is the lower cost and size of the digester (Jain et al., 2015).

A mesophilic digester can be loaded with concentrated organic substrates when the digester is operated at sufficiently long retention times. On the other hand, thermophilic digesters have higher conversion rates and allow shorter retention times than mesophilic digesters. Though, thermophilic digesters can behave more sensitively with ammonia, becoming more toxic at lower concentrations (Aramrueang et al., 2016).

2.3.6. Retention Time

Retention time (RT) is the time required for the complete degradation of the organic matter, and differs with process parameters such as type of feedstock and temperature (Jain et al., 2015; Matheri et al., 2017). This parameter is directly proportional to the degradation rate, the shorter the RT, the lower the degradation rate (Matheri et al., 2017).

Retention time includes hydraulic retention time (HRT) and solids retention time (SRT). SRT is defined as the average residence time of microorganisms in the reactor (Aramrueang et al., 2016) and HRT is the average time an input organic matter stays inside the digester before it comes out (El Achkar et al., 2017), defined by **Equation 1**,

$$HRT = \frac{V}{Q}$$ **Equation 1**

where V is the reactor volume and Q the influent flow rate (Mao et al., 2015; Ziganshin et al., 2016).

SRT determines the time available for substrate degradation and microbial growth (Vanwonterghem et al., 2015). A low SRT does not allow enough time for the methanogens to consume VFA and produce CH_4 and CO_2, because the growth rate of methanogens is slower when compared with acidogens. Hence, a SRT shorter than the optimum value can cause VFA accumulation, washout of the methanogens (Khan et al., 2016) and significant shifts in the microbial population, as bacteria and archaea present became less diverse (Manser et al., 2015). On the opposite, acidogens require a minimum SRT to perform the hydrolysis of the substrates (Khan et al., 2016).

A long SRT provides sufficient time for the methanogens, enables more biogas production (Khan et al., 2016), allows a decrease in toxicity and maintain digester stability through microbial adaptation and acclimation (Aramrueang et al., 2016). However, longer SRT may also increase the capital and operational cost (Chen et al., 2018).

According to El Achkar and colleagues, hydraulic retention time is one of the most important parameters affecting significantly microbial ecology (El Achkar et al., 2017). The microorganisms need a specific time to consume the substrate and synthetize the products, so if the process could not be maintained at its optimum HRT, unfavorable metabolic activity of microorganisms and undesirable products will result (Neshat et al., 2017).

Low values of HRT affects the microbial community composition (Ziganshin et al., 2016), can result in accumulation of VFA and has a potential risk of biomass washout from the system, leading in the end to a low methane yield (Khan et al., 2016; Neshat et al., 2017). Differently, long HRT values can lead to the death of microorganism due to the shortage of nutrients (Neshat et al., 2017) and need large digester sizes (Mao et al., 2015).

2.3.7.   pH

Between all the parameters with influence on anaerobic digestion process, pH is one of the most important. The pH value is a measurement of acid or basic concentration in aqueous substances (Matheri et al., 2017) and affects the chemical reactions and the activity of the microbial consortia (Montañés et al., 2014). The microorganisms are sensitive to pH variations and each anaerobic phase shows a different pH sensitivity (Braguglia et al., 2017).

Due to the direct impacts on microbial activity and community stability, pH influences the organic biodegradation and biogas production (Mao et al., 2017). The growth of each type of microorganism happens only within a characteristic pH range and the maximum growth rate occurs at an optimum pH value (Montañés et al., 2014). In **Table 3** are represented the optimal intervals of some bacteria involved in AD process, according to the literature.

Table 3: Optimum pH range to different types of microorganisms involved in anaerobic digestion process.

Microorganisms	Optimum Range	References
Hydrolytic	5.5 - 6.5	(Hagos et al., 2017)
Acidogenic	4.0 - 8.5	(Braguglia et al., 2017; Hagos et al., 2017; Piatek et al., 2016)
	5.5 - 6.5	(Khan et al., 2016; Mao et al., 2015)
Methanogenic	6.5 - 7.2	(Braguglia et al., 2017; Piatek et al., 2016; Zhang et al., 2014)
	6.5 - 7.5	(J. Zhou et al., 2017)
	6.5 - 8.2	(Khan et al., 2016; Mao et al., 2015)

The optimum pH for anaerobic digestion process changes in the various stages. As can be observed in the **Table 3**, the optimum pH for methanogenic bacteria is higher than

the optimum pH for hydrolytic and acidogenic bacteria. It can also be noticed that there is a greater discrepancy in the literature values for acidogenic values comparatively with the methanogenic bacteria. According to **Table 3**, methane formation takes place within a relatively narrow pH interval, from about 6.5 to 8.2 and, according to Hagos and co-workers, 7.0 is the optimal value (Hagos et al., 2017). The pH increases due to ammonia accumulation, while the accumulation of VFA decreases the pH value and reduces the methane production (Neshat et al., 2017; Weiland, 2010). Accordingly, if the pH value is outside the optimum range the process is severely inhibited (J. Zhou et al., 2017).

The growth rate of acidogenic bacteria is much higher than that of the methanogenic microorganisms and, as it can be seen in **Table 3,** the acidogenic bacteria are active in lower pH values, whereas methanogenic archaea are not active (Kim et al., 2017). Thus, the pH of anaerobic digestion is typically maintained between methanogenic limits to prevent the predominance of the acid forming bacteria, which may cause VFA accumulation (Rajeshwari et al., 2000).

The different optimum pH value is one at the main reason to separate the process into two-phase digesters as acidogenic phase and methanogenesis phase (Hagos et al., 2017). Also, co-digestion, the simultaneous anaerobic digestion of a mixture of two or more substrates (Montañés Alonso et al., 2016), can facilitate stable pH by avoiding extreme acidification conditions.

As previously described, the microbial activity may alter the pH values during the AD process. For this reason pH regulation is essential and one of the appropriate ways to increase methane yield (Yang et al., 2015). However, the initial pH adjustment may be insufficient to analyze the relationship between biogas production and microbial communities (Zhou et al., 2016).

The pH can be maintained at a desired level manually or automatically. Examples of automatic methods can be programmable logic control (Latif et al., 2017) or the use of a alkali and acid pump (Strik et al., 2006). Manually, chemical products such as hydrochloric acid (HCl) or sodium hydroxide (NaOH), among others (Chen et al., 2012; Tomaszewski et al., 2017) can be added. However, the use of chemicals may have some side effects, for instance, the ionization of these chemicals and the production of certain ions, can inhibit the process. As an example, the presence of Na^+ at high concentrations can inhibit AD and may decay the whole process (Neshat et al., 2017). A recent method of pH control is the use of geopolymers with high buffer capacity (Novais et al., 2016b).

2.4. Geopolymers

Geopolymers, also known as alkaline cements or inorganic polymers, are materials with a tri-dimensional aluminosilicate structure that result from the chemical interaction between a strongly alkaline solution and a source of aluminosilicates (Aguirre-Guerrero et al., 2017). Their microstructure consists of chains or networks of inorganic molecules linked by covalent bonds (Nikolov et al., 2017). These materials, that emerged as a result of attempts to model the geological formation of zeolites, have good mechanical and physical properties (Majidi, 2009) and have been attracting increased attention due to the low CO_2 emissions associated with their production (Novais et al., 2016b).

A variety of aluminosilicate materials can be used as solid raw materials in the geopolymerization technology, such as, metakaolin, kaolinite, feldspar and industrial solid residues like fly ash, metallurgical slag or mining wastes (Singh et al., 2015). To develop stable geopolymers, the source materials should be highly amorphous, possess sufficient reactive glassy content, low water demand and be able to release aluminum easily (Singh et al., 2015). The geopolymers are considered as a green construction material since in its production it can be used, as main ingredients, solid wastes such as fly ash and industrial slags (Panda et al., 2018).

Depending on the raw material selection and processing conditions, geopolymers can exhibit a wide variety of properties and characteristics. Their main advantages are production technology, non-flammable and high temperature stability, safe for humans, highly resistant towards freezing/thawing cycles, high compressive strength and low shrinkage (Feng et al., 2015; Novais et al., 2016a).

Despite this wide variety of attributes, these properties are not necessarily inherent to all geopolymeric formulations (Duxson et al., 2007). The properties of geopolymers can be optimized by a proper selection of the raw materials, correct mix and processing design to suit an appropriate application (Singh et al., 2015). Among the wide range of applications, geopolymers can be used in the construction industry as an alternative to Portland cement (building materials, concrete, fire resistant coatings, fiber reinforced composites), waste immobilization solutions for the chemical, nuclear industries and bulk materials for military applications, automotive sector and marine applications (Palmero et al., 2015; Singh et al., 2015).

A type of geopolymers uses fly ash from porous biomass burning to energy production (Novais et al., 2016b). The main products of biomass combustion are H_2O, CO_2, combustion air and excess O_2. However, other compounds can also be formed

depending on the constitution of the fuel and the conditions of combustion (Nunes et al., 2017). The use of biomass as fuel generates large amounts of residual ash which can cause serious environmental problems. Fly ash particles are highly contaminating due to their enrichment in potentially toxic trace elements which condense from the flue gas, thus research on the potential applications of these wastes has environmental relevance and industrial interest (Ahmaruzzaman, 2010). Accordingly, the production of this type of geopolymers will contribute to the material valorization of fly ash, decreasing the need of its disposal in landfills and associated environmental problems (Novais et al., 2018).

Porous biomass fly ash containing geopolymers, when immersed in water, have the ability to control pH over time (Novais et al., 2016b), by leaching significant amounts of OH^- from their structure (Novais et al., 2018). Hence, geopolymers can be used as pH regulators in applications where high buffer capacity is required, for instance wastewater treatment systems and in biogas reactors (Novais et al., 2016b).

The use of fly ash based geopolymers instead of commercial alkaline materials in AD process results in process simplicity, since no continuous pH adjustment is required. Besides, according to Novais and colleagues, the biogas production was also enhanced by geopolymers addition, which can be related with the release of suitable alkali metals that improve the process stability and performance (Novais et al., 2018).

Chapter 3. Materials and Methods

3.1. Experimental Set-up

A schematic representation of the experimental set-up used in the laboratory to study the long-term utilization of geopolymers for pH control in anaerobic reactors for methane production is shown in **Figure 2**.

1. Graduated cylinder
2. Separation funnel
3. Bubbler
4. Sampling point of biogas
5. Clamp
6. Bath
7. Anaerobic reactor
8. Thermostat
9. Sampling syringe
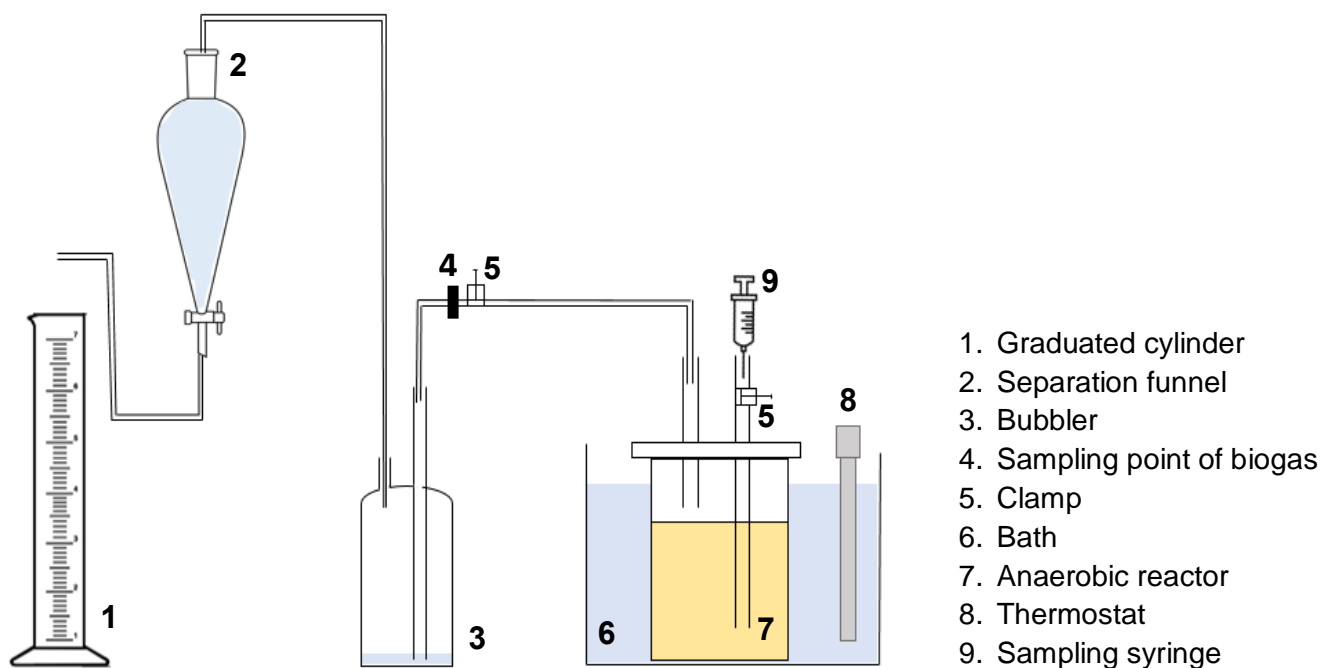
Figure 2: Representation of the experimental set-up.

The set-up was composed by an anaerobic glass reactor in a bath with controlled mesophilic temperature of 37 ºC, using a thermostat. The batch reactor had one sampling point to take samples with a syringe for analysis. From the reactor until the bubbler lies

the gas circuit, where samples were frequently taken to analyze the biogas composition by gas chromatography. After the bubbler lies the water displacement circuit, where the produced biogas volume was measured by water displacement method, using a graduated cylinder.

3.1.1. Operation Mode

The experimental work was divided in two studies with different objectives, yet both experiments were carried out in batch mode using anaerobic reactors with 1 L of working volume. At the beginning of the experiment was added anaerobic sludge, cheese whey as carbon source, distilled water, 2 mL/L of macronutrients and 1 mL/L micronutrients to each reactor (**Table 4**). Macronutrients are indispensable constituents for biomass development and micronutrients are crucial cofactors in numerous enzymatic reactions involved in the biochemistry of methane formation (Romero-Guiza et al., 2016).

Table 4: Micro and macro nutrients added to the reactors, as described by (van Lier et al., 1997).

		Element of interest	Stock Solution Concentration (mg/L)
Micronutrients	NH_4Cl	N	86.802
	KH_2PO_4	P	16.456
	$CaCl_2.2H_2O$	Ca	4.248
	$MgSO_4.7H_2O$	Mg	1.733
Macronutrients	$FeCl_2.6H_2O$	Fe	0.403
	$CoCl_2.6H_2O$	Co	0.483
	$MnCl_2.4H_2O$	Mn	0.135
	$CuCl_2.2H_2O$	Cu	0.011
	$ZnCl_2$	Zn	0.023
	H_3BO_3	B	0.009
	$(NH_4)_6Mo_7O_{24}.4H_2O$	Mo	0.048
	$Na_2SeO_3.5H_2O$	Se	0.029
	$NiCl_2.6H_2O$	Ni	0.012
	EDTA ($C_{10}H_{16}N_2O_8$)	EDTA	0.976

The volume of anaerobic sludge and cheese whey added to each reactor was determined in order to obtain a concentration of 2 g/L VSS of biomass and 8 g/L COD of

substrate, based on previous anaerobic tests (Silva et al., 2013). In the reactors with addition of alkalinity a buffer solution of $NaHCO_3$ and $KHCO_3$ it was used to promote pH autoregulation, to achieve 4 g/L of alkalinity measured as $CaCO_3$. Before sealing, the reactors were purged with nitrogen gas to remove any residual oxygen.

The objective of the first study was to evaluate the influence of the concentration and the type of geopolymers in AD process and it were operated four reactors for 87 days. A schematic representation of the study is displayed in **Figure 3.**

Figure 3: Representation of the conditions applied in the first study.

At one of the anaerobic reactors it was added 4 g/L of alkalinity concentration measured as $CaCO_3$ (A1) whereas to the other three assays it was add two types of geopolymer spheres, with the same composition but different porosity (type L- low porosity and type H- high porosity). Two assays use geopolymer spheres with the lowest porosity and two distinct concentrations (12 g/L (L1) and 16 g/L (L2)) and the other assay uses 16 g/L of geopolymer spheres with the highest porosity (H1). After 56 days of operation most of the organic matter had already been removed, so cheese whey (substrate) was again added in order to evaluate the ability of the geopolymers to control pH after a long incubation time. Thus, the first study was composed by two phases, where the first phase began in day 0 and ended in day 55, and the second phase began with the substrate addition at the day 56 until the end of the study (day 87).

In the second study the objective was to evaluate the long-term geopolymers performance and reproducibility. So, accordingly with the first phase results, it was used one anaerobic reactor with 4 g/L of $CaCO_3$ (A2) and two reactors with 16 g/L of geopolymer spheres with the highest porosity (type H) (H2 and H3), as shown in **Figure 4**.
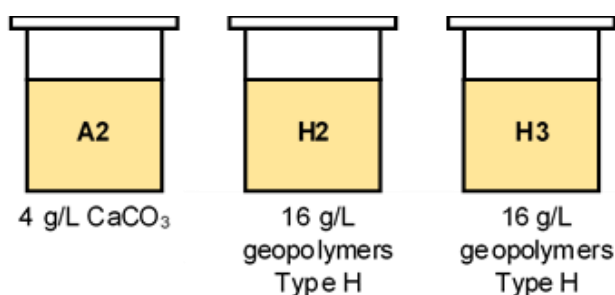

Figure 4: Representation of the conditions applied in the second study.

This study had the duration of 128 days, during which it were made three additions of cheese whey at days 48, 79 and 104 of operation, according to the observed removal of organic matter. Therefore, the study was divided into four phases: from day 0 until day 47 (phase 1), from day 48 until day 78 (phase 2), from day 79 until day 103 (phase 3) and from day 104 until day 128 (phase 4). Before the samples were taken for analysis, the reactors were manually stirred.

3.2. Inoculum and Substrate Characterization

The anaerobic microbial mixed culture used as inoculum in the laboratory stet-up was obtained from a wastewater treatment plant (WWTP) located near Aveiro. This WWTP performs the treatment of urban and industrial effluents from Ílhavo, Mira, Vagos and part of Aveiro city (Águas do Centro Litoral, 2018). The volume of inoculum to be added to the reactors was determined before in order to obtain an initial volatile suspended solid (VSS) concentration of 2 g/L in each reactor.

The substrate used was cheese whey powder, an industrial by-product with high acidogenic potential. A concentrated solution of cheese whey was prepared, and the volume of substrate used was determined to achieve an initial substrate concentration corresponding to a soluble chemical oxygen demand (COD) of 8 g/L. The inoculum and substrate characterizations are presented in **Table 5.**

Table 5: Characterization of the inoculum and substrate used in the experimental work.

Parameters	Inoculum	Substrate
pH	7.37	5.72
Total Suspended Solids (g/L)	33.40	11.36
Volatile Suspended Solids (g/L)	23.57	10.09
Total Chemical Oxygen Demand (g/L)	NA	144.67
Soluble Oxygen Demand (g/L)	0.00	128.25
Total Organic Carbon (g/L)	NA	24.83

Note: NA- not analysed

3.3. Geopolymers Preparation

The geopolymer spheres were produced following a procedure described by Novais and colleagues (Novais et al., 2017). The mixture composition of the two types of geopolymers (with lower and higher porosity) is represented in **Table 6.**

Table 6: Mixture composition utilized in geopolymers preparation.

Geopolymers Type	Mixture proportion (g)				
	FA	MK	Alkaline activator	H_2O	Foaming agent
Type L	10.00	10.00	24.38	4.15	0.59
Type H					0.75

The activating solution (mixture of sodium hydroxide and sodium silicate) was added to the solid components (fly ash (FA) and metakaolin (MK)) and to a constant water amount. The components were mixed thoroughly during 2 min and the foaming agent (Sodium dodecyl sulphate) was added to slurry and blended for another 5 min to ensure that a homogeneous paste was produced. The foaming agent was added in different amounts, since one of the objectives of this work was study the influence of spheres porosity in the geopolymers pH buffer

Finally, the geopolymeric slurry was injected into a polyethylene glycol medium (PEG) to produce the geopolymeric spheres. Afterwards, the spheres were immediately collected, washed with distilled water and cured for 24h in controlled conditions (at slightly elevated temperature, 40 ºC, and at 65% relative humidity). Lastly, the spheres were cured at room temperature and humidity. In **Figure 5**, is shown the obtained geopolymer spheres.

Figure 5: Geopolymer spheres used in the experimental work.

3.4. Analytical Procedures and Calculations

During the assays, samples were taken to control the reactors operation and performance. It was analyzed pH, alkalinity, total and volatile suspended solids (TSS and VSS) content, total and soluble chemical oxygen demand (tCOD and sCOD), volatile fatty acids (VFA), total organic carbon (TOC) and biogas. All analyses were performed in accordance with Standard Methods (APHA, 2012).

In the first study, biogas volume and composition were monitored daily; pH, sCOD, TOC, VFA, TSS and VSS were analyzed twice a week. During the second study, the biogas volume and composition were also monitored daily; pH, sCOD and VFA were analyzed weekly and the solids were analyzed only when substrate was added to the reactors.

3.4.1. pH and Alkalinity

The pH was measured in accordance with electrometric method 4500-H$^+$ B (APHA, 2012), using a portable equipment Consort P602.

Alkalinity, the capacity of an aqueous solution to neutralize acids (Neshat et al., 2017), were was measured according to titration method 2320 B (APHA, 2012), using a portable equipment Consort P602. It was titrated 50 mL of sample with hydrochloric acid (HCl) 1 N and the pH was monitored until reach the value of 4.5. The alkalinity concentration was obtained through **Equation 2**.

$$Alkalinity, mg\ CaCO_3/L = \frac{A \times B \times 50\ 000}{mL\ sample}$$ **Equation 2**

A represents the volume of standard acid used and B represents the normality of the standard acid.

3.4.2. Total and Volatile Suspended Solids

The total suspended solids analysis was performed according to the method 2540 B and the volatile suspended solids according to the method 2540 E (APHA, 2012). It was used an analytic scale Precisa^{TM} XB 120A, a drying oven WTC^{TM} Binder E28 and a muffle furnace Termolab^{TM} Fuji PXR-9. Two replicates were made to each reactor and sample and the determination of the solids contents was calculated via **Equations 3 and 4.**

$$g \; total \; suspended \; solids/L = \frac{(A-B)}{sample \; volume, L} \qquad \textbf{Equation 3}$$

A is the weight of dried residue and filter and B is the weight of filter, in g.

$$g \; volatile \; suspended \; solids/L = \frac{(A-C)}{sample \; volume, L} \qquad \textbf{Equation 4}$$

A is the weight of residue and filter before ignition and C is the weight of residue and filter after ignition, in g.

3.4.3. Total and Soluble Chemical Oxygen Demand

The COD test is used to measure the oxygen equivalent of the organic material in wastewater that can be oxidized chemically, using dichromate in acid solution (Tchobanoglous et al., 2003). tCOD was determined according to Raposo and colleagues (Raposo et al., 2008) and sCOD was measured in accordance with colorimetric method 5220 D (APHA, 2012), using an Aqualytic^{TM} AL125 thermoreactor and a spectrophotometer equipment Aqualytic^{TM} COD Vario PC compact (which presents the results in mg/L). The COD concentrations were obtained through **Equation 5.**

$$g \; COD/L = \frac{1}{Dilution \; Factor} \times absorbance \qquad \textbf{Equation 5}$$

The organic matter removal, considering the initial value of COD, was calculated via **Equation 6**.

$$\% \; removal \; of \; organic \; matter = \frac{(COD_{initial} - COD_{final}) \times 100}{COD_{initial}} \qquad \textbf{Equation 6}$$

3.4.4. Volatile Fatty Acids

VFA quantification was made by gas chromatography, injecting 0.5 µL of filtered sample containing 10 % (v/v) of formic acid (Panreac^{TM}) in a gas chromatograph PerkinElmer^{TM} Clarus 480 with an injector set to 300 °C, a flame ionization detector set to 240 °C, a 25 m × 0.53 mm SGE^{TM} ID-BP1 5.0-µm column, and helium as carrier gas.

The temperature program used was based on the work of Gameiro and co-workers (Gameiro et al., 2015), as follows: 1 min at 70 °C, rise of 20 °C min^{-1} to 100 °C and then kept for 2 min; rise of 10 °C min^{-1} to 140 °C, and kept for 1 min; and rise of 35 °C min^{-1} to 235 °C, and kept for 6 min (18.21 min of total running time). The calibration curves (**Appendices**) were obtained by injecting nine standard solutions of acetic, propionic, iso-butyric, n-butyric, iso-valeric, n-valeric, iso-caproic, and n-caproic acids (Riedel-de Haën^{TM}), in a range of concentrations between 0.05 and 5 g/L. Acids concentrations obtained from calibration measured in grams per liter were converted into COD according to the oxidation stoichiometry represented in **Table 6**.

Table 7: Values of oxidation stoichiometry for VFA in mg COD mg^{-1}.

Acetic acid	Propionic acid	i-Butyric acid	n-Butyric acid	i-Valeric acid	n-Valeric acid	n-Caproic acid
1.067	1.514	1.818	1.818	2.039	2.039	2.207

The degree of acidification (DA) was the main parameter used to evaluate the acidogenic potential of the substrate and was calculated via **Equation 7.**

$$Degree\ of\ Acidification\ (\%) = \frac{\sum VFA_{produced}}{COD_{initial}} \times 100 \qquad \textbf{Equation 7}$$

The VFA yield was determinate by the quotient between the VFA produced and the substrate consumed, as can be observed in **Equation 8.**

$$y_{VFA/COD}\ (g\ COD^{-1}\ g\ COD) = \frac{tVFA_{final} - tVFA_{initial}}{(sCOD_{initial} - tVFA_{initial}) - (sCOD_{final} - tVFA_{final})} \textbf{Equation 8}$$

3.4.5. Total Organic Carbon

Total organic carbon is a more convenient and direct expression of total organic content than COD (APHA, 2012). For TOC determination, the samples were acidified with HNO$_3$ and keep in the fridge until analysis. Posteriorly, the samples were diluted as

necessary and TOC was measured using a TOC/-TN$_b$ Analyzer Analytik Jena^{TM} multi N/C 3100, at CESAM laboratories.

3.4.6. Biogas

The volume of biogas produced was measured by water displacement method and the biogas composition was analyzed by a gas chromatograph SRI^{TM} 8610C with thermal conductivity detector set to 75 °C using 80/10 × 2.5 m CRS Hayesep^{TM} column set to 61 °C and helium as the carrier gas.

The equipment provided values of methane, carbon dioxide and air; the variable called air includes various gaseous components. Through the integration software PeakSimple three different peaks were obtained. The first peak was the area of air in the sample, the second peak, to a retention time of approximately 1.2 min, was the CH_4 area and the peak at a retention time of approximately 2 min was the area of CO_2. The percentages of methane and carbon dioxide in the biogas were calculated via **Equations 9 and 10**, respectively.

$$\% \, CH_4 = 0.9896 \times \left(\frac{x_1}{x}\right) \times 100 \qquad \textbf{Equation 9}$$

$$\% \, CO_2 = 0.9924 \times \left(\frac{x_2}{x}\right) \times 100 \qquad \textbf{Equation 10}$$

x_1 represents the CH_4 area, x_2 the CO_2 area and x the sum of the two areas.

The methane yields, considering the initial COD values, were calculated via **Equations 11 and 12**.

$$\gamma \frac{CH_4}{COD_{removed}} = \frac{CH_4 \, produced}{COD_{initial} - COD_{final}} \qquad \textbf{Equation 11}$$

$$\gamma \frac{CH_4}{COD_{initial}} = \frac{CH_4 \, produced}{COD_{initial}} \qquad \textbf{Equation 12}$$

The degree of methanization, which expresses the COD conversion into methane, was calculated through **Equation 13.**

$$Degree\ of\ methanization\ (\%) = \frac{COD_{CH_4}}{COD_{initial}} \times 100 \qquad \textbf{Equation 13}$$

The methane production in COD units, COD_{CH4}. This conversion was calculated via **Equation 14,** based on the equation of perfect gases.

$$COD_{CH_4} = \frac{Q_{biogas} \times f_{CH_4} \times 101325 \times ThOD_{CH_4}}{R \times T} \qquad \textbf{Equation 14}$$

f_{CH4} represents the methane content in biogas, Q_{biogas} the volume of biogas, R the perfect gases constant, T the temperature of experimental conditions and $ThOD_{CH4}$ the theoretical oxygen demand of methane obtained from the oxidation stoichiometry (64 g O_2 mol^{-1}).

Chapter 4. Results and Discussion

4.1. Optimization of the concentration and type of geopolymers

4.1.1. pH Evolution

The main objective of the first study was the optimization of the concentration and type of geopolymers for pH control in anaerobic reactors for methane production. Due to the importance of pH level to the microorganisms responsible for methane production, its evolution is a crucial parameter for an anaerobic process. In light of this, the pH variation inside the four anaerobic reactors during the 87 days of operation is represented in **Figure 6**.
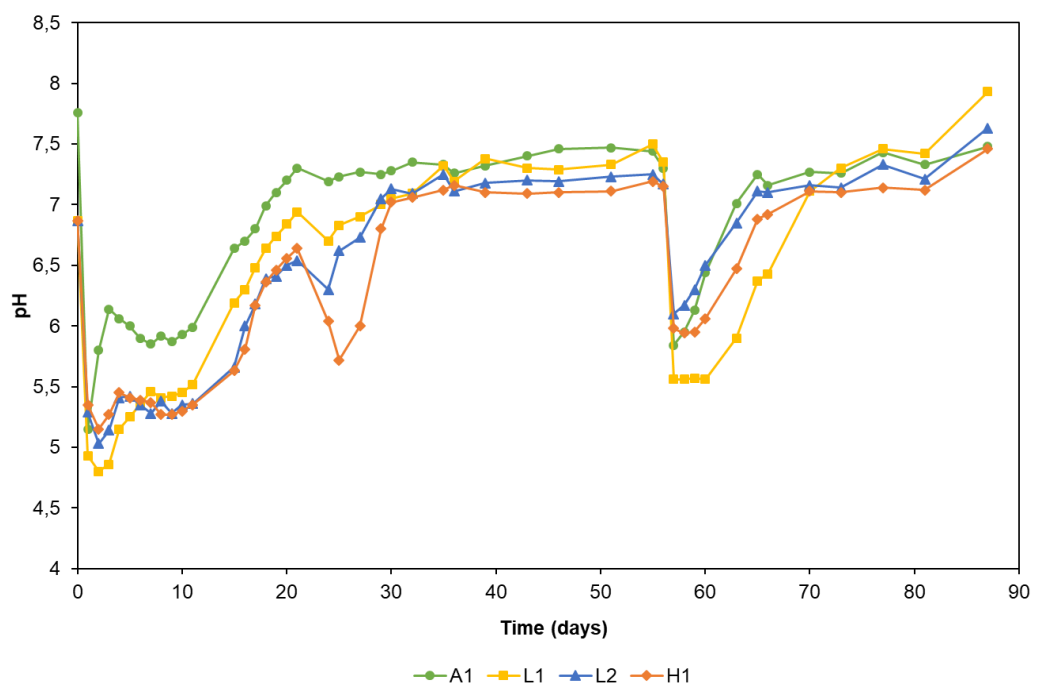

Figure 6: pH variation as a function of time in anaerobic reactors with the addition of chemical alkalinity and different concentrations and types of geopolymers.

As can be observed in **Figure 6**, in the beginning of the study, the pH value of the reactor with chemical alkalinity (A1) was higher (7.76) than the values of the reactors with geopolymers (6.87). Nevertheless, in all reactors the pH dropped in the first day of incubation to values in the range 4.80 - 5.15 probably due to the rapid substrate degradation, followed by a pH increase. At around the 15th day, pH reached favorable values for the development of the methanogenic archaea (between 6.5 and 7.5), which is in accordance with the beginning of methane production (**Figure 9**). Until the end of the first phase (day 55 of operation), pH was favorable for the methanogenic archaea, with the exception of day 24 in reactor H1 (6.04). However, as this pH level just occurred for a short period of time, it didn't affect the methane production.

In the 56th day of operation most of the organic matter had already been removed (**Figure 7**). Based on this, an amount of substrate equal to the beginning of the assays (8 g COD/L) was added to all reactors (Phase 2). After this addition, pH values suffered again a considerable decrease, although not so high as in the first phase, to values between 5.56 and 6.10, which confirmed an adaptation of the biomass. Three of the reactors (A1, L2 and H1) reached favorable values for methane production 8 days after the addition of the substrate (cheese whey), whereas reactor L1 (with the lowest spheres concentration) was at a low pH (5.56) for 4 days, after which the pH value also increased. This fact most probably affected the behavior of this reactor, with a much lower methane production, as can be seen in **Figure 9.**

Comparing the performance of the reactors with the same type of geopolymers (L1 and L2) and different spheres concentration it can be observed that the pH behavior during both assays was similar in the first phase, although different in the second phase, with the reactor with a lower concentration of spheres experienced more fluctuations in its values. On the other hand, the two reactors with different types of spheres and the same concentration, displayed different behavior in the first phase and a similar behavior in the second phase. However, globally, the pH for the reactor with spheres with higher porosity (type H) varied between 5.15 and 7.46, whereas the pH of the reactor with lower porosity (L2) varied between 5.03 and 7.63. The pH obtained in the reactors with spheres with lower porosity (type L) and lower concentration (L1) had greater fluctuations, ranging between 4.80 and 7.93. The reactor with chemical alkalinity (A1) was the fastest to increase pH to values favorable to methane formation. Nevertheless, after a start-up period, it presented similar values to the reactors with geopolymers spheres and, during the study, the pH ranged between 5.15 and 7.76.

4.1.2. Soluble Chemical Oxygen Demand Evolution

Figure 7 presents the evolution of COD soluble (sCOD) inside the reactors. As can be seen in this figure, in the beginning of the assays, the sCOD values were close to 8 g COD/L, which is in accordance with the amount of substrate added to each reactor. After this start-up period, the sCOD values decreased in the reactor with chemical alkalinity (A1). In opposition, in the three reactors with geopolymers the sCOD values increased significantly, being that increase higher in the reactors with higher amounts of spheres (L2 and H1). This can be due to the lixiviation of polyethylene glycol (PEG), which is used in the preparation of geopolymeric spheres and is attached in the spheres surface. Only after 11 days in the reactors with lower porosity (L1 and L2) and 15 days in the reactor with higher porosity (H1), the sCOD values decreased.
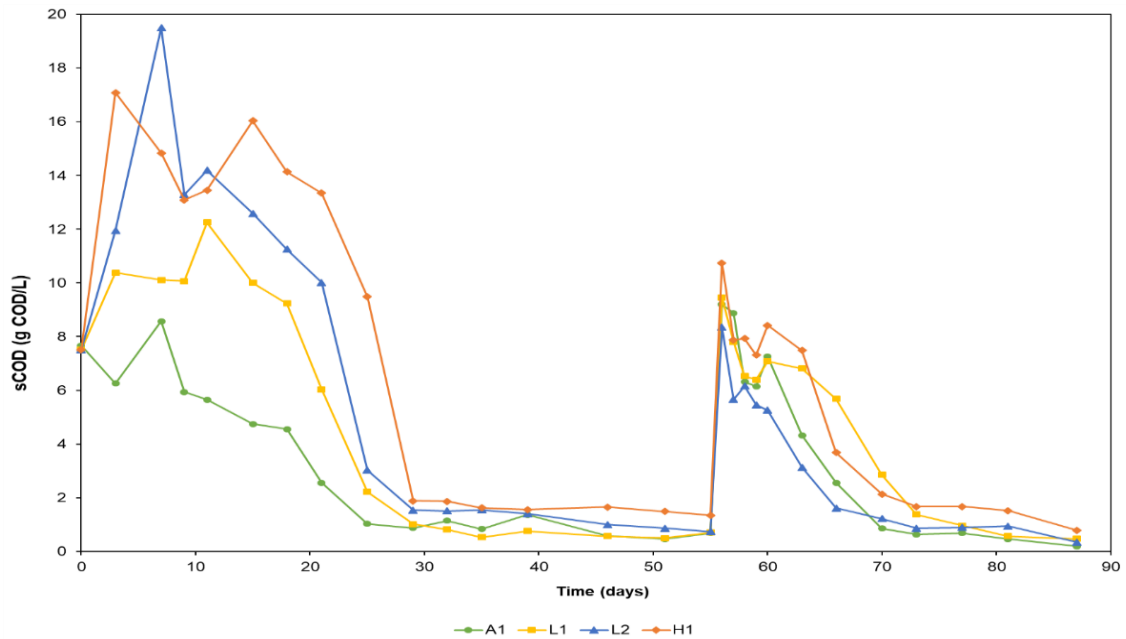
Figure 7: sCOD evolution in anaerobic digesters as a function of time, with addition of chemical alkalinity and different concentrations and types of geopolymers.

The reactor A1 reached a concentration at around 1 g COD/L after 25 days of operation, and the reactors L1 and L2 after 29 days. The reactor H1 stabilized at values close to 1.5 g COD/L after 29 days since the beginning of the assays. Despite the time needed to decrease the COD concentration, the organic matter removal performance in the anaerobic reactors was not significantly affected. After the second subtract addition in the 56th day of operation, the sCOD reached concentrations close to 1 g COD/L in a shorter period (15 days), which demonstrated the adaptation capacity of the system for

organic matter removal. In fact, as observed for pH, the COD increase at the beginning of the second phase was lower than the observed in the first phase. In the second phase, it was not observed a different COD increase between the reactors with and without spheres, which supported the theory that the increase in the first phase was due to lixiviation of polyethylene glycol (PEG) from the spheres.

The percentages of organic matter removal for all reactors, calculated with **Equation 6**, for phase 1 and phase 2 (**Table 8**), allows to conclude that the removal percentages were higher after the second addition of substrate to the anaerobic reactors, which supported the theory of biomass adaptation.

Table 8: Organic matter removal (%) in the two phases of the study.

Reactor		Organic matter removal (%)
A1	Phase 1	91.98
	Phase 2	97.90
L1	Phase 1	90.62
	Phase 2	95.00
L2	Phase 1	90.14
	Phase 2	95.85
H1	Phase 1	82.08
	Phase 2	92.67

4.1.3. Total Organic Carbon Evolution

During the study, TOC analysis was also performed in order to verify any similarity with the COD performance. Through **Figure 8**, it can be observed that the TOC values displayed a similar behavior as the sCOD values, increasing in the start-up period in the reactors with the addition of geopolymers (L1, L2, and H1), supporting the theory that there was lixiviation of organic matter present in the geopolymers spheres.
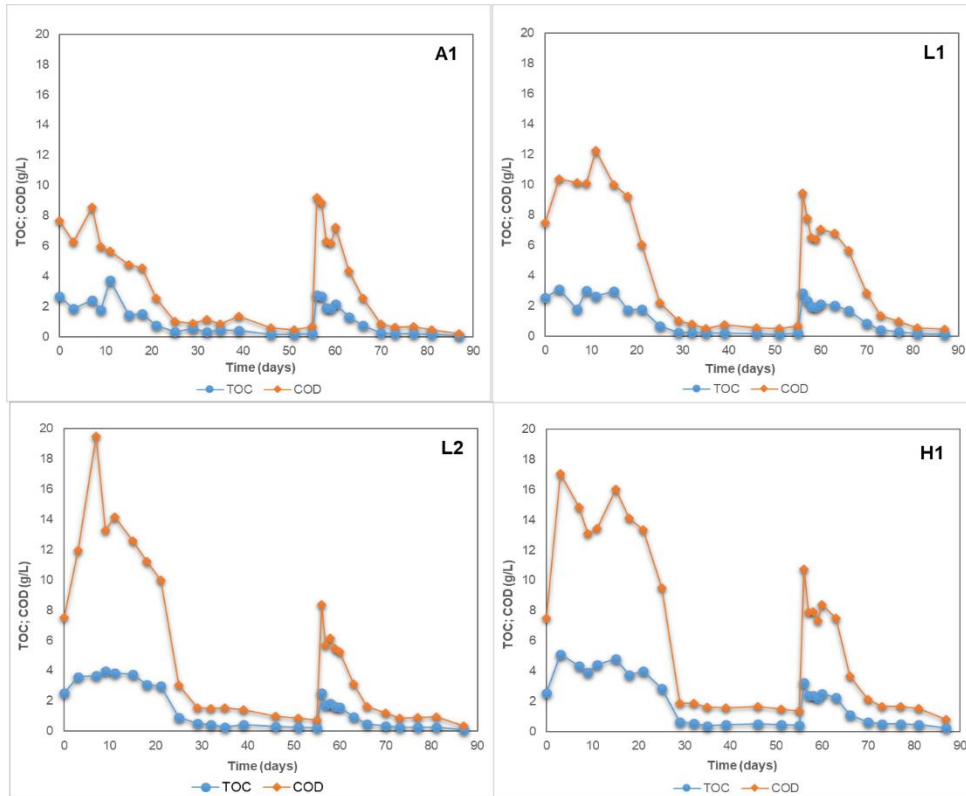
Figure 8: TOC and COD concentrations during time in anaerobic digesters.

4.1.4. Methane and Biogas Production

As previously described, pH for all assays reached values higher than 6.5, which favored the methanogenesis step after 15-20 days. The evolution of the methane production during the 87 days of the study is illustrated in **Figure 9**.

In the previous figure, it can be seen that reactor H1 had the highest accumulated volume of methane produced, whereas reactor L1 produced the lowest accumulated volume. Reactors A1 and L2 demonstrated a similar behavior during the study. The results herein obtained suggest that, in these experimental conditions, regardless the porosity of the geopolymers selected, higher concentrations of spheres (L2 and H1) lead to higher productions of methane.

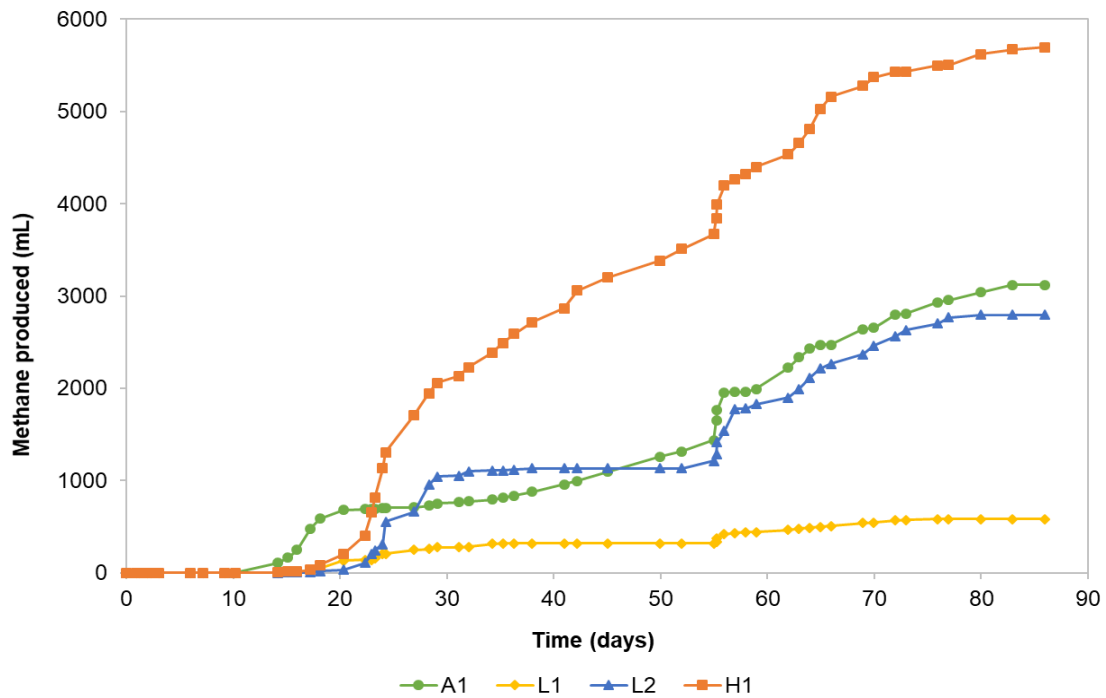**Figure 9:** Cumulative methane produced in anaerobic reactors, with the addition of chemical alkalinity and different concentrations and types of geopolymers.

As can be observed in **Table 9**, reactor L1 produced the lowest methane production in both phases, most probably due to the lowest pH values achieved after substrate addition, showing a lower recovering capacity of this reactor for methane production, suggesting that the amount of speres was not sufficient. Reactors A1 and L2 produced similar methane accumulated volumes, with an average of 1350 mL in phase 1 and 1600 mL in phase 2, showing a 17% increase, most probably due to biomass adaptation. Reactor H1 presented the highest values, 3670 mL (phase 1) and 2020 mL (phase 2), where the difference obtained in phase 1 most probably result from PEG (spheres lixiviation) degradation. Considering just the values obtained in phase 2 (without spheres lixiviation), it was obtained values between 1690 e 2029 mL, being the highest one for the reactor H1.

The methane production yields ($Y_{CH4/CODremoved}$ and $Y_{CH4/CODinitial}$) and the degrees of methanization considering the COD initial, for both phases of the study, are presented in **Table 10**.

Table 9: Accumulated volume of methane and biogas produced in the two phases of the study.

Reactor		Volume (mL)	
		Methane	Biogas
A1	Phase 1	1438.0	3806.3
	Phase 2	1685.0	3367.8
	Average value	**1561.5**	**3587.1**
L1	Phase 1	321.7	1393.8
	Phase 2	261.6	1049.0
	Average value	**291.7**	**1221.4**
L2	Phase 1	1287.6	3783.5
	Phase 2	1509.3	3230.5
	Average value	**1398.5**	**3507.0**
H1	Phase 1	3673.5	7960.8
	Phase 2	2021.9	4227.5
	Average value	**2847.7**	**6094.2**

Table 10: Methane production yields and degrees of methanization for both phases of the study.

Reactor		$Y_{CH4/CODremoved}$ (L_{CH4}/g_{COD})	$Y_{CH4/CODinitial}$ (L_{CH4}/g_{COD})	% methanization ($g_{CH4\text{-}COD}/g_{COD}$)
A1	Phase 1	0.182	0.168	42.60
	Phase 2	0.187	0.183	46.51
L1	Phase 1	0.047	0.043	10.89
	Phase 2	0.029	0.028	7.02
L2	Phase 1	0.190	0.172	43.59
	Phase 2	0.189	0.182	45.90
H1	Phase 1	-	-	-
	Phase 2	0.203	0.188	47.79

Similarly to what happened to methane production, reactor L1 produced the lowest methane production yields and degrees of methanization in both phases, supporting the lowest recovering capacity of this reactor for methane production. Reactors A1 and L2 produced similar methane yields, with an average of 0.186 $L_{CH4/gCODrem}$ in phase 1 and 0.188 $L_{CH4/gCODrem}$ in phase 2. In reactor H1, in the first phase, much higher values were

obtained, as a result of COD removed that was converted into methane from the lixiviation of organic compounds present in geopolymers in the beginning of the study (phase 1). So, considering just the values obtained in phase 2 for reactors A1, L2 and H1, not having spheres lixiviation, it was obtained similar values between 0.187 and 0.203 $L_{CH4/gCODrem}$, being the highest value for the reactor H1.

Regarding the degree of methanization, similar conclusions can be drawn. Reactor L1 presented the lowest percentages in both phases (7-10%), which is in accordance with the lowest methane productions (**Figure 9**). Likewise, reactors A1 and L2 presented similar percentages, ranging in average, 43% in phase 1 and 46% in phase 2. Considering just phase 2, the degree of methanization ranged from 46-48%, being the highest value for reactor H1. Hence, it can also be verified, that the reactor H1, which had geopolymers with higher porosity, had a higher degree of lixiviation of polyethylene glycol than in the reactors with addition of geopolymers with lower porosity (L1 and L2).

4.1.5. Volatile Fatty Acids Production

The VFA determined in the four reactors by gas chromatography, are represented in **Figure 10**. All reactors presented similar behaviors in terms of VFA production, with higher concentrations after both additions of substrate, reflecting the easily biodegradability of the substrate (cheese whey). The most produced acid was n-butyric with concentrations higher than 3000 mg COD/L, followed by acetic and propionic acids. The other acids (i-butyric, i-valeric, n-valeric and n-caproic acids) were detected at lower concentrations, and mainly in the first phase of the study (until day 56 of operation). Although the n-butyric acid reached the highest concentrations (c.a. 3500 mg COD/L) in the four reactors, it was rapidly consumed afterwards. In contrast, acetic and propionic acids maintained higher concentrations during some time, acetic (1000-1500 mg COD/L) and propionic (500-750 mg COD/L), being the propionic acid the last being consumed.

As can be expected, VFA production was related to the pH and sCOD evolution in the system (**Figure 11**). For all reactors, the highest concentrations of accumulated VFA were achieved when the pH values were lower in the first days of each phase, i.e., when a new addition of substrate was made to the reactors. In the same way, when the pH values increased, the VFA concentrations decreased and the methane production increased. The soluble COD concentration displayed a similar behavior as the VFA concentration.
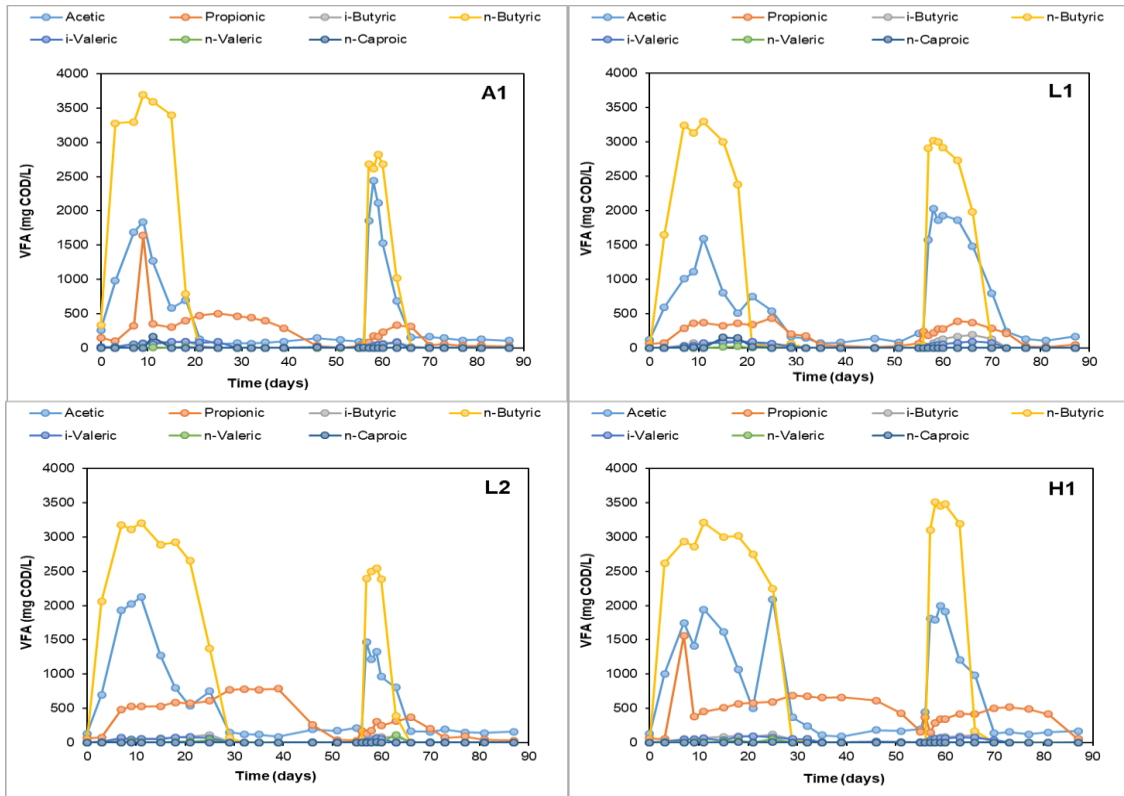
Figure 10: VFA composition during time in anaerobic digesters for the distinct systems, in the first study.
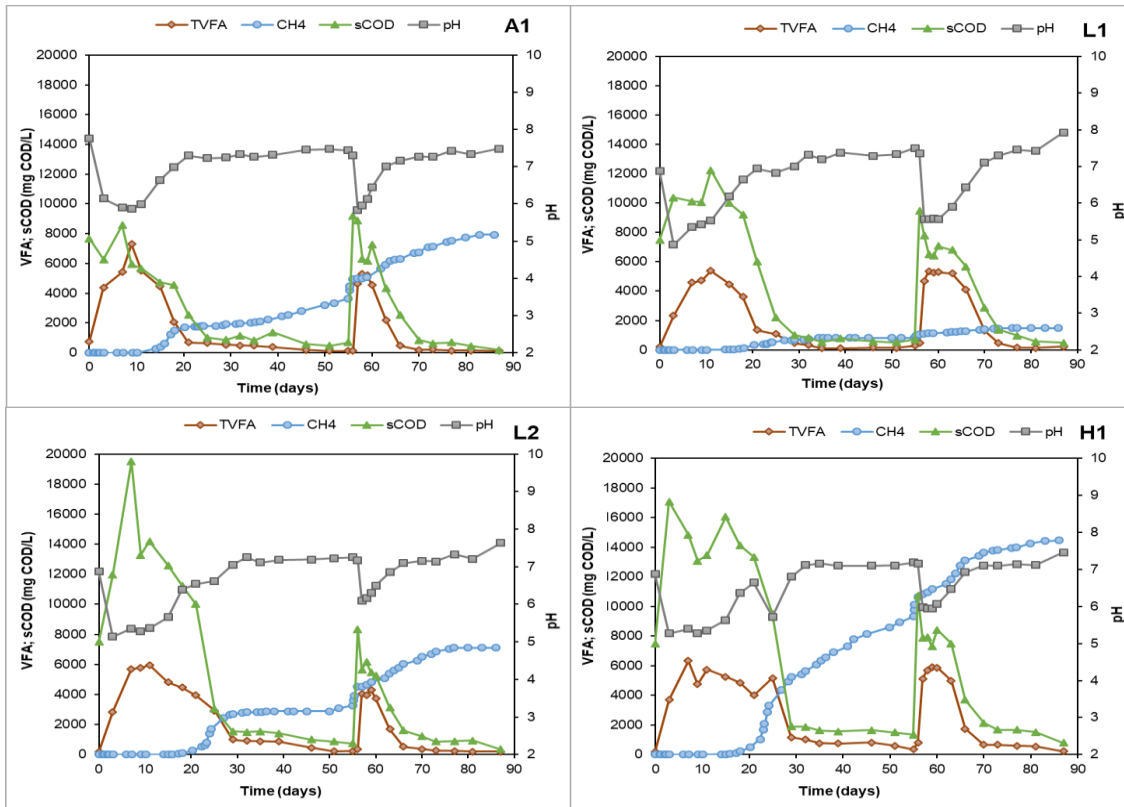

Figure 11: pH, VFA, sCOD and methane evolution during time in anaerobic digesters for the distinct systems, in the first study.

In **Table 11** are indicated the degrees of acidification and VFA yields obtained during the two phases of the study. As expected, it can be observed that the first phase was the most favorable to the system acidification, showing higher percentages of maximum acidification in all reactors (72-85%). After the second substrate addition (phase 2) the maximum degrees of acidification obtained were much lower (52-58%) suggesting a good adaptation of the microbial culture, especially the methane production ones. Hence, it was observed a decreased system capacity to maintain low pH values that favor the VFA production in detriment of methane production, in accordance with the main objective of the study.

Table 11: Maximum degrees of acidification and VFA yields in the two phases of the first study.

Reactors		Degree of Acidification (%)	$Y_{VFA/COD}$ max (g_{VFA}/g_{COD})
A1	Phase 1	71.90	0.98
	Phase 2	57.47	0.84
L1	Phase 1	71.94	0.49
	Phase 2	56.74	0.83
L2	Phase 1	79.55	0.97
	Phase 2	51.62	0.79
H1	Phase 1	84.50	0.61
	Phase 2	55.06	0.81

4.1.6. Total and Volatile Suspended Solids

The total and volatile suspended solids evolution during the study is indicated in **Figures 12 and 13**, respectively. TSS and VSS concentrations exhibited similar behavior, as expected, because they reflect mostly the amount of biomass inside the reactors. The solids concentrations increased after each addition of substrate, which reflected the microbial growth, the solids input from the substrate and the batch mode of the experiment. The reactor H1, with 16 g/L of geopolymers with higher porosity, in most of the experimental period, had a higher solids concentration than the other reactors during the study, probably due to the COD input from the spheres lixiviation, which may had resulted in higher biomass growth.
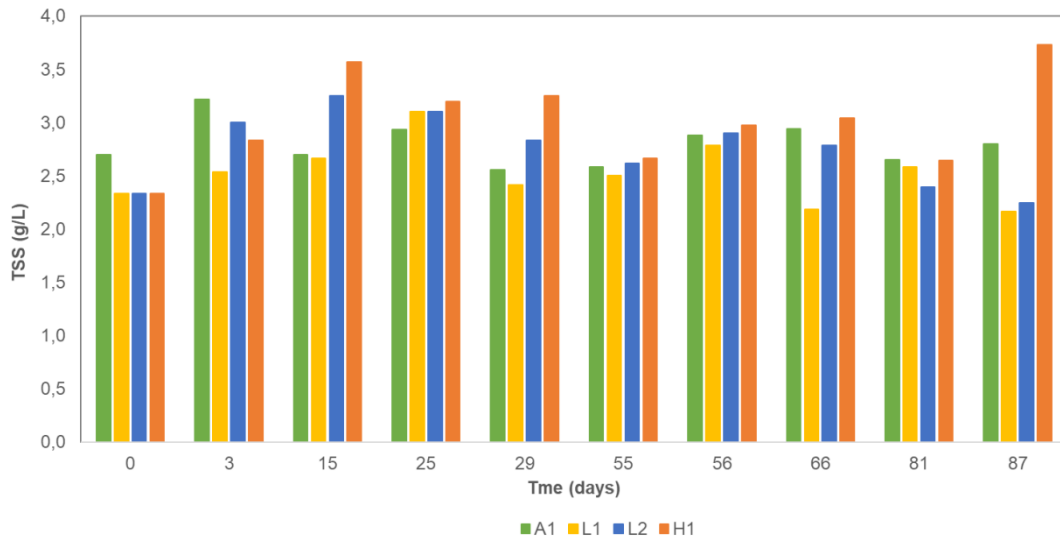
Figure 12: Total suspended solids concentration obtained in anaerobic reactors during the first study.
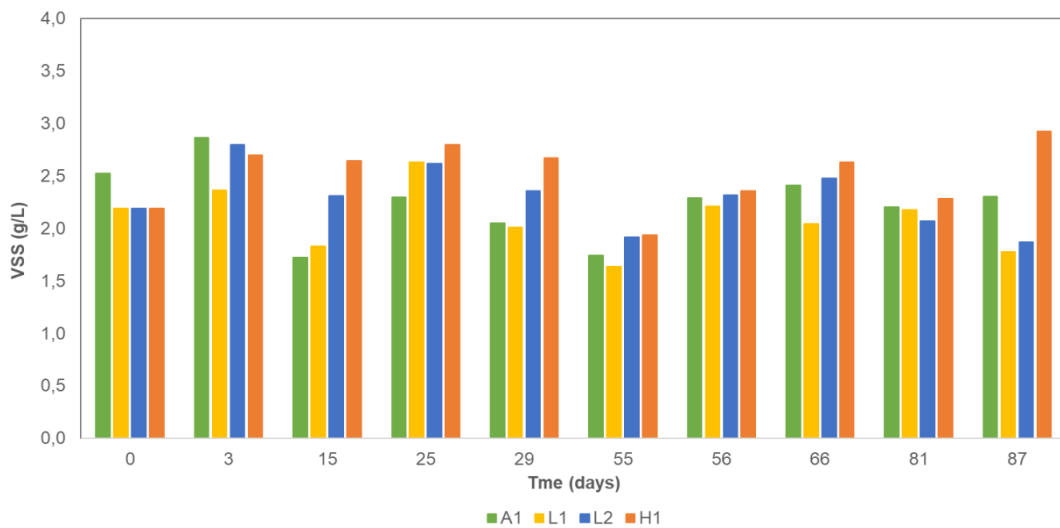
Figure 13: Volatile suspended solids concentration obtained in anaerobic reactors during the first study.

4.1.7. Geopolymers Mass Loss

The mass of geopolymer spheres in the reactors after 87 days of operation was lower than the initial one (**Table 12**). The reactor with geopolymer spheres with higher porosity (H1) that produced the highest accumulated methane volume during the study, exhibited the highest mass loss, 48% of the initial one. The reactors with lower porosity spheres (denominated L1 and L2), displayed an identical loss mass percentage (38 – 40%), being,

as expected, slightly higher in the reactor with a higher mass of spheres added. The observed mass loss was due to the lixiviation of compounds from the geopolymers, which allowed the pH control during the assays and favored the methanogenic archaea activity.

Table 12: Difference in geopolymers mass between the start and the end of the first study.

Reactors	$m_{geopolymers}$ initial (g)	$m_{geopolymers}$ final (g)	Mass loss (g)	Mass loss (%)
L1	12.03	7.45	4.58	38.07
L2	16.01	9.60	6.41	40.04
H1	16.01	8.27	7.74	48.34

4.2. Long term geopolymers performance

Considering the results obtained in the first study, the conditions chosen for further studies where those of the reactor with 16 g/L of geopolymer spheres with higher porosity (H1), which produced the highest accumulated methane volume. Along these lines, and as previously mentioned, a reactor with 4 g/L of $CaCO_3$ (A2) was also considered, as reference, besides two reactors with 16 g/L each of geopolymers type H (H2 and H3).

4.2.1. pH Evolution

Similar to the previous study, in the first day after substrate addition, the pH value of the reactor with chemical alkalinity (A2) was higher (8.60) than the values of the reactors with geopolymers (7.59). Immediately after, as can be seen in **Figure 14**, the pH values for all reactors decreased, reaching the lowest values on the third day of operation (6.09 for reactor A2, 5.22 for reactor H2 and 5.14 for reactor H3). After this decrease, the pH increased, and reactors A2 and H3 reached favorable pH values for the methanogenic archaea development at day 13 and the reactor H2 at day 16 of operation. The methane production started at day 13 in reactor A2, at day 21 in reactor H2 and at day 20 in reactor H3 (**Figure 16**).
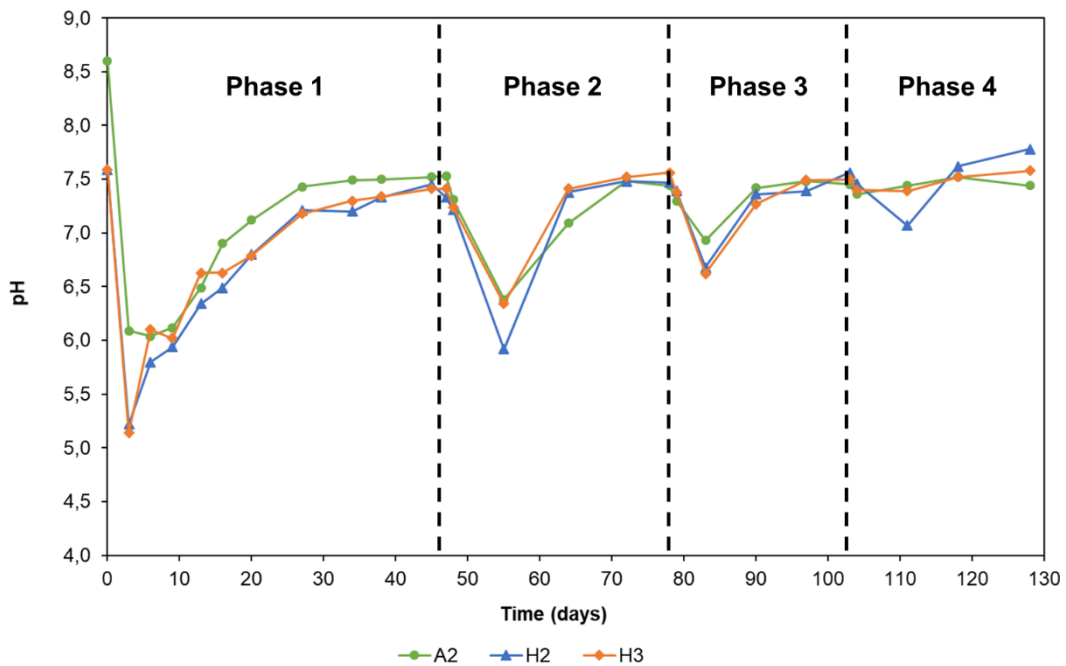

Figure 14: pH evolution in the different phases during the second study as function of time, with addition of chemical alkalinity and geopolymers spheres with higher porosity.

In phase 2, after the second substrate addition (day 48), the pH values of all reactors decreased as expected, but with a lower decrease (**Figure 14**), with the reactor H2 achieving the lowest pH value (5.92). Yet, all reactors reached favorable values for methane production 16 days after the cheese whey addition, showing much better recovery than in phase 1. In phases 3 (from day 79 until 103) and 4 (from day 104 until 128), after new substrate addition, pH values decreased as in previous phases but reaching higher values, and achieving more rapidly favorable range for methanogenic microorganisms development. In the fourth phase, the reactor H2 reached a lower value than the other reactors (7.07), although in a neutral range, similar to what had happened in phase 2.

In terms of pH evolution, the reactors with the same concentration of geopolymer spheres (H2 and H3) demonstrated a similar behavior during the study, except in day 55 and 111, when the reactor H2 reached a lower pH value. The pH values ranged between 7.78 and 5.23 in reactor H2 and between 7.59 and 5.14 in reactor H3. The reactor with the addition of chemical alkalinity (A2) exhibited higher pH values in the first phase than the reactors H2 and H3, but during the remained study, it displayed a similar tendency as the reactors with geopolymers. The pH values of this reactor during all study varied between 8.60 and 6.04.

4.2.2. Soluble Chemical Oxygen Demand Evolution

The temporal evolution of sCOD in all anaerobic reactors is represented in **Figure 15**. In day zero the sCOD values were near 8 g COD/L, which corresponded to the quantity of substrate initially added to each reactor. After the initial period, the sCOD decreased in the reactor A2 and increased in the reactors H2 (15.72 g COD/L) and H3 (14.14 g COD/L). As previously described, this increase resulted from the polyethylene glycol lixiviation of the geopolymers. Only after 20 days, the sCOD values started to decrease in reactors H2 and H3, reaching low values equivalent to reactor A2 at around day 34.

Through the four different phases, it can be observed that the system was requiring less time to consume the organic matter, and from phase 2 on the behaviour of the three reactors became very similar. In the first phase, it took for all reactors around 34 days to reach a sCOD concentration below 1 g COD/L, and this time was successively decreased to 24, 18 and 14 days in the subsequent phases (phases 2, 3 and 4, respectively). The multiple substrate additions illustrated the increasing recovery capacity of the anaerobic system for the organic matter removal, along the operation of the system for a long period

of time (128 days). The percentages of organic matter removal in the different phases, represented in **Table 13**, were very high and similar for all reactors, ranging mostly from 91–95%. However, it can be emphasized, that reactor H3 had the highest average efficiency (92.5%), most probably due to the fact that it had lower pH fluctuations.
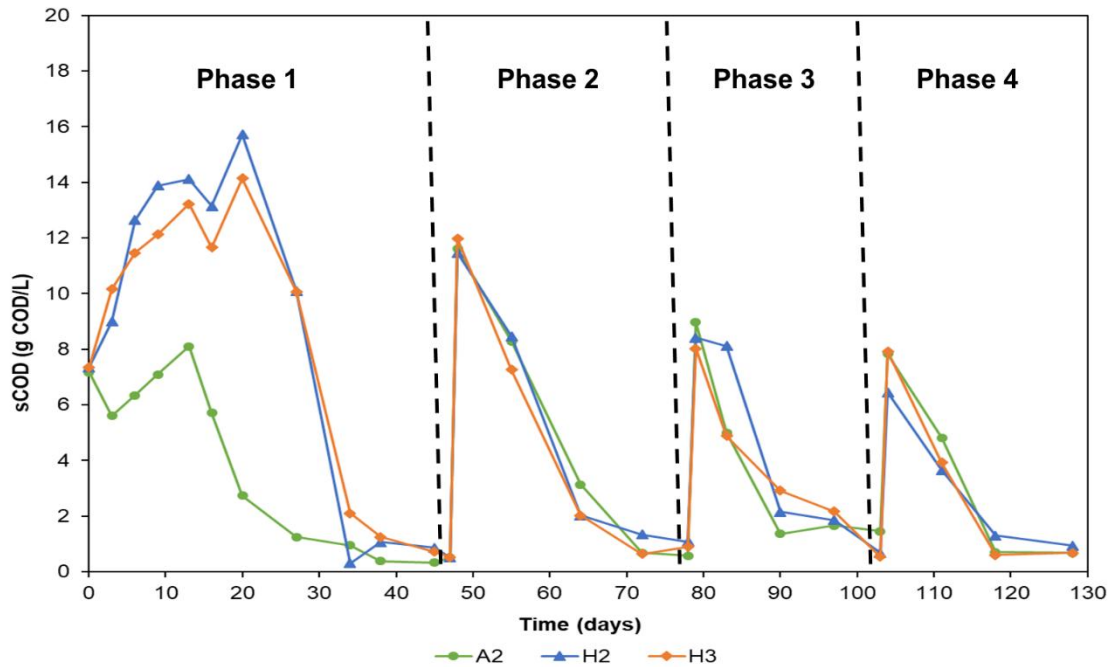

Figure 15: sCOD evolution in the different phases during the second study, as a function of time, with addition of chemical alkalinity and geopolymers spheres with higher porosity.

Table 13: Organic matter removal (%) in the four phases of the study.

Reactor		Organic matter removal (%)
A2	Phase 1	92.69
	Phase 2	95.05
	Phase 3	83.81
	Phase 4	91.24
H2	Phase 1	93.04
	Phase 2	90.52
	Phase 3	91.89
	Phase 4	85.38
H3	Phase 1	92.78
	Phase 2	92.50
	Phase 3	93.22
	Phase 4	91.45

4.2.3. Methane and Biogas Production

Figure 16 represents the cumulative methane production during the study, where it can be observed that reactor H2 produced the highest and reactor H3 the lowest accumulated methane volume.
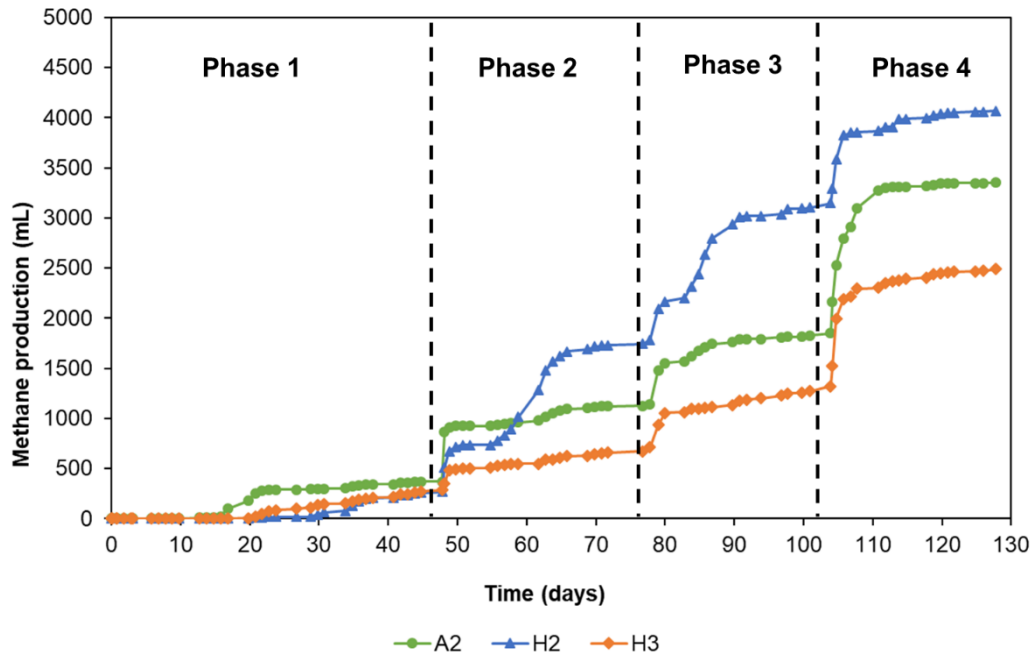

Figure 16: Cumulative methane produced in the different phases during the second study, with addition of chemical alkalinity (A2) and geopolymers spheres with higher porosity (H2 and H3).

The cumulative volumes of methane and biogas produced in the four phases of the study for each reactor are represented in **Table 14**. In the first phase, the reactors with the addition of geopolymers (H2 and H3) presented a lower pH (lower than 6), than the reactor with chemical alkalinity (**Figure 14**), which required more time to recover, hence resulting in a higher cumulative methane volume produced in reactor A2. The first phase was the phase of biomass adaption, which can explain the lower methane volume produced for al reactors (**Table 14**, when compared to the values obtained in the remaining phases.

After the first phase, the reactors H2 and H3, despite having the same concentration of geopolymer spheres displayed a different performance, with reactor H2 producing larger methane volumes in phases 2 and 3. Reactor A2 displayed a higher volume in phase 4 (1500 mL). However, in average (excluding first phase), reactor A2 had a value of 993.6 mL, reactor H2 of 1265.3 mL (the highest) and reactor H3 of 734.5 mL (the lowest).

Compared with reactor H1, which was under the same conditions, reactors H2 and H3 produced a much lower average cumulative methane volume (c.a. of half). It should be referred that the anaerobic sludge used in both studies was the same. However, as it was stored for a long time between both assays, the microorganisms would no longer be active, requiring a longer time to adapt, which could explain the difference in the produced methane volumes obtained in both sequential studies.

Table 14: Volume of methane and biogas produced in the four phases of the study.

Reactor		Volume (mL)	
		Methane	Biogas
A2	Phase 1	370.4	2674.0
	Phase 2	769.3	2958.5
	Phase 3	711.7	1993.8
	Phase 4	1499.7	2508.0
	Average value	**837.8**	**2533.6**
H2	Phase 1	269.2	1536.8
	Phase 2	1513.6	3273.3
	Phase 3	1362.9	3309.8
	Phase 4	919.3	1395.3
	Average value	**1016.3**	**2378.8**
H3	Phase 1	284.2	1022.0
	Phase 2	426.0	1195.5
	Phase 3	611.9	1622.8
	Phase 4	1165.5	1883.0
	Average value	**622.15**	**1430.8**

The methane production yields and degrees of methanization for all reactors in the different phases of the study are presented in **Table 15**. According to the cumulative produced volume, the higher methane production yields were obtained in phase 4 in reactors A2 and H3 and in the phases 3 and 4 in reactor H2. Excluding the yields obtained in phases 1 and 2 for reactor A1, and phase 1 for reactor H2, and phases 1, 2 and 3 for reactor H3 (with degrees of methanization lower than 30%), due to the longer adaptation of biomass, it was obtained similar yields for all reactors, respectively 0.210, 0.167 and 0.161 $L_{CH4/gCODremoved}$ for reactors A2, H2 and H3. The calculated methane yields in the second study were lower than the ones obtained in the first study, by either comparing

reactors A1 with A2 or reactors H1 with H2 or H3, which support the idea of biomass with a lower activity in the second study.

Generally, the highest degrees of methanization were obtained in the last phase of the study (phase 4), which suggests also that the biomass required longer times for adaptation in all reactors, with or without the addition of geopolymers.

Table 15: Methane production yields and degrees of methanization in the four phases of the study.

Reactor		$Y_{CH4/CODremoved}$ (L_{CH4}/g_{COD})	$Y_{CH4/CODinitial}$ (L_{CH4}/g_{COD})	% methanization $(g_{CH4-COD}/g_{COD})$
A2	Phase 1	0.056	0.052	13.12
	Phase 2	0.070	0.066	16.38
	Phase 3	0.095	0.079	30.64
	Phase 4	0.210	0.192	73.61
H2	Phase 1	0.039	0.037	9.30
	Phase 2	0.146	0.132	33.54
	Phase 3	0.176	0.162	49.26
	Phase 4	0.167	0.142	95.73
H3	Phase 1	0.042	0.039	9.81
	Phase 2	0.038	0.036	9.02
	Phase 3	0.082	0.076	28.37
	Phase 4	0.161	0.147	51.06

4.2.4. Volatile Fatty Acids Production

In **Figure 17** is represented the VFA concentrations identified in the anaerobic reactors. In phase 1 all reactors presented higher concentrations of n-butyric acid (higher than 3500 mg COD/L) followed by acetic acid (1000-1500 mg COD/L). In reactors with the addition of geopolymers (H2 and H3), n-caproic acid was also detected. In phase 2 the three reactors displayed high concentrations of acetic (c.a. 1500 mg COD/L) and propionic (c.a. 500 mg COD/L) acids. In reactors H2 and H3 n-butyric was also presented in high concentrations (c.a. 3500 mg CD/L), although being rapidly consumed during the phase. In phase 3, all reactors show an identical performance, with n-butyric, acetic and propionic acids presented in higher concentrations, respectively 2000, 1500 and 500 mg COD/L, than the remaining acids. In phase 4, acetic acid exhibited higher concentrations in reactor H2 (about 1700 mg COD/L). Consequently, in this phase, the accumulated volume of the methane produced in this reactor was lower (**Table 13**) than the values obtained in the reactors with a lower concentration of acetic acid (about 500 mg COD/L in A2 and 700 mg COD/L in H3).
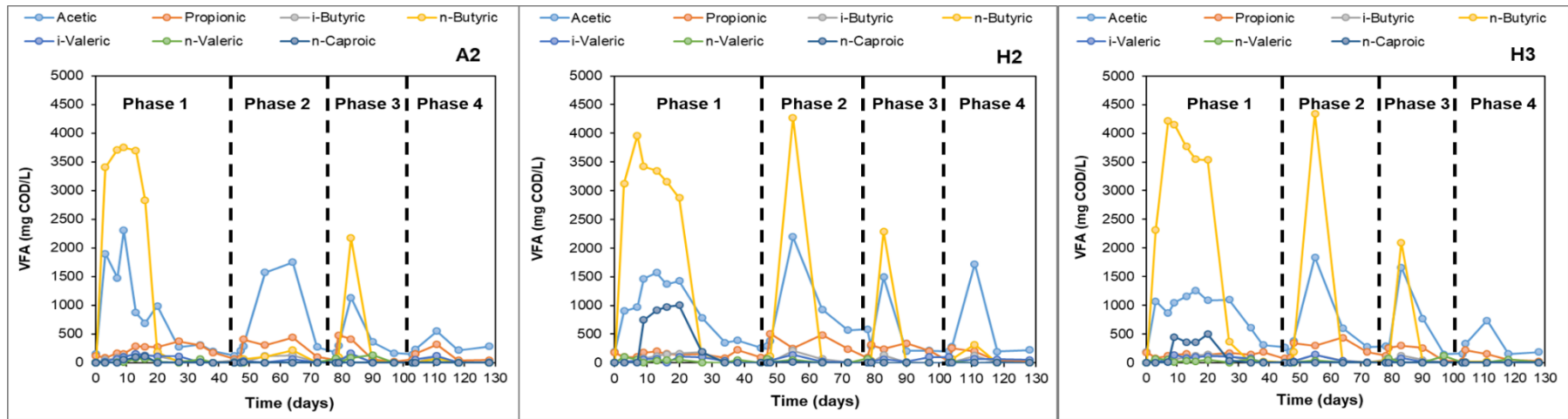
Figure 17: VFA composition during time in the different phases for the distinct systems, in the second study.
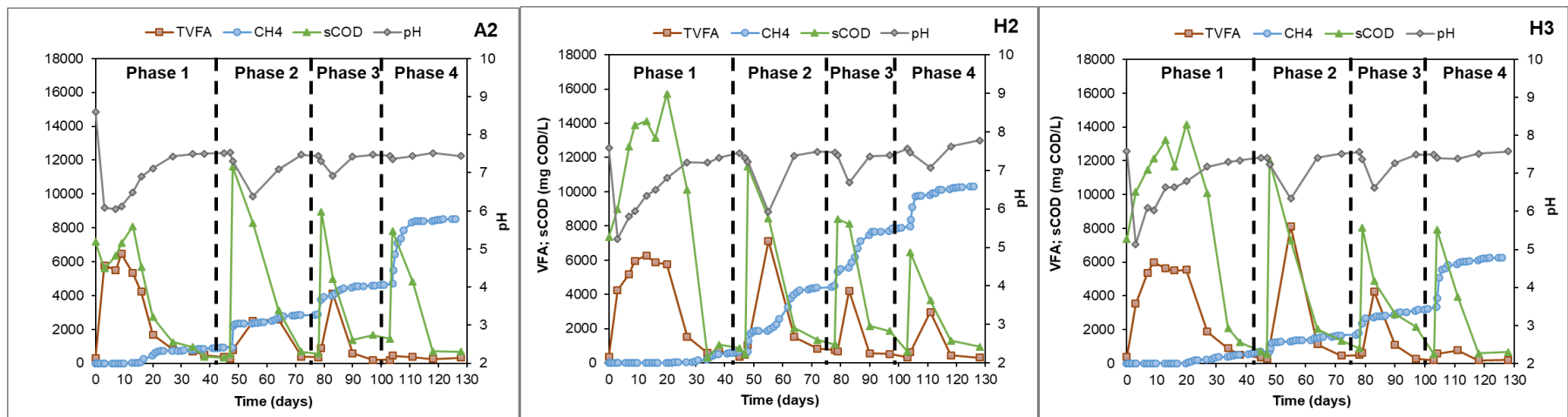

Figure 18: pH, VFA, sCOD and methane evolution during time in anaerobic reactor for the distinct systems, in the second study.

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In **Figure 18** is represented the relation between pH, VFA, sCOD and methane evolution during the study. In the start-up period of this study, and after the different substrate additions, the pH reached low values due to high concentrations of accumulated VFA. Likewise, when the pH increases to values close to 7.5, the VFA concentrations decrease and the methane production became favored (**Figure 18**). The sCOD presented a similar performance as the VFA concentrations. Comparing the results obtained for reactors H2 and H3 with the ones obtained for reactor H1 from the first study (**Figure 11**), it can be observed that, in general, the reactors performance was identical, despite the higher cumulative methane volume produced in the first study.

Table 16: Maximum degrees of acidification and VFA yields in the different phases of the second study.

Reactors		Degree of Acidification (%)	$Y_{VFA/COD}$ max (g_{VFA}/g_{COD})
A2	Phase 1	90.15	0.98
	Phase 2	22.32	0.82
	Phase 3	45.76	0.82
	Phase 4	15.01	0.49
H2	Phase 1	85.28	0.90
	Phase 2	62.26	0.84
	Phase 3	50.11	0.52
	Phase 4	37.64	0.66
H3	Phase 1	81.40	0.55
	Phase 2	56.61	0.93
	Phase 3	53.06	0.87
	Phase 4	11.68	0.42

According to **Table 16**, the higher degrees of acidification in all reactors were obtained in the first phase of the study, which demonstrates that this phase was the most favorable to the system acidification. Like in the first study, the percentages of acidification decreased in the remaining phases, which may be a result to the microbial culture adaption to methane production. Hence, it was observed a decreasing capacity of the system to maintain low pH values that favor the VFA production, in detriment of methane production.

4.2.5. Total and Volatile Suspended Solids

The total and volatile suspended solids concentrations in the start-up and conclusion of each of the four phases of the study are displayed in **Figures 19 and 20**, respectively. The solids concentrations increased immediately after the substrate was added to the reactors (time zero) and decreased with the evolution of each phase, probably due to the consumption of particulate substrate biodegradation. Before the second substrate addition (phase 2), it was necessary to add more inoculum to the reaction media, because volatile solids concentration was near or even lower than the pre-set conditions (2 g SSV/L). H2 was the reactor that exhibited the higher increase in the solids concentrations. In phase 4, reactors A2 and H3 presented an increase in TSS and VSS concentrations, probably due to biomass growth, being reactor H2 the one presenting the highest concentration.
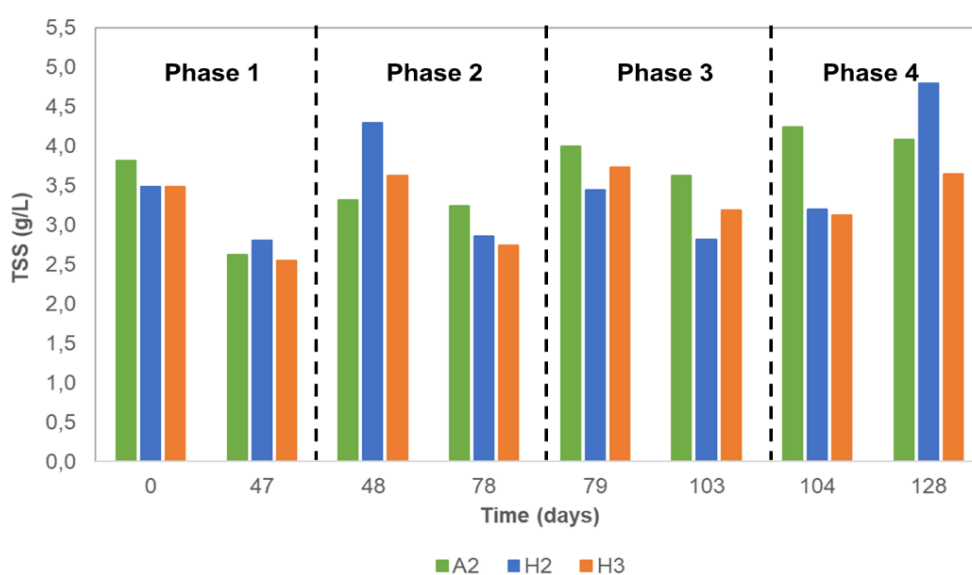

Figure 19: Total suspended solids concentration obtained in the different phases during the second study.

According to **Figure 20**, and in opposite to what had happened in the other three phases, there was an increase on VSS in all reactors during phase 4, most probably due to the increase of methanogenic biomass, which is in accordance with the much lower acidification degrees achieved in this phase. This fact suggests also that it was necessary a longer period for the biomass to adapt for methane production, when compared to the first study.
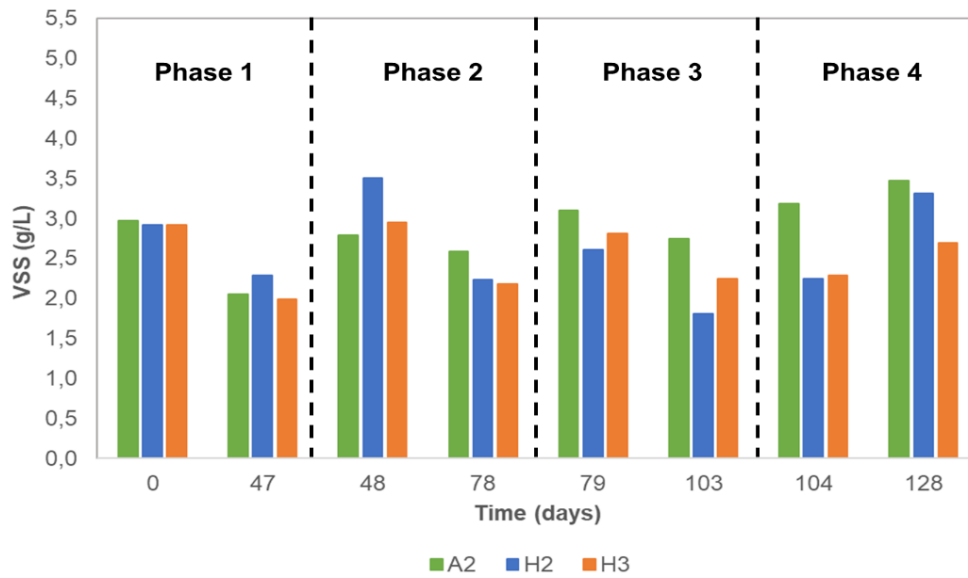
Figure 20: Volatile suspended solids concentration obtained in the different phases during the second study.

4.2.7. Geopolymers Mass Loss

Such as in the previous study, the geopolymers spheres lost mass during the experimental trial (**Table 17**), due to the lixiviation of compounds present in the spheres, which allowed the pH control during the assays. Both H2 and H3 lost approximately 32% of their initial mass during the 128 days of operation. Reactor H1 had lost c.a. of 48% of geopolymers mass (**Table 12**) in less time (87 days) of operation than the reactors H2 and H3. This may be related with the lower methane volume obtained in the second study; meaning that it was required a smaller contribution to pH control of the geopolymers spheres in reactors H2 and H3, which had resulted in a smaller dissolution of these materials.

Table 17: Difference in geopolymers mass in the start and end of the second study.

Reactors	$m_{geopolymers}$ initial (g)	$m_{geopolymers}$ final (g)	Mass loss (g)	Mass loss (%)
H2	15.99	10.84	5.15	32.23
H3	16.00	10.79	5.21	32.57

Chapter 5. Conclusions

The main objective of this work was the use of waste-based geopolymers for pH control in anaerobic reactors for methane production, treating easily acidifiable substrates. The experimental work was divided in two studies, with different goals. The first study, that had two phases and four batch anaerobic reactors, was done to optimize the concentration and type (level of porosity) of the two geopolymers under study, to achieve a higher methane production. After this selection, the second study was divided in four phases and performed with three anaerobic reactors, in order to evaluate the long-term geopolymers performance and reproducibility.

In the first study, pH evolution was similar in all reactors with and without spheres, reaching favorable values for methane production after an initial period where it was observed a decrease in this parameter. In addition, all reactors presented a similar sCOD performance, except in the start-up period (phase 1) in the reactors with the addition of geopolymers, where it was observed an increase of this parameter, due to the leaching of some of the organic components present in these materials. In the second addition of substrate (phase 2) it was observed a decrease in the sCOD values for all reactors, reaching very low values in smaller times than in phase 1, which showed the recovery capacity of the system for organic matter removal. The VFA production in the beginning of each phase were rapidly consumed, which suggest a good adaptation of the microbial culture to methane production.

Regarding the methane production, in the first study, the reactor with the addition of geopolymers with higher porosity (H1) produced the highest volume of about 2020 mL of CH_4 in phase 2, correspondent to a methane yield of 0.203 $L_{CH4/gCOD}$ removed. Comparing the reactors with geopolymers with lower porosity and different concentrations (L1 and L2), it was observed that the reactor with the highest concentration (16 g/L) produced a

superior methane volume of about 1510 mL of CH_4 in phase 2, correspondent to a methane yield of 0.190 $L_{CH4/gCOD\ removed}$.

Thus, considering the results from the first study, the geopolymer spheres with higher porosity (type H) and the higher tested concentration (16 g/L) were the conditions selected to the second study of the experimental work.

In the second study, the pH performance was similar in all reactors, immediately decreasing after substrate addition followed by an increase to favorable values for the development of the methanogenic archaea in 13 days (A2 and H3) and 16 days (H2) in phase 1. Like in the first study, the sCOD values of the reactors with addition of geopolymers increased in the start-up period due to the geopolymers leaching. The time that the reactors needed to remove the organic matter between phases decreased, which, once again, proved the increased recovery capacity of the system.

The methane volume produced in reactors H2 and H3 in the second study was expected to be close to the obtained in the first study with the reactor H1, which did not occur. In the same conditions, in the first study reactor H1 produced 2020 mL of CH_4 in phase 2, and in the second study reactor H2 1360 mL of CH_4 in phase 3 and reactor H3 1170 mL in phase 4. This could be due to the anaerobic microbial mixed culture used as inoculum, since it was stored for a long period between studies, which could have resulted that the microorganisms could no longer be active, needing more time to adapt. Hence, it was observed that in the second study it was necessary to wait 3-4 phases for the system to adapt and to respond to methane improvement. This fact happened with and without polymers addition for pH control.

During both studies, it was observed a mass loss in the geopolymers. In the reactors H2 and H3 (second study), approximately 32% of the initial mass was lost during the 128 days of operation. On the other hand, in the first study, reactor H1 had lost 48 % of geopolymers mass in the 87 days of operation. The higher dissolution of geopolymers in reactor H1 can be related with the higher required contribution to pH control, which may be explained by the higher methane volume obtained.

The results obtained demonstrated the usefulness of geopolymers in anaerobic digestion processes. It was confirmed that it is possible to control the pH for methane production in anaerobic processes at around 6.5 - 7.5, using geopolymer spheres containing fly ash. Besides, using these sustainable waste-based materials instead of commercial alkaline materials in AD processes result in environmental sustainability.

In conclusion, the goals of this thesis were successfully achieved and the obtained results bring new insights that can contribute to solve the current problems associated

with pH control in AD, and also to contribute to the valorization of fly ash, thus decreasing the needs for its landfill disposal and the associated environmental problems.

Chapter 6. Future Perspectives

To confirm the feasibility of using fly-ash based geopolymers for pH control in anaerobic digestion process, further studies are required. As a continuation of the experimental work carried out in this thesis, it can be suggested:

- Identification of the compounds that are leachate from the geopolymeric spheres, which promoted the COD and TOC increasing in the start-up period of the studies, and assessment of their biodegradability.

- Study of the reuse of the geopolymer spheres. Is was confirmed that it was possible to perform several series, of approximately 30 days each. However, due to the geopolymers mass loss during the assays, the number of series may be limited. In this sense, it is necessary to perform more studies to verify their capacity to maintain the pH values in the desired range, promoting the methane production.

- Determination of the geopolymers concentrations and/or compositions that allow to obtain, in alternative, an anaerobic acidogenic process, reaching pH values favorable to the accumulation of volatile fatty acids (below 6.5).

- Study the anaerobic process in a continuous mode, using the geopolymers spheres as buffer materials, reducing the cost of chemicals and promoting a sustainable process.

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Appendices

The calibration curves used to volatile fatty acids analysis by gas chromatography are represented in the following figures.
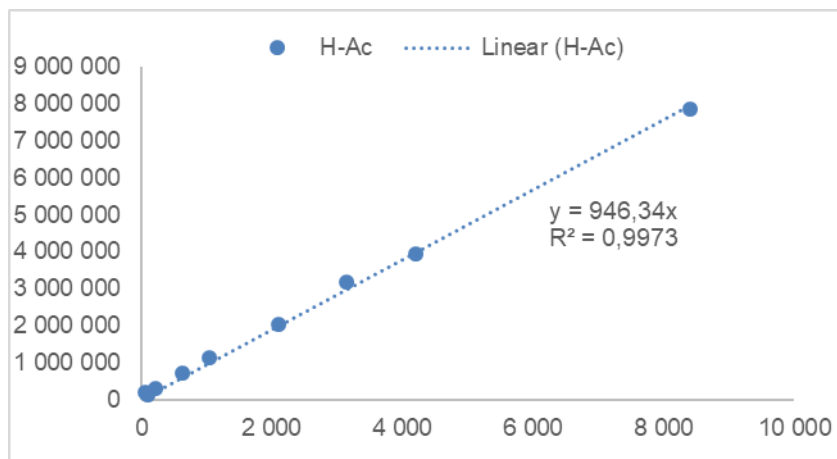

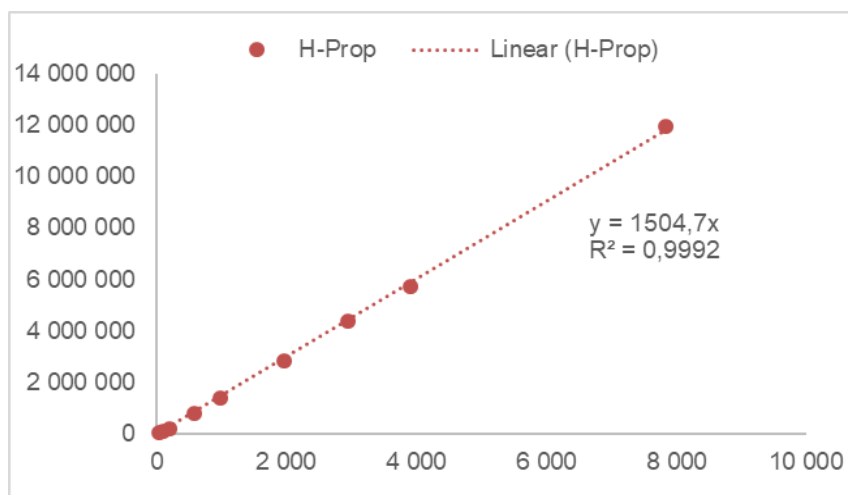**Figure 21:** Calibration curve of acetic acid.
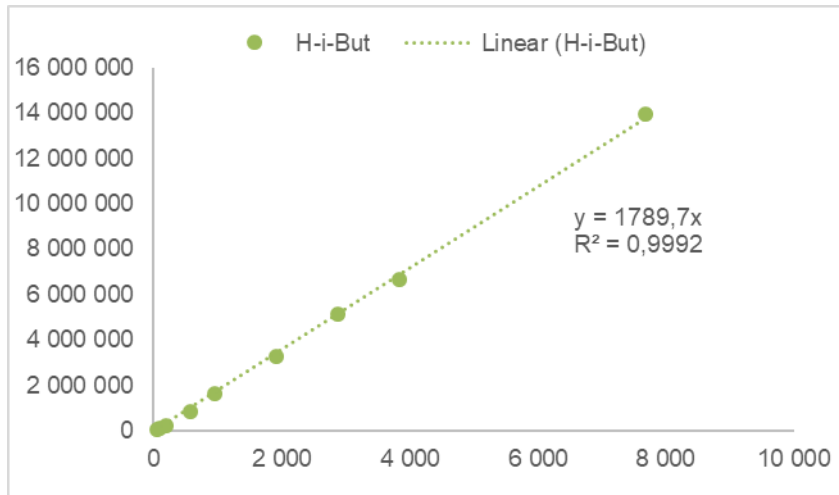

Figure 22: Calibration curve of propionic acid.

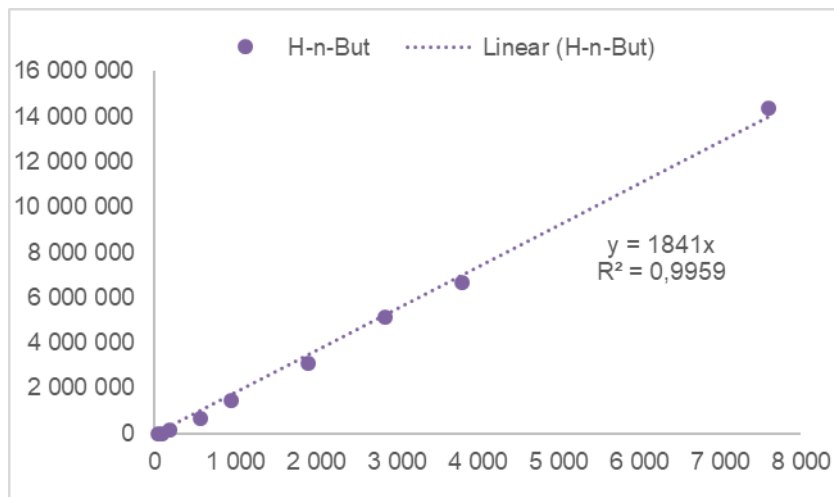**Figure 23:** Calibration curve of i-butyric acid.
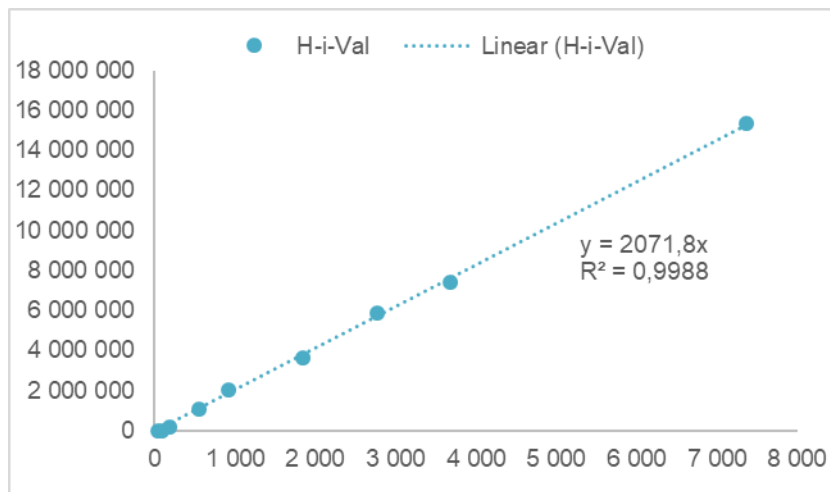

Figure 24: Calibration curve of n-butyric acid.

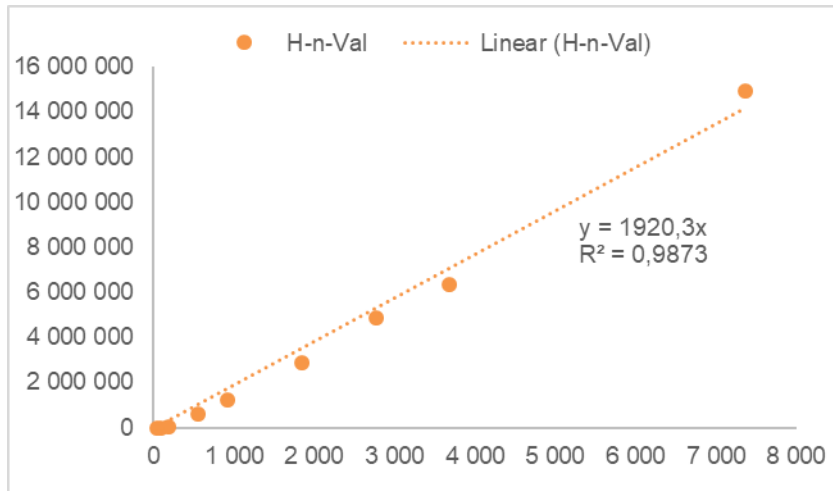**Figure 25:** Calibration curve of i-valeric acid.
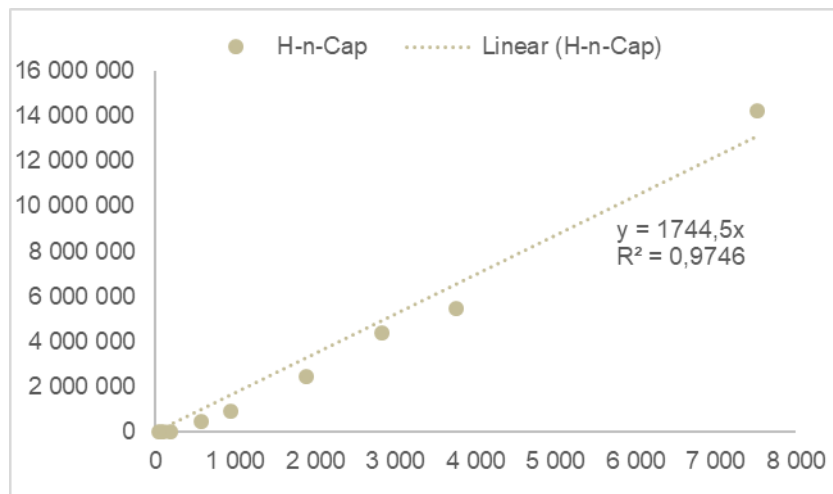
b

Figure 26: Calibration curve of n-valeric acid.

Figure 27: Calibration curve of n-caproic acid.