Universidade de Aveiro
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Ana Lúcia Oliveira Costa

Valorização biotecnológica de biorresíduos utilizando biochar

Biotechnological valorisation of biowaste using biochar

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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 do Professor Doutor Flávio Gonzaga Castro Santos Silva, Professor Auxiliar, e co-orientação científica da Professora Doutora Maria Isabel Aparício Paulo Fernandes Capela, Professora Associada, do Departamento de Ambiente e Ordenamento da Universidade de Aveiro.

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Dedico este trabalho aos meus pais e ao Francisco, pelo incansável apoio em todas as horas.

o júri

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palavras-chave biochar; digestão anaeróbia; biogás; biorresíduos; resíduos alimentares

resumo

Historicamente, a digestão anaeróbia (DA) é um processo com grande relevância na gestão e tratamento de resíduos biodegradáveis, permitindo simultaneamente a geração de combustíveis de origem renovável (biometano e bio-hidrogénio). Atualmente tem-se procurado a optimização deste processo em contexto de Economia Circular. Uma das possibilidades com elevado interesse é a utilização de biochar - material carbonáceo produzido a partir da pirólise de biomassa - em processos de DA. O presente trabalho tem como principal objetivo aferir os benefícios do biochar em processos de digestão anaeróbia de biorresíduos de origem alimentar. Para o efeito foram realizados ensaios experimentais em reatores descontínuos, variando os seguintes parâmetros: razão food-to-microorganisms (F/M = 0,5; 1; e 2 g/g); concentração de biochar (0; 2,5 e 5 g/L) e tamanho de partícula do biochar (<2 mm – S; e >2 mm – L). Verificou-se que a degradação de sólidos voláteis ocorreu na maioria dos ensaios, tendo as maiores eficiências de remoção sido observadas nos ensaios à razão F/M = 2 g/g, não tendo a adição de biochar tido influência significativa na remoção. Já na remoção de carência química de oxigénio solúvel (CQOs), o efeito da adição de biochar foi visível, em particular em condições de operação F/M = 2 g/g e concentração de biochar de partícula S a 5 g/L, produzindo uma eficiência de remoção de substrato de 62%. A quantificação de fósforo biodisponível e azoto Kjeldahl no digestato após os ensaios demonstrou o seu potencial agronómico, atingindo concentrações de até 54 mg P /L e 55 mg N /L, nomeadamente nas razões F/M = 0,5 e 1 g/g. No entanto, a adição de biochar não se mostrou relevante na concentração destes elementos no digestato. A produção de biogás aumentou linearmente com a razão F/M, em especial com concentrações menores de biochar (2,5 g/L), sendo o fator granulométrico pouco relevante. Recomenda-se que testes com razões F/M elevadas devem ser prolongados para além de 21 dias para se maximizar a degradação de CQOs e produção de biogás produzidos.

keywords biochar; anaerobic digestion; biogas; bio-waste; food waste

abstract

Historically, anaerobic digestion (AD) is a process with great relevance in the management and treatment of biodegradable waste, simultaneously allowing the generation of fuels from renewable sources (biomethane and bio-hydrogen). Currently, efforts have been made to optimize this process in the context of the Circular Economy. One of the possibilities with great interest is the use of biochar - carbonaceous material produced from the pyrolysis of biomass in AD processes. The main objective of this work is to assess the benefits of biochar in anaerobic digestion processes of bio-waste from food origin. For this purpose, experimental tests were carried out in batch reactors, varying the following parameters: food-to-microorganisms ratio (F/M = 0.5; 1; and 2 g/g); biochar concentration (0, 2.5 and 5 g/L) and biochar particle size (<2 mm – S; and >2 mm – L). It was found that the degradation of volatile solids occurred in most of tests, with the highest removal efficiencies being observed in tests at the F/M ratio = 2 g/g, with the addition of biochar not having a significant influence on removal. Regarding the removal of soluble chemical oxygen demand (sCOD), the effect of adding biochar was quite visible, in particular under operating conditions of F/M = 2 g/g and particle biochar concentration S at 5 g/L, yielding a 62% substrate removal efficiency. The quantification of bioavailable phosphorus and Kjeldahl nitrogen in the digestate after the tests demonstrated its agronomic potential, reaching concentrations of up to = 54 mg P /L and 55 mg N /L. However, the addition of biochar was not relevant in the concentration of these elements in the digestate. Biogas production increased directly with the F/M ratio, especially at lower concentrations of biochar (2.5 g/L), with the particle size factor being of little relevance. It is recommended that tests with high F/M ratios should be extended beyond 21 days to maximize COD degradation and biogas production.

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List of abbreviations and symbols

AD – Anaerobic Digestion

sCOD – Soluble Chemical Oxygen Demand

EU – European Union

OFMSW – Organic Fraction of Municipal Solid Waste

FW – Food Waste

VOA – Volatile Organic Acids

VFAs – Volatile Fatty Acids

I/S or ISR – Inoculum to Substrate Ratio

GHG – Greenhouse gases

STP – Standard Temperature and Pressure

PNAER – Plano Nacional de Ação para as Energias Renováveis

PNEC – Plano Nacional Energia e Clima

HRT – Hydraulic Retention Time

SRT – Solids Retention Time

TS – Total Solids

VS – Volatile Solids

N-K – Kjeldhal Nitrogen

N-org – Organic Nitrogen

TAN – Total Ammonia Nitrogen

FAN – Free Ammonia Nitrogen

DIET – Direct Interspecies Electron Transfer

1. Introduction

Due to population growth in recent decades, there has been a proportional increase in the consumption of resources that the planet has to offer. This consumption of resources leads to a huge amount of solid waste of different types. It has therefore become a necessity to manage this waste so that, due to its high volume, it does not become harmful to the environment.

According to the European Environment Agency (2020), bio-waste is the largest component (34 %) of all municipal waste in Europe and about 60 % of this bio-waste comprises food waste. Along the entire value chain, food waste amounts to about 173 kilograms per EU citizen per year, corresponding to about one fifth of all food that is produced. As part of the organic fraction of municipal solid waste, food waste can be used to produce energy through fermentative processes such as anaerobic digestion (AD).

Anaerobic digestion is well-known process that is already competing for the place of the most efficient multipurpose technology in the environment field of the century (Deena et al., 2022). AD can generate biogas – an approximately 3:1 mixture of methane and carbon dioxide – which has been known to be a clean fuel since the late 19th century (Abbasi et al., 2012). With this, AD may address synergistically the two most challenging problems of waste disposal and clean renewable energy generation (Deena et al., 2022).

Even though anaerobic digestion is a promising technology, it is also very complex and depends on several factors that may can cause imbalances to the process. Two major challenges of the AD process are: long-term operational stability and the quality of the digestate produced, that may retain the nutritive value of the digestate. Several factors, such as pH and the presence of ammonia, can cause disturbances, which is why many studies have been carried out to optimize the process (Fagbohungbe et al., 2017). One of the approaches is the split of the process into two stages, since acidogenic and

acetogenic bacteria perform better in more acidic conditions and methanogenic archea in more basic conditions (Algapani et al., 2018).

A recent approach that has been studied to overcome these problems is the application of biochar in AD. Biochar is a carbonaceous material produced via thermochemical conversion of biomass, and its use in diverse environmental applications reflects the principles of circular economy (Kumar et al., 2021). The addition of biochar to bioreactors has been gaining attention in order to provide stability to the system, either by adsorption of ammonia from the reacting medium or by immobilizing the bacteria onto a larger surface (Fagbohungbe et al., 2017).

The rationale behind the present work relies in the application of biochar to anaerobic batch reactors, with different particle size and concentrations, by using food waste as substrate.

The structure of this dissertation is organized as follows:

- The introductory chapter provides an overview of the research problem and its significance, setting the stage for the subsequent chapters.
- The second chapter presents the literature review, which covers three main topics: the organic fraction of municipal solid waste, anaerobic digestion and biochar.
- The third chapter states the research objectives that guided the study.
- The fourth describes the methodology, the materials used, the experimental setup and the analytical techniques.
- In the fifth chapter the main results are presented and discussed.
- In the final chapter the main conclusions are summarized, as well as suggestions for future research on this topic.

2. Literature review

2.1. Organic Fraction of Municipal Solid Waste

According to the Portuguese Decree-Law 178/2006, Municipal Waste is "waste from households as well as other waste that, by its nature or composition, is similar to waste from households". The organic fraction of municipal solid waste is the biodegradable waste, that accordingly to the Portuguese Decree-Law 183/2009 is "waste that can be subjected to anaerobic or aerobic decomposition, namely food and garden waste, paper and cardboard".

The Directive (EU) 2018/851 of the European Parliament and of the Council of 30 May 2018 amended Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 (also known as the Waste Framework Directive - "WFD") on various matters related to waste, including changes to the obligation for separate collection of municipal waste in the "material" and bioresidues.

For transposing of such Directive, the Portuguese Decree-Law no. 102-D/2020 of 10 December establishes the timetable for the separation, selective collection and forwarding for recycling of bio-waste by the end of 2023. To help achieve this, the Dispatch no. 2623/2021, of 9 March, creates the second phase of the Support Program for the Elaboration of Municipal Studies for the Development of Bio-waste Collection Systems.

According to the Portuguese Environmental Agency (APA, 2022), Portuguese waste production *per capita* stood at 511 kg/inhabitant per year, which is similar to the average of 517 kg/inhabitant per year for the EU27 Member States in 2020. This report from APA shows that, in 2021, the dominant fraction in the waste generated consists of bio-waste (37.42 %), followed by plastic waste (10.37 %).

As mentioned in the Municipal Waste Annual Report, in 2021, 24 facilities were able for the treatment of the organic recovery for biowaste (19 for biowaste from undifferentiated collection and 5 for biowaste from selective collection). These facilities

are distributed across 19 Municipal Waste Management Systems. Regarding the type of treatment at these facilities, half (12 facilities) carry out composting, while the other half carry out anaerobic digestion followed by composting (APA, 2022).

Despite the efforts to achieve the European and national targets for the selective collection of biowaste, it worth note that this is a very recent type of collection in Portugal, and that it is not yet fully disseminated at national level, as in some cases it is still operating in a pilot test format. The results of bio-waste collection show high levels of contamination with non-degradable fractions, thus suggesting that collection mechanisms need to be strengthened in terms of awareness-raising and participation and monitoring programs by the citizens (APA, 2022).

More recently, the Strategic Plan for Municipal Waste 2030 (PERSU 2030) was approved by Resolution of the Council of Ministers no. 30/2023, of 24 March, which incorporates some important objectives within the scope of bio-waste management. This plan defines how to achieve the goals for waste management, by setting targets for Municipal Waste Management Systems. In 2021, more than half of the waste produced in mainland Portugal (53 %) was landfilled, while the PERSU targets for 2035 are just 10 %. Regarding the preparation for reuse and recycling target, the next milestone to be achieved will be in 2025, with a rate of 55 %, contrasting with just 32 % in 2021. This indicator assesses the amount of waste prepared for reuse and recycling as compared to the amount of waste generated (APA, 2021).

PERSU 2030 also brings great relevance to the European Biomethane Strategy and the contribution of municipal bio-waste treatment for achieving its goals. Compost, digestate and materials that can be transformed into waste-based fuels represent the main outflows from the sorting, organic recovery, mechanical treatment and mechanical biological treatment units, after the separation of recyclable waste (APA, 2021).

Since this work studies the anaerobic digestion of food waste with the aim of obtaining biogas, it contributes in a certain way to the scientific study of the implementation of the above mentioned strategic objectives.

2.2. Fundamentals of Anaerobic digestion

The decomposition of the organic fraction of the solid waste, such as food waste, with microorganisms can occur in either anaerobic or aerobic conditions. Under aerobic conditions, i.e. in the presence of oxygen, the engineered process is named composting, while under anaerobic conditions, i.e. in the absence of oxygen, it is called anaerobic digestion.

The composting process has been traditionally used for many years. It is a simple and stable process, although it generates mainly CO_2 and water, without added value besides the agronomic value of the compost. Moreover, CO_2 emission is not compatible with carbon neutrality principles. On the other hand, the anaerobic digestion process generates methane-rich biogas that can be used as renewable energy vector. Similarly to composting, anaerobic digestion also produces a digestate that retains key nutrients of agronomic relevance.

AD is a complex biological process divided into several stages and dependent on different types of microorganisms working together to metabolize the organic substrate. The process can be divided into four main stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. In the Figure 1 the main steps of AD are presented:

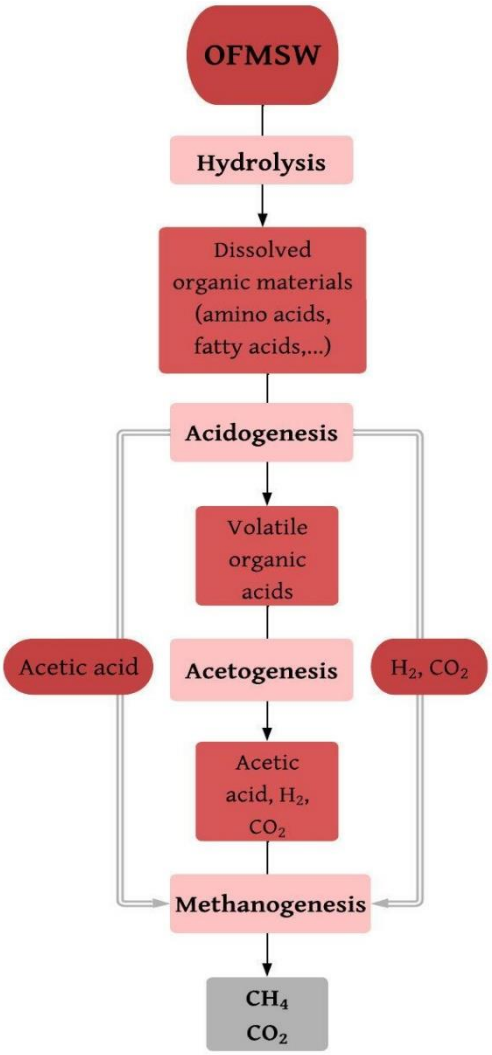

Figure 1 - Main stages of the AD process

Adapted from Fagbohungbe et al. (2017)

2.2.1. Stages of the anaerobic digestion process

2.2.1.1. Hydrolysis

During hydrolysis, the complex organic material with high molecular weight such as carbohydrates, proteins, lipids, and fibers are transformed by the action of exoenzymes (e.g. amylase, protease, lipase, and cellulase) secreted by microorganisms into dissolved organic materials consisting of simpler components (monosaccharides, amino acids, glycerol, long-chain fatty acids, etc.), which can then be assimilated by other microorganisms (Bajpai, 2017).

The breakdown of carbohydrates takes place over a period of hours, while lipids and proteins can take several days to be hydrolyzed. Hydrolysis is therefore the limiting step in the whole AD process, as it takes place relatively slowly compared to the other stages (Kondusamy & Kalamdhad, 2014).

2.2.1.2. Acidogenesis

Acidogenesis is the second stage on the AD process. Acidogenic bacteria convert the hydrolysis products into simple molecules with low molecular weight, such as volatile fatty acids (acetic, lactic, propionic, butyric, caproic acids), alcohols, aldehydes and gases such as CO_2, H_2 and NH_3. The presence of oxygen at this stage is extremely toxic and an acidic pH (around 5.5) favors the thriving of the acidogenic bacteria, while it is harmful to methanogenic archaeobacteria, which thrive under neutral pH (Kondusamy & Kalamdhad, 2014).

2.2.1.3. Acetogenesis

In the third stage of AD process, two types of microorganisms play a fundamental role: homoacetogenic bacteria and syntrophic bacteria. Homoacetogenic bacteria consume the products of the previous stages (VFA, amino acids, purines, fatty acids, among others) to produce acetate, formate and methanol and are H_2 consumers. In turn,

syntrophic bacteria consume different types of substrates such as ethanol, propionic acid or butyric acid to produce H_2 (Abbasi et al., 2012). The partial pressure of H_2 at this stage inevitably rises to levels that can be toxic for acetogenic microorganisms. For this reason, it is important to coexist in symbiosis with methanogenic archaeobacteria that consume H_2 and CO_2 to produce methane, keeping the H_2 pressure low. The acetate and hydrogen molecules from the oxidized volatile fatty acids are the precursors of methanogenesis, the final stage of the AD process for biogas production.

2.2.1.4. Methanogenesis

The last stage of AD process, methanogenesis, occurs through three different pathways: acetotrophic pathway (through acetic acid metabolization), hydrogenotrophic pathway (through hydrogen ions) and methylotrophic pathway (through methanol) (Abbasi et al., 2012).

The acetotrophic pathway is the main one and accounts from 65 – 70 % of the methane produced in AD. On the other hand, methanogenesis from CO_2 and H_2 also plays a relevant role, by maintaining a low H_2 pressure in the medium and thus supporting the growth of microorganisms that carry out the anaerobic oxidation of acetic acid. Methanogenic archea are extremely sensitive to temperature, loading rate and pH fluctuations, and they can also be inhibited by toxic compounds (Gavala et al., 2003).

2.2.2. Parameters that affect anaerobic digestion

To maximize the conversion of organic matter into biogas ideal conditions must be applied to the bioreactor. Factors such as temperature, pH, food/microorganisms ratio (F/M), residence time, nutrients, toxicity and mixing shapes the activity of the bacteria and the production of biogas. The following subtopics explain the importance of these factors with more detail.

2.2.2.1. Temperature

Temperature has been considered one of the most critical factors regarding performance and stability of the AD process, and so, adequate temperature control is essential to mitigate any oscillations during digester operation, which may therefore affect the microbial structure and its growth kinetics (Labatut et al., 2014).

The performance and stability of the AD process significantly affects the production of biogas. Methane production occurs over a wide temperature range (0 °C to 97 °C), with the mesophilic (30 °C to 40 °C) and thermophilic (50 °C to 60 °C) ranges showing the highest levels of production (Bouallagui et al., 2004).

Despite the energy cost of constant heating, the operation of thermophilic anaerobic reactors has some advantages over mesophilic ones. Since the activity of the microorganisms is high in this temperature range, a greater reduction of volatile solids and pathogenic microorganisms can be achieved, and a higher biogas yield can be expected (Mao et al., 2015). However, great attention must be paid to process monitoring, as thermophilic microorganisms are sensitive to small temperature fluctuations and therefore the efficiency of AD can be compromised. Usually, anaerobic digestion is carried out under mesophilic temperatures due to its high stability and lower economic burden (Deena et al., 2022).

2.2.2.2. pH

The pH values for the anaerobic digestion process can lie in a relatively narrow interval between 6.0 and 8.0 (Aragaw et al., 2013). A value outside this range can lead to an imbalance. Each microbial group predominant in the various stages of AD has an optimum pH and a specific range in which it is allowed to grow. While methanogenic microorganisms require pH conditions between 6.8 and 7.3, acid producing bacteria develops better activity at pH ranging from 5.0 to 6.0 (Sakar et al., 2009).

The accumulation of volatile fatty acids (VFAs) leads to a decrease in pH, acidifying the environment and causing an imbalance in AD. However, the accumulation of VFAs will not always be accompanied by a decrease in pH, due to the buffering capacity of some substrates, namely its inherent alkalinity. There are many factors that influence pH, such as organic acids and carbon dioxide, which reduce its value, while ammonia contributes to its increase. Some compounds contribute to the buffering capacity, among them hydrogen sulfide and phosphates (Angelidaki et al., 2003).

2.2.2.3. Food to Microorganisms ratio

Anaerobic inoculation consists of inserting a certain amount of a given microbial community with known methanogenic activity into a reactor in order to speed up the anaerobic digestion process, such as active anaerobic reactor sludge from wastewater treatment plants (Malinowsky, 2016).

The food to microorganisms ratio (F/M ratio) can be expressed as the amount of volatile solids (VS) originating from the substrate (food) per the amount of VS in the inoculum. In bioreactors that work in batch mode, the F/M ratio becomes important since all the substrate is placed into the bioreactor at once, requiring the microbial community to be able to degrade all the organic matter until the end. Although the F/M ratio depends on the type of substrate and the inoculum used, several studies have been carried out to find out the ideal F/M ratio.

As stated by Gandhi et al. (2022) in AD of food waste, the maximum methane production was F/M ratio between 0.5 and 1, and further increases in F/M were found to result in system acidification. The study carried out by Rahmani et al. (2022) showed that the F/M of 0.5 g/g leads to the highest biogas yield and VS removal over other F/M ratios studied. According to Ambaye et al. (2020), the best F/M ratio range values with the addition of biochar was 1, 0.67 and 0.5 g/g.

With the substrate being algal residues, Li et al. (2014) indicated that the highest methane production was for an F/M of 0.33 g/g and that methane production was significantly decreased as the F/M ratio was higher than 1 g/g, which was resulting from the poor methanogenesis inhibited by ammoniacal nitrogen.

2.2.2.4. Solids Retention Time (SRT) and Hydraulic Retention Time (HRT)

The time needed for microorganisms consume and synthesize the substrate may be defined as the solids retention time (SRT) when the bioreactor has a sludge recirculation system inside. When the bioreactor does not have a sludge recirculation system, the SRT equals the hydraulic retention time (HRT), which is defined as the average time interval over which the substrate is kept inside the digester.

The adoption of a suitable HRT is extremely important, especially when the production system aims at digestate utilization as soil amendment and/or fertilization for crops. Usually longer HRT are required to achieve an adequate sanitation of digestate, i.e. removal of pathogenic microorganisms. It is important to emphasize that to set up a HRT it should be taken into account not only the type of organic substrate, but also the type of digester that will be used. In the case of continuous reactors, the HRT must be set up with caution, since there is a constant organic load and effluent outlet of processed material with high buffering power, thus, the equilibrium between the entrance and exit of material must be maintained in order to allow proper anaerobic conditions for constant activity of anaerobic microorganisms (Braun, 2007).

A long HRT can lead to the death of microorganisms due to a lack of nutrients. A short HRT can lead to (i) the elimination of microorganisms before their duplication, if their growth rate is not respected, or (ii) the accumulation of VFAs due to the increase in the applied organic load, leading to inhibition of AD (Siddique & Wahid, 2018).

Rossi et al. (2021) studied the HRT in dry-anaerobic digestion of OFMSW in order to achieve the optimal biogas production. The study compared the results of two experimental tests operating with an HRT of 23 and 14 days. The highest specific biogas production and the highest volatile solids removal were achieved when the HRT was set to 23 days; this indicated that 23 days was a suitable HRT. Several studies indicate that in anaerobic digestion of food waste, the HRT usually used is between 10 to 40 days.

2.2.2.5. Nutrients

The macronutrients C, N, P, S are constituents of living biomass and are necessary for the activation or functioning of many metabolic processes of microorganisms involved in AD. However, microorganisms also need micronutrients, such as Fe, Ni, Mo, Co, Se and W, for their growth, as well as for enzymatic and chemical reactions (Mao et al., 2015).

Along with C, N is the most important macronutrient necessary for cell growth, so the C/N ratio in the substrate is an important parameter for the AD process (Surra et al., 2019). Substrates with an optimum C/N ratio of 20 to 30 provide enough nutrients for microorganisms to maximize biogas production. Lower C/N values lead to build-up of ammonia and hinder microbial growth. When the C/N ratio is higher than the optimum value in the fermentation process, large quantities of VFAs are produced, thus acidifying the process. Therefore, maintaining an appropriate C/N ratio is important for biogas generation. The optimum C/N ratios of the various substrates used in AD are different. Substrate co-digestion (mixing a substrate with one or more co-substrates) is often carried out to maintain the C/N ratio in digesters (Siddique & Wahid, 2018).

Micronutrients (Fe, Ni, Mo, Co, W and Se) are responsible for supporting the bacterial metabolism. Several studies have shown that the supplementation of these nutrients, in low concentrations, has been successful in promoting the performance of DA with different types of substrates, including the organic fraction of municipal solid waste. Methanogenic archaeobacteria are the main methane formers and they need micronutrients to maintain their metabolism and to perform optimally and stably (Di et al., 2016). Therefore, an ideal balance between micro and macro-nutrients is essential to maintain stable AD and efficient biogas production.

2.2.2.6. Toxicity

There are various compounds that are toxic to the microorganisms present in AD. Methanogenic archaeobacteria are the most sensitive to toxins. However, the process can acclimatize and tolerate higher concentrations of the toxic agents after adaptation.

The most common inhibitor for the anaerobic process is ammonia. The results concerning ammonia-N inhibitory level are conflicting, as they depend on parameters such as pH, temperature and acclimation of the inoculum. It is generally accepted that the non-ionized form of ammonia is the main responsible for inhibition. In addition, pH has a significant effect on the level of ammonia inhibition, as the pH value determine the degree of ionization. Free ammonia inhibition result in VFA accumulation, which in turn lower pH and decrease the amount of free ammonia, with the result that free ammonia inhibition is relieved. Due to this self-stabilizing mechanism, processes can be maintained in a stable ammonia inhibited state, where a balance between VFA concentration and ammonia loading exist (Angelidaki et al., 2003). Information on tolerated limits of ammonia concentration varies between authors and it is not a parameter with clear results. Thus, the level of ammonia inhibition strongly depends on the degree of acclimatization of the inoculum, which can tolerate very different concentrations.

Mineral ions, especially of heavy metals (Cu, Ni, Cr, Zn, and Pb), and detergents are among the materials that inhibit the normal growth of bacteria in an anaerobic digester. Small quantities of minerals (Na, K, Ca, Mg, and S) stimulate the bacterial growth, but higher concentrations may be inhibitory (Abbasi et al., 2012).

2.2.2.7. Stirring

In the anaerobic digestion process the purpose of stirring is to maintain the temperature and uniformity of the mixture, reduce the formation of foam inside the digester and evenly distribute substrates, microorganisms and nutrients. Stirring brings benefits to AD but consumes a large amount of energy. It is estimated that stirring processes consume between 29 % and 54 % of the energy required in a biogas producing unit. Therefore, a good mixing strategy can significantly reduce the energy requirement in the system (Wang, 2018). Depending on the set-up, the agitation can be continuous or intermittent.

According to Kariyama et al. (2018), continuous mixing does not improve AD efficiency, while optimized intermittent mixing improves AD efficiency. Some authors indicate that intermittent stirring increases biogas production when compared to continuous mixing. This is because propionate oxidative microorganisms and methanogenic microorganisms live in close communities, using H_2 and formate as electron carriers, so in the intermittent mixing there is enough time for electrons to be transported from one community to the other, increasing the biogas production (Wang, 2018).

2.2.3. Valorization through anaerobic digestion

Anaerobic digestion processes are of high environmental value and fall into the circular economy concept, because it has the advantage of using organic wastes as a raw material, resulting in products that can be used in different applications. The products of the AD process, biogas and digestate, and their applications are described in the following subtopics.

2.2.3.1. Biogas

The main product of anaerobic digestion is biogas which is an excellent source of renewable energy. Biogas is a mixture of methane gas (CH_4), carbon dioxide (CO_2), ammonia gas (NH_3), hydrogen sulfide (H_2S) and nitrogen (N_2). However, its composition varies with factors such as type of substrate, temperature, HRT, mixing, use of additives and pre-treatment of the organic material. Overall, when operated under stable conditions, the AD process yields between 50 – 75 % CH_4 and 25 – 50 % CO_2 in volume, while gases such as NH_3, H_2S and N_2 are present in small concentrations (Surendra et al., 2013). For the organic fraction of municipal solid waste, the average composition of biogas for methane varies between 60 – 70 % v/v, while CO_2 is between 30 – 40 % v/v (Fernandes De Carvalho, 2019).

Biogas has a high calorific value due to its concentration of methane and can be used for a variety of purposes: fuel for boilers and furnaces, electricity generation for local use or sale to the electricity grid, alternative fuel for injection into the natural gas grid or for use in vehicles. Depending on the type of use, biogas has to be treated differently (Malinowsky, 2016). In order to be used as a fuel for vehicles and for injection into the natural gas grid, biogas must first be upgraded, where CO_2 and other contaminants are removed to produce biomethane. This is a promising way of meeting energy needs in urban and rural environments, while providing environmental and economic benefits (Al Seadi et al., 2008; Mao et al., 2015).

According to Abbasi et al. (2012), the advantages of biogas production are due to:

i. a relatively high calorific value (Table 1) and, after upgrading, biomethane has a calorific value close to that of natural gas;
ii. low cost, since it is produced from biodegradable waste substrates;
iii. reduction in greenhouse gases emissions (around 23 times less than natural gas) (Table 1);
iv. energy recovery from waste;
v. recycling of nutrients contained in the liquid and solid fractions of the digestate.

Table 1 - Calorific value of various fuels and their CO_2 equivalent emissions (Abbasi et al., 2012)

Fuel	Calorific value (CV)	Indirect emission factor (kg CO_2e/GJ, net CV basis)
Petrol	10800 kcal/m^3	12.51
Natural gas	8600 kcal/m^3	5.55
Liquefied natural gas	13140 kcal/m^3	20.00
Liquefied petroleum gas	10800 kcal/m^3	8.00
Kerosene	10300 kcal/m^3	13.34
Diesel	10700 kcal/m^3	14.13
CNG (Compressed natural gas)	8600 kcal/m^3	8.36
Biogas	5000 kcal/m^3	0.246

As stated in the report of the EurObserv'ER (2022) primary biogas energy output across the EU increased slightly in 2021, by 1.6 % year-on-year according to Eurostat, to reach 14928.9 kilotonne of oil equivalent (ktoe). Methanation biogas from non-hazardous waste or raw plant matter dominates this output (at 83.5 % in 2021), outstripping sewage sludge gas (7.8 %), landfill biogas (7.7 %), and thermal biogas (0.9 %). In 2021 Germany was the main producer of biogas in the EU with 7518.2 ktoe, followed by Italy (2078.1 ktoe), France (1404.2 ktoe) and Denmark (625.6 ktoe). Portugal was the 16th on the EU-27 list, with 87.2 ktoe biogas produced in 2021.

According to the National Renewable Energy Action Plan (PNAER, 2020) that was in action from 2009 to 2020 in Portugal, the main biogas production clusters are the

agricultural sector, wastewater treatment and, more recently, municipal solid waste. In Portugal, biogas is mostly used to produce electricity, but this type of solution is not very efficient when compared to cogeneration plants, which are becoming more beneficial in terms of energy production. However, it is not always possible to utilize the heat generated due to the remote location of the biogas generation units. For this reason, injecting biogas into the natural gas network is an interesting way of utilizing it. The PNAER also clarifies the duties and ways of connecting biogas production to the country's natural gas network.

The National Integrated Energy and Climate Plan (PNEC, 2023) is the main energy and climate policy instrument for the 2021-2030 period, replacing the PNAER, and is also geared towards Portugal's long-term goals of carbon neutrality. In the PNEC draft submitted to the European Commission in June 2023, there are measures to promote the production of renewable gas, such as: enabling of support mechanisms to increase the installed capacity of biodigesters; the creation of the Biomethane Action Plan; simplifying licensing and regulatory procedures for applications towards injection into the national gas network.

2.2.3.2. Digestate

During anaerobic digestion, there is a biological transformation of the substrate through the fermentative process, leading to generation of digestate which volume is approximately the initial volume of material that is fed into the bioreactor.

This digestate has physical-chemical properties of agronomic interest, being useful as soil amendment and fertilizer (Rufino Andrade, 2019). The application of digestate as fertilizer in agriculture is one of the simplest management solutions to avoid or minimize negative environmental impacts and improve the economic sustainability of biogas production. Digestates contain plant nutrients and it is hygienic, microbially stable, and rich in ammonium, as compared to undigested organic waste (Lamolinara et al., 2022).

However, Holm-Nielsen et al. (2009) reported that improper handling, storage and application of digestate as fertilizer can cause ammonia emissions, nitrate leaching and phosphorus overload.

In order to sanitize the digestate and to concentrate its nutrients in the liquid fraction for subsequent biological treatment, various technologies could be deployed for the inactivation/ removal of pathogens and adjustment of nitrogen/ phosphorous content. Digestate treatments include a broad spectrum of physical (e.g., screw press, belt dryer, drum dryer, solar dryer, ultrasound), chemical (e.g., coagulation/flocculation), and biological (e.g., composting) technologies. Other treatments such as settling and conditioning could improve the rheological behavior of digestate (e.g., stability and density) (Dutta et al., 2021).

Besides the agronomical application, Dutta et al. (2021) proposes other paths to use the digestate such as:

- Composting and compost additive:
    - Bio-stabilization;
    - Enhance composting reaction;
    - NH_3 volatilization;
- Hydrothermal process / Pyrolysis:
    - Hydrochar/ biochar production:
        - Solid fuel;
        - Functional materials for catalysis and remediation;
        - Soil amendment/ fertilizer;
        - Anaerobic digestion additive;
    - Energy application:
        - Bio-oil/ bio-fuel production;
        - Microalgae cultivation.

2.3. Biochar

Biochar has emerged as a promising and innovative environmental solution, offering a range of ecological and agricultural benefits through its unique properties and applications. Biochar is a type of charcoal produced by the pyrolysis of biomass, such as wood chips, crop residues, or manure, in the absence of oxygen and at temperatures on a range of 400 to 950 ºC.

To ensure that biochar production does not contribute to air pollution and GHG emissions, it must be produced in a facility that captures and burns the gases released when the biomass is heated. The heat produced by the combustion of these gases can be used as a form of renewable energy in the pyrolytic biochar production process itself (Fernandes De Carvalho, 2019).

In agricultural applications, biochar increases the storage capacity of soils for water and plant nutrients, reduces GHG emissions, and to improve crop yields. Furthermore, a relevant carbon offset potential is expected because of its high biological stability (Mumme et al., 2014).

According to Mumme et al. (2014), the relatively high costs of producing biochars prevent them from being used more widely. One way to overcome this barrier is to obtain more economic benefits by expanding the biochar value chain. One possible application for biochars is their use as an additive in AD. In addition, digestate from AD has been shown to be a suitable raw material for the production of hydrochar (similar to biochar but produced by hydrothermal carbonization at lower temperatures). Thus, the integration of the biogas and biochar systems promises several synergies and biochar as an additive to AD has been studied, in order to provide more stability and increase efficiency in biogas production. As a dissertation in Environmental Engineering, the present study aims at contributing to develop more environmental and economic synergies between AD and biochar fields.

2.3.1. Biochar application in anaerobic digestion

Due to the high cost of producing biochar, it is necessary to unravel new ways of adding value to this material. More recently, its application as an additive in AD has been studied, in order to make biochar more economically viable, as well as helping with the AD process stability and increasing efficiency in biogas production.

Several studies revealed that biochar has suitable conditions for microbial growth and microbial immobilization (biofilms), and to mitigate inhibition (ammonia and VFAs) in anaerobic digestion, which is partially credited to the high porosity of biochar (Saif et al., 2022). In addition, Saif et al. points that other important roles of biochar during AD are increasing buffering capacity, acceleration of process kinetics, CO_2 adsorption and nutrient retention.

The main biochar properties that may contribute for more efficient AD processes are summarized by Kumar et al. (2021):

- Porosity – As one of the most important characteristics, the pore size of biochar can provide microhabitats to aerobic and anaerobic microbes to proliferate and facilitate biofilm formation, which acts as a shield for the selective enrichment efficient microorganisms involved in the AD process under acid stress condition;
- Specific surface area - is considered one of the key factors along with others in the adsorption of environmental contaminants, besides, a large SSA of biochar may promote the colonization of bacteria and archaea, resulting in an improved AD performance;
- Cation exchange capacity (CEC) - When the concentration of NH_4^+ and NH_3 are maintained under an optimized range for an AD system, it can add buffer capacity for balanced bacterial growth, while the surplus amount of total NH_3/freeNH_3-N can lead to AD failure. It has been observed that biochar could efficiently alleviate NH_3 inhibition, enhancing CH_4 production by reducing the lag phase in the reactors due to its strong CEC. Therefore, depending on the operating conditions of AD (e.g., temperature and pH), substrate, and biochar,

it may achieve NH_3 mitigation by indirect pathways (via microbe immobilization and Direct Interspecies Electron Transfer - DIET) and/or direct adsorption effects (depending on surface functional groups, CEC, etc.);

- Electrical conductivity (EC) - The syntrophic activities conducted by microbes largely depend upon the EC. Some studies demonstrated the significance of biochar's redox properties (e.g. electron-accepting capacity - EAC) in the generation of CH_4 and VFAs degradation in the AD process. However, it has been shown that even though the EC of biochars from distinct feedstocks may be similar, they act differently: while the some were found to promote microbial activities and VFAs degradation via DIET, there was no productivity observed in AD on the addition of different biochars.
- Redox properties - The redox properties of biochar have been considered as a key parameter in the AD process and they are governed by its surface functional groups, presence of free radicals, metals and metal oxides. For instance, phenolic C-OH fractions have been recognized as the key functional groups accountable for their electron-donating capacity (EDC), whereas quinoid C=O fractions for its EAC. Collectively, they govern the electron exchange capacity (EEC) (EEC=EDC+EAC) of the biochar. The improvement of surface functional groups of the biochar can be achieved by an oxidation process, yet the oxidation procedure should be adequate to acquaint with new functionalities but not too strong to induce transformation to the redox-inactive COOH functional group or even elimination as CO_2. Free radicals affect the redox tendency of biochar, such as aryl radicals (carbon-centred) or as semi-quinoid radicals (intermediate of the phenolic and quinoid groups). Regarding the inorganic components of biochar, redox-active metals such as Fe and Mn oxides are generally present in the feedstock and exist in an array of different oxidation states that can serve as electron donors and acceptors. Their degree of involvement in the EEC depends on the metal types, variations in the oxidation states, distribution of the metal oxides, and the coordination as organo-mineral complexes on the biochar surface. As told in previous properties, due to conductive properties, biochar

could facilitate DIET during the AD process. Despite all this, there are relatively few studies proving the mechanisms involved in the biochar redox-facilitated AD process, in which a knowledge gap still exists and requires more mechanistic studies in the future.

- pH - The biochar pH values generally fall within the alkaline range due to ash content and volatilization of acidic functional groups, and biochar pH often increases with the increase in the pyrolysis temperature; Biochar was found to increase the alkalinity of AD, promoting better microbial action for quick CH_4 production and adaptability to initial loading shocks. Under acidic stress, biochar can effectively facilitate methanogenesis phase, which could improve the operating capacity with higher organic loadings and increased total solids.
- Surface functional groups - The composition of biochar surface includes variable functional groups such as -OH, C-O, -COOH, C=O, -NH_x, etc., which boost its multiple functionalities, including nutrient retention and toxics removal. Biochar showed promising results in the NH_3 adsorption from digestate and wastewater because of its porous structure and relatively high SSA (physical adsorption), however, they were not considered as the prominent parameters in NH_4^+ adsorption in some studies. For example, ion exchange can also take place between NH_4^+ and acidic functional groups on the biochar surface, and CEC can play a role in elevating the biochar's NH_4^+ adsorption capacity. Some studies shown that in H_2S (hydrogen sulphide acid) removal using biochar indicated that OH radical and COOH groups were found accountable for H_2S adsorption. Unlike physical adsorption, H_2S adsorption involves chemical reactions with surface functional groups of biochar.

3. Objectives

The motivation for this work is related to the current need to find renewable fuels that are not derived from oil, the management of solid urban waste generated by growing consumption and the conversion of forest biomass waste into a carbonaceous material that can be used in different applications.

The overarching aim of this dissertation is to evaluate the benefits of adding biochar to the AD process using food waste as a substrate. Its specific objectives are as follows:

- To assess the behavior of biogas production during AD upon the addition of biochar in different concentrations and particle sizes.
- To evaluate the process behavior of AD, with different substrate to inoculum ratios.

The innovative nature of this work is the use of biochar directly in the AD to optimize the process and increase the production of biogas. This approach is recent and the work carried out reports divergent results, when it comes to biochar bringing benefits to AD.

4. Methodology

The study presented in this work was carried out in the laboratories of the Department of Environment and Planning (DAO-UA), in the period between December 2022 and August 2023.

4.1. Materials: Inoculum, substrate, biochar

The inoculum used consisted in anaerobic sludge collected from a wastewater treatment facility (SIMRIA - Saneamento Integrado dos Municípios da Ria, S.A) located in Gafanha da Encarnação, Ílhavo.

The substrate used was simulated to correspond to the food waste of the organic fraction of the municipal solid waste (OFMSW) and it was prepared in laboratory according to the formula for European countries which is as percentage by weight: fruit and vegetables (72 %), cooked pasta and rice (10 %), bread and bakery (5 %), dairy products (2 %), meat and fish (8 %) and snacks (3 %) (Ghimire et al., 2016). The mass of the different components of the substrate were weighed on a balance (acADAM, model CKT 8H) making up to the total of 1 ± 0.1 kg of food waste substrate. Afterwards, this food waste mixture was ground on a meat grinder then divided in small plastic bags and stored in a freezer at -11 ºC. The substrate and inoculum were characterized on the following parameters: moisture, total solids (TS), fixed solids (FS) and volatile solids (VS).

The biochar used in this experimental work was prepared from eucalypt wood chips by pyrolysis with a temperature of 580 °C and a residence time of 4.2 minutes. In an initial stage all the biochar was sieved in a 2 mm sieve in order to separate these two particle sizes. The portion of the biochar particles smaller than 2 mm was named “S” (small) and the biochar particles portion larger than 2 mm was named “L” (large) to differentiate them.

At the beginning and end of each experimental assay, the mixture inside of the reactors was characterized. For the initial mixture, it was performed the characterization of TS, VS, total suspended (TSS) and volatile suspended solids (VSS) and chemical oxygen demand (COD). For the final mixture, the same characterization was carried out and the quantification of phosphorus and Kjeldahl nitrogen was also carried out in order to assess the agronomic quality of the digestate.

4.2. Experimental design

Taking in consideration the literature review, the experimental design relies in the operation of batch bioreactors, at 35 ºC, single stage, with intermittent stirring and operating for approximately 21 days.

According to the literature review, the following ranges were chosen:

- F/M ratio of 0.5, 1 and 2 gVS/gVS;
- Biochar concentrations of 0, 2.5 and 5 g/L;
- Biochar particle size of 0 (negative control), S (smaller than 2 mm) and L (larger than 2 mm).

The reactors used had a working volume of 1 L and the target for initial mass of volatile solids were 2 g VS/L.

All the test conditions are depicted in Table 2, and the experimental set-up is presented in Figure 2. The masses and volumes of the components used in the anaerobic digestion tests are described in Appendix A.

Table 2 - Experimental matrix

F/M (gVS/gVS)	Biochar particle size	Biochar concentration (g/L)
0.5	0	0
0.5	S	2.5
0.5	S	5
0.5	L	2.5
0.5	L	5
1	0	0
1	S	2.5
1	S	5
1	L	2.5
1	L	5
2	0	0
2	S	2.5
2	S	5
2	L	2.5
2	L	5

Figure 2 - Experimental set-up. 1-thermostat, 2-reactor inside water bath, 3-bubbler, 4-separating funnel, 5-graduated cylinder

The reactors were round bottomed flasks with a working volume of 1 liter. The container with the water bath could stand 4 reactors at the same time.

The water bath was kept at a temperature of 35 ºC. All pipe connections were sealed with Parafilm M and regularly checked to guarantee no gas leaks. The gas quantification system consisted on the principle of communicating vessels. The water inside the separating funnel and the tube was pressurized by the biogas formed, then falling into the graduated cylinder, allowing the biogas formed to be quantified in milliliters.

4.3. Analytical techniques

The inoculum, substrate, biochar, initial and final mixtures from the reactors were analyzed in several parameters by following specific laboratory procedures.

The parameters analyzed in the inoculum and substrate were the bulk density, moisture content, total solids content and volatile solids content – subtopics 4.3.1 and 4.3.3.

The parameters analyzed in the biochar were the pH, the bulk density, moisture, total solids content, volatile solids content and ash content – subtopics 4.3.2, 4.3.3 and 4.3.4.

The parameters analyzed in the initial mixtures from the reactors were the total and volatile solids content, total suspended solids and volatile suspended solids content and soluble chemical oxygen demand (sCOD) – subtopics 4.3.1, 4.3.5 and 4.3.6.

The parameters analyzed in the final mixtures from the reactors were the total and volatile solids content, total suspended solids and volatile suspended solids content, soluble chemical oxygen demand (sCOD), phosphorus and Kjeldahl nitrogen – subtopics 4.3.1, 4.3.5, 4.3.6, 4.3.7 and 4.3.8.

4.3.1. Moisture, total solids and volatile solids

The proximate analysis carried out consisted in determination of moisture content, total solids content and volatile solids content. These parameters were measured according the Protocol 2540 B. Total Solids Dried at 103 - 105 ºC and the Protocol 2540 E. Fixed and Volatile Solids Ignited at 550 ºC, both from Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 2017).

4.3.2. pH

The pH value of the biochar was obtained according to the standard method by European Biochar Certificate (EBC), DIN ISO 10390 ($CaCl_2$). The procedure consisted

in placing a minimum of 5 mL of the biochar sample in a glass vessel and five times the volume (25 mL) of a 0.01 M $CaCl_2$ solution was added. After shaking for 1 hour, the suspension obtained was directly analyzed with a pH meter (Consort, C535). The pH was read three times and the value calculated is the average of these three readings.

4.3.3. Bulk density

The bulk density is the density of a volume of material that takes in account the air spaces between the particles of that material. To determine the bulk density, a certain mass was weighed in a graduated cylinder and then the weighed mass and the respective volume obtained were verified. Thus, the bulk density (in g/L) is calculated through the quotient of the weighed mass (in grams) with the obtained volume (in liters). It was important to calculate the bulk density of the biochar considering that this parameter allowed to calculate the volume occupied by the biochar inside the reactor.

4.3.4. Proximate analysis

The proximate analysis of the biochar was performed according to the methods CEN/TS 14774-3:2004 for moisture content, CEN/TS 14775:2004 for ash content and CEN/TS 15148:2005 for volatile solids. All these methods are from the Slovenian Institute for Standardization (SIST).

To determine the moisture, total solids and ash content of the biochar all the weights were measured on an analytical balance (Sartorius, model B120s). Three 3 replicates were made for each size of biochar particles (S and L). To determine the moisture, the replicates were put on an oven (WTB binder 7200) at 105 $\pm$ 5 °C for 24 hours.

To determine the ash content, the replicates that were took from the oven were placed on a muffle (Nabertherm, model N3). This muffle had a program to keep the right temperatures at the right times according to the method: raising the temperature evenly to 250 °C over a period of 50 minutes (a rise of 5 °C/min); maintain this temperature for

60 min and continue to raise the furnace temperature evenly to 550 ± 10 °C over either a period of 60 minutes, or a rise of 5 °C/min, and keep this temperature level for at least 120 min. When the program ended, the replicates were removed from the muffle, cooled and their mass was weighted.

For the determination of volatile solids, the used crucibles were smaller than the previous ones and had a lid. Then, was added on the crucibles around 1 g of biochar, where 3 replicates were made for each size of biochar particles (S and L). The replicates were placed on a previous heated furnace (Eurotherm, Carbolite) at 900 ± 10 ºC for 7 minutes. After this, the replicates were removed from the furnace, cooled and their mass was weighted, calculating the volatile solids.

In the end, the fixed carbon content was calculated through the subtraction of the values obtained for moisture, ash and volatile solids:

$$Fixed\ carbon\ (\%) = 100 - (Moisture + Ash + Volatile\ solids).$$

4.3.5. Total Suspended Solids and Volatile Suspended Solids

The total suspended solids and volatile suspended solids were determined on the initial and final mixtures from the reactors. The protocols used were the Protocol 2540 D. Total Suspended Solids Dried at 103 – 105 ºC and the Protocol 2540 E. Fixed and Volatile Solids Ignited at 550 ºC, both from Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 2017). The procedure for TSS and VSS is similar to the procedure used for the proximate analysis of biochar, however, the analyzed sample of the initial and final mixtures is filtered through glass fiber filters. The mass that is filtered and the glass fiber filters are dried in the oven (WTB binder 7200) for 24 hours at 105 ± 5 °C to determine the TSS. To determine the VSS, after being weighed and cooled, the TSS filters are placed in the muffle (Eurotherm, Carbolite) for 2 hours at 550 ± 10 °C. The respective masses are weighed on an analytical balance (Sartorius, model B120s).

4.3.6. Soluble Chemical Oxygen Demand (sCOD)

The Soluble Chemical Oxygen Demand (sCOD) was determined on the initial and final mixtures from the reactors. The protocol used was the Protocol 5220 D. Closed Reflux, Colorimetric Method from Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 2017). Chemical Oxygen Demand is the measurement used to assess the amount of oxygen needed to oxidize a given volume of a sample. In the method used, oxidation is carried out using potassium dichromate, and after digestion the samples are colored and measured colorimetrically in a spectrophotometer. The analyzed COD was the soluble fraction since the initial and final samples from the reactors had been filtered. In this process, standards in the range of 0 - 900 mg O_2/L are prepared using the standard solution of potassium hydrogen phthalate, so that the values of the samples can be found out graphically later. Three replicates of the samples were made by transferring 2.5 mL of the sample into a capped digestion tube. All the tubes (samples and standards) were digested at 150 ºC for 2 hours in a heating block (Selecta Multiplaces). After digestion, the absorbance of the digestion tubes is read at 500 nm on a spectrophotometer (Aqualytic PC compact COD vario).

4.3.7. Phosphorus

The phosphorus content was only determined on the final mixtures from the reactors. The protocol used was the Protocol 4500-P E. Ascorbic Acid Method from Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 2017).

4.3.8. Kjeldahl Nitrogen

The Kjeldahl nitrogen was only determined on the final mixtures from the reactors. The protocol used was the Protocol 4500-N_{org} C. Semi-Micro-Kjeldahl Method from

Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 2017).

4.3.9. Biogas composition

Sampling for biogas was carried out by puncturing a rubber septum in the biogas passage pipe of each reactor with the aid of a syringe with a useful volume of 2 mL.

To determine the composition of the biogas, a gas chromatograph (SRI, model 8610-C), with a thermal conductivity detector was used set at 75 °C using an CRS Hayesep^{TM} column set at 61 °C and helium as a carrier gas. By analysing the chromatogram obtained, it was possible to calculate the CH_4 and CO_2 values in percentage terms (v/v).

5. Results and discussion

5.1. Inoculum and substrate characterization

The parameters analyzed in the inoculum and substrate were the bulk density, moisture content, total solids content and volatile solids content. Since the substrate was prepared and frozen, it was characterized once before the first test and the values were assumed to be conservative for the further tests. The inoculum (anaerobic sludge) was stored in a container and was characterized immediately before every assay in order to ensure that the same VS content was tested consistently.

Table 3 presents the bulk density values of the inoculum and substrate, and Table 4 presents the proximate analysis of the food waste substrate.

Table 3 - Bulk density of inoculum and substrate

	Bulk density (g/L)
Inoculum	1015.0 ± 0.1
Substrate	1214.8 ± 0.1

Table 4 - Proximate analysis of substrate (food waste)

	Moisture (%)	**Total solids (%)**	**Volatile solids (%)**	**Fixed solids (%)**
Substrate	78.39	21.61	20.58	1.03

According to the study by Waqas et al. (2018), the food waste physicochemical characteristics reported were 83.6 % of moisture and 18.1 % of total solids, similar to those presented above. Deena et al. (2022) study also reports the content of total and volatile solids for several types of food waste, being the total solids and volatile solids values of around 10 to 25 %, close to the presented above.

Table 5 presents the proximate analysis of the inoculum in content of moisture, total solids, volatile solids and fixed solids.

Table 5 - Proximate analysis of inoculum (sewage sludge)

Inoculum	Moisture (%)	Total solids (%)	Volatile solids (%)	Fixed solids (%)
27/2/2023 – Preliminary characterization	97.80	2.20	1.48	0.72
7/3/2023 –test 1	97.99	2.01	1.32	0.69
13/4/2023 –test 2	98.07	1.93	1.28	0.65
9/5/2023 –test 3	98.36	1.64	1.04	0.60
7/6/2023 –test 4	98.00	2.00	1.28	0.72

The values remained close throughout the experimental period, except before test 3, when the moisture value was slightly higher and the total and volatile solids values were slightly lower. This change was counteracted in test 3, in which more inoculum was added to achieve the target volatile solids value.

5.2. Biochar characterization

As previously mentioned, the biochar used in this experimental work was prepared from eucalypt wood chips by pyrolysis with a temperature of 580 °C and a residence time of 4.2 minutes. The parameters analyzed to characterize the biochar were the moisture, total solids content, volatile solids content, ash content, bulk density and pH.

5.2.1. pH

The pH of the biochar was the only parameter determined before the sieving of the biochar into two different particle sizes. The value obtained for the pH for the biochar used was 7.5 ± 0.1 at 18 ºC. The value obtained for wood biochar by Zhang et al. (2014) was 8.63, which is slightly higher than the value obtained, however, when compared to bamboo or rice husk biochar and rice husk ash, wood biochar has the lowest pH, as the type of biomass feedstock may influence this value.

5.2.2. Bulk density

The values of the bulk density for the two types of biochar particle size are presented in Table 6.

Table 6 - Bulk density of biochar

Biochar particle size	Bulk density (g/L)
Biochar S	233.5 ± 0.1
Biochar L	157.3 ± 0.1

The bulk density is different for the two types of biochar particle because the same volume of S particles has lower apparent porosity than the equivalent volume of L particles.

5.2.3. Proximate analysis

The proximate analysis of the biochar consisted in determination of the moisture, volatile matter, ash and fixed carbon content in percentage. Table 7 presents the values obtained for the biochar proximate analysis.

Table 7 - Proximate analysis of biochar

Biochar type	Moisture (%)	Volatile matter (%db)	Ash content (%db)	Fixed carbon (%db)
Biochar S	10.59	25.10	14.24	50.07
Biochar L	10.26	25.78	6.48	57.48

Fernandes et al. (2019) obtained a moisture content of 9.67 % for biochar produced from eucalyptus waste forest biomass, which is close to the value obtained in this study. However, the value obtained by Fernandes et al. for volatile matter was 7.76 %, for ash was 1.67 %, and for fixed carbon was 80.9 %. It is believed that the high volatile matter obtained in the eucalypt biochar in this study, leading to a relatively low fixed carbon value, is due to the fact that the biochar was not pyrolyzed for long enough, thus resulting in a relatively low fixed carbon content.

Zhang et al. (2014) reported the ash content of various biochars in their study, and the biochar produced from wood had an ash content of 5.49 %, slightly lower than that obtained in this study. The difference can be explained by the type of wood used and pyrolysis parameters.

5.3. Anaerobic digestion assays

Since the set-up available only allowed to have 4 reactors at a time, the anaerobic digestion tests were conducted through the following timeline (Table 8). All the tests had a duration of approximately 21 days.

Table 8 - Anaerobic digestion tests held

Test	Period of time	F/M (gVS/gVS)	Biochar particle size	Biochar concentration (g/L)
1	9/3/2023 to 30/3/2023	0.5	0	0
		0.5	S	2.5
		0.5	S	5
		0.5	L	2.5
3	17/5/2023 to 7/6/2023	0.5	L	5
		1	0	0
		1	S	2.5
		1	S	5
4	13/6/2023 to 4/7/2023	1	L	2.5
		1	L	5
		2	0	0
2	19/4/2023 to 10/5/2023	2	S	2.5
		2	S	5
		2	L	2.5
		2	L	5

5.3.1. Characterization of the initial and final mixture

The parameters analyzed in the initial mixtures from the reactors were the total and volatile solids content, total suspended solids and volatile suspended solids content and soluble chemical oxygen demand (sCOD). In the final mixtures from the reactors the parameters analyzed were the same as the initial mixtures plus the phosphorus and Kjeldahl nitrogen. The outputs obtained experimentally will be discussed taking into account the parameters that varied in the experimental matrix: F/M ratio, biochar concentration and biochar particle size.

5.3.2. Total solids

Figure 3 presents graphically the results of the Total solids, for the initial and final mixtures from the reactors.

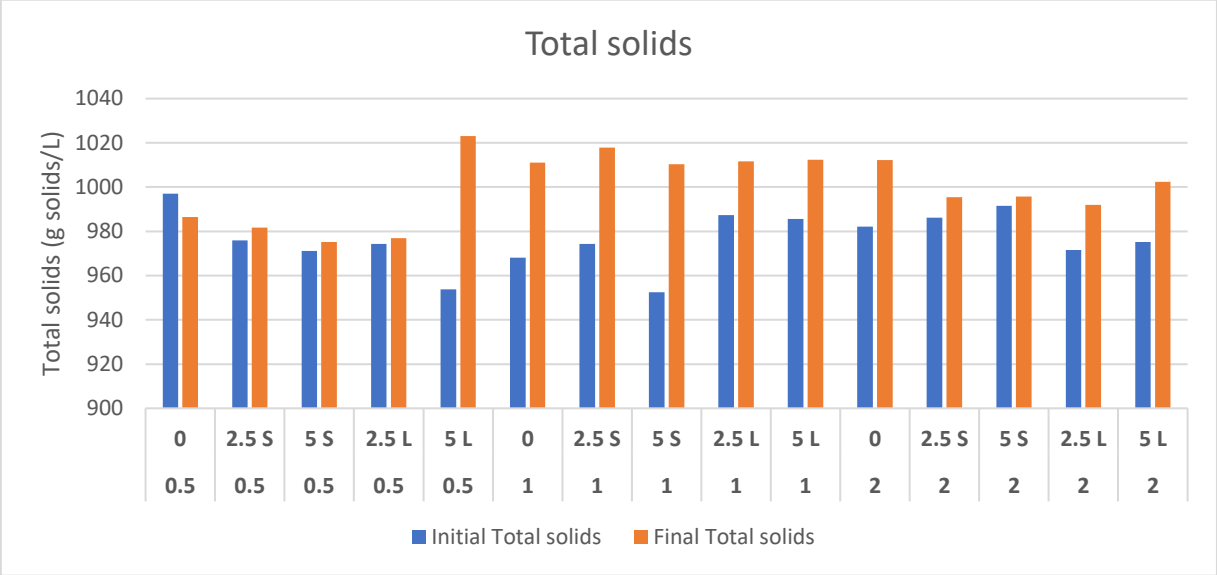

Figure 3 - Initial and final values of Total solids content. X-axis above: Biochar concentration and particle size (0, 2.5 and 5 g/L; S-smaller than 2 mm; L-larger than 2 mm). X-axis bellow: F/M ratio (0.5, 1 and 2 g/g)

It can be seen from Figure 3 that, in general, the initial total solids contents varied between 900 and 1000 g/L. The final total solids values did not fall out much from this range either, except for some assays where the final value was higher than the initial one. It would be expected that the reactors containing biochar would contain more total solids than the control reactors, but this did not happen, since in all ratios there are reactors with biochar with a lower total solids value than the control.

Assays in which the final value of total solids was higher than the initial value may indicate growth of the microbial community, thus influencing the quantity of solids at the end of the process. It should be noted that quantification of solids is lumped, so that it cannot screen between solids contribution from biochar and from sludge. This is a limitation of all studies regarding AD processes loaded with biochar, further research must be considered to counteract this fact.

F/M ratio comparison

Evaluating F/M = 0.5 g/g ratio, the graph shows that the first four reactors presented similar initial and final total solids contents, with no relevant variation among them. However, the assay with biochar particle size L and concentration 5 g/L showed a higher value at the end as compared to the beginning. It should be noted that this reactor was operated in test 3, while the first four reactors operated in test 1. This fact may have some influence on these solids values, since before test 3 the total solids content in the inoculum (sludge) was lower than the total solids content in the inoculum before test 1; this meant that, for the same F/M ratio, a higher mass of sludge was placed in this reactor so that the initial volatile solids value was the same.

The total solids values of the F/M = 1 g/g reactors are similar, with the final value being higher than the initial value in all assays. In the assay with the biochar S concentration 5 g/L, the increase in total solids was higher than in the other reactors, but its initial value was a bit lower in comparison with the other reactors.

The total solids values of the reactors with the F/M = 2 g/g ratio are similar, with the final value being higher in all reactors than the initial value. However, in comparison with the reactors from the F/M = 1 g/g ratio, the initial and final values of the F/M = 2 g/g ratio are closer than in the F/M = 1 g/g ratio. It is possible to see in the table that the initial total solid values in this F/M = 2 g/g ratio are also higher than those in the F/M = 1 g/g ratio, this is due to the fact that it has a higher amount of inoculum (sludge) added, which would also have led to a higher solids degradation.

Biochar concentration and particle size comparison

The biochar factors under study were concentration and particle size, which consisted in three biochar concentrations (0 g/L, 2.5 g/L and 5 g/L) and two particle sizes (S and L). It is noticed through Figure 3 that the biochar variables did not play a major role when it comes to the values of total solids, in comparison, for example, with the F/M ratio parameter.

It would be expected that the assays containing biochar would have a higher total solids value than the control ones, however, as seen in Figure 3, the control reactor from F/M = 0.5 g/g ratio had a higher total solids value than the others with biochar. This assay is also the only one in which the final total solids value was lower than the initial value. In all other assays the opposite was observed, and in reactors with biochar the difference is even greater. This difference may indicate a greater growth of the microbial community, thus influencing the amount of solids at the end of the process. A greater development of the microbial community can also be noted in the following reactors: F/M = 0.5 g/g ratio and biochar 5 g/L particle size L, F/M = 1 g/g ratio and biochar 5 g/L particle size S, and F/M = 2 g/g ratio and biochar 5 g/L particle size L; where there is a relevant increase in total solids at the end of the test, when compared to the other reactors.

5.3.3. Volatile solids

The evaluation of the obtained values of volatile solids is important in anaerobic digestion since it is expected their conversion into biogas during the process.

Figure 4 presents graphically the results of the Volatile solids, for the initial and final mixtures from the reactors.

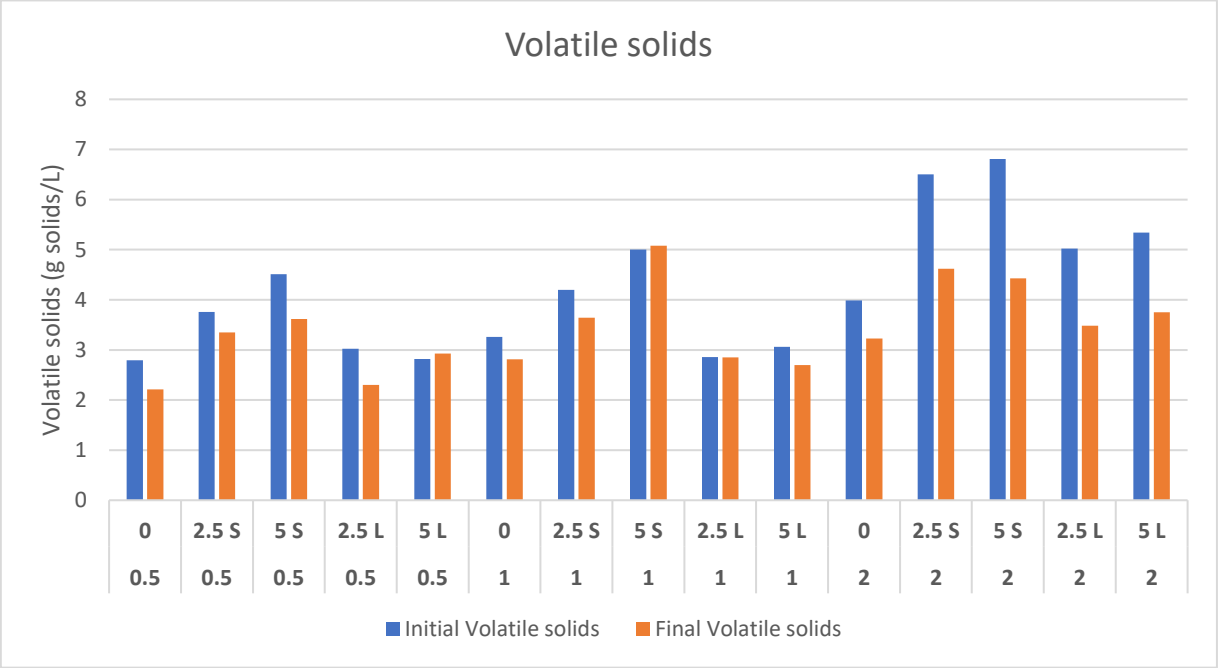

Figure 4 - Initial and final values of Volatile solids. X-axis above: Biochar concentration and particle size (0, 2.5 and 5 g/L; S-smaller than 2 mm; L-larger than 2 mm). X-axis bellow: F/M ratio (0.5, 1 and 2 g/g)

In general, the initial volatile solids values were in the range 2 - 7 g/L. The final volatile solids values did not vary much from this range either. The initial volatile solids values were higher than the final ones, except for assays F/M = 0.5 g/g ratio with biochar 5 g/L particle L and F/M = 1 g/g with biochar 5 g/L particle S.

F/M ratio comparison

Figure 4 shows in the F/M = 0.5 g/g ratio that the first four reactors (control reactor, reactor with biochar concentration 2.5 g/L of particle size S; reactor with biochar concentration 2.5 g/L of particle size L; reactor with biochar concentration 5 of particle size S) have very similar decreases in initial and final volatile solids, with the removal efficiencies for these four reactors varying between 10 and 24 %. However, the last reactor in this ratio (reactor with biochar concentration 5 g/L of particle L) showed a higher value at the end compared to the beginning, with its removal efficiency being -3.90 %. Despite this, the initial value (2.82 g solids/L) was close to the final value (2.93 g solids/L) showing that the process failed to degrade the volatile solids on this reactor as it did with the others.

In the values obtained in F/M = 1 g/g ratio, it can be seen that in most assays the final value of volatile solids is lower than the initial one, with the exception of the reactor with biochar concentration 5 g/L of particle S. Even so, the initial value is very close to the final one (5.00 and 5.08 g solids/L, respectively).

Unlike the values of the above-mentioned ratios, the F/M = 2 g/g ratio was the experimental condition that yielded the best set of removal efficiencies. This can be seen in Figure 4, as the initial values of volatile solids in the reactors are substantially higher than the final values, which shows that this F/M ratio had induced relevant conversion of volatile solids.

Biochar concentration and particle size comparison

Comparing the results based on the biochar concentration and particle size, it can be seen that the results of the control reactors (without biochar) are very similar. In these assays the volatile solids contents increased proportionally to the F/M ratio.

It was also observed that, in all reactors that received biochar particle size S, the volatile solids content increased from the reactor with biochar concentration of 2.5 g/L to the reactor with biochar concentration of 5 g/L. However, when considering the reactors with biochar concentration 2.5 g/L of particle L and the reactors with biochar concentration 5 g/L of particle L, the volatile solids values decrease.

5.3.4. Soluble Chemical Oxygen Demand (sCOD)

Figure 5 presents graphically the results of the sCOD, for the initial and final mixtures from the reactors.

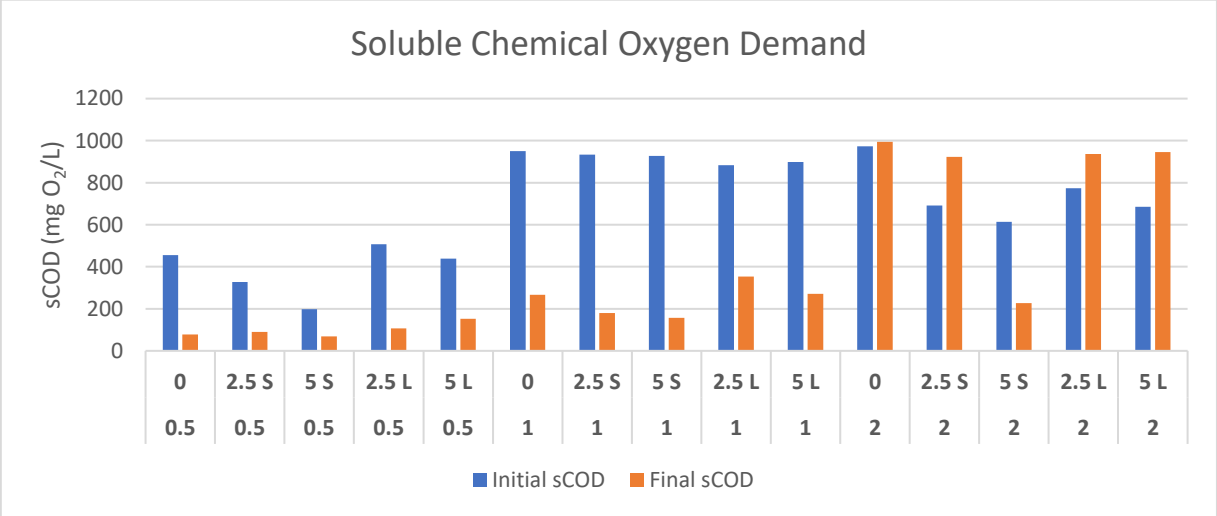

Figure 5 - Initial and final values of Soluble Chemical Oxygen Demand. X-axis above: Biochar concentration and particle size (0, 2.5 and 5 g/L; S-smaller than 2 mm; L-larger than 2 mm). X-axis bellow: F/M ratio (0.5, 1 and 2 g/g)

The sCOD concentrations were much higher at the beginning than compared to the end of the tests, however, this decrease did not occur in all reactors, thus expressing different methanation behaviors.

F/M ratio comparison

It is possible to verify from Figure 5 that at F/M = 0.5 g/g ratio and at F/M = 1 g/g ratio there was a decrease in sCOD initial to final value in all reactors.

At the F/M = 0.5 g/g ratio, it can be seen that the assay that yielded a higher sCOD removal efficiency was the control and, at the F/M = 1 g/g ratio, it was the one containing biochar with concentration 5 g/L of particle size S.

The decrease observed in the F/M = 0.5 g/g ratio and F/M = 1 g/g ratio did not occur in full in F/M = 2 g/g ratio, because four reactors (control reactor; reactor with biochar concentration 2.5 g/L of particle S; reactor with biochar concentration 2.5 g/L of particle L; reactor with biochar concentration 5 g/L of particle L) present higher sCOD values at the end, compared to the initial ones. Comparing the initial and final values for sCOD in these four reactors, it can be stated that these conditions are not ideal for sCOD degradation (as for example, an higher solids retention time as compared to the others). Thus, at the F/M = 2 g/g ratio, the only reactor that obtained a positive sCOD removal efficiency was the reactor with biochar concentration 5 g/L of particle size S.

Biochar concentration and particle size comparison

About the concentration of biochar and its particle size, it can be observed that there is a decrease in the sCOD values from the control reactor to the reactor with biochar concentration 2.5 g/L of particle S and then to the reactor with biochar concentration 2.5 g/L of particle L, increasing in the reactor with biochar concentration 2.5 g/L of particle L and in the reactor with biochar concentration 5 g/L of particle L, but also decreasing between them. In other words, at the F/M = 0.5 g/g ratio, the reactors with biochar concentration 2.5 g/L (for both particle sizes S and L) were those that yielded the higher results in sCOD removal. At the F/M = 1 g/g ratio, in general, the reactors with biochar particle size S yielded higher results in the removal of sCOD, regardless of the concentration.

In general, it can be stated that reactors with biochar particle size S yielded better results in sCOD removal, except in F/M 0.5, where the control reactor had a better performance in removing sCOD. No prior studies were found that related sCOD removal to biochar concentration or particle size. Even so, Wambugu et al. (2019) found a higher sCOD removal efficiency in the bioreactor that received biochar (65 – 75 %) than in the control bioreactor (33 - 59 %).

5.3.5. Phosphorus and Kjeldahl Nitrogen

Both P and Kjeldahl N were measured in the final digestates. As previously mentioned, the agronomic properties of digestate have been studied in recent years so that this product of anaerobic digestion can also have an economic value. Thus, both P and Kjeldahl N are two important nutrients from an agronomic point of view.

Figure 6 presents graphically the values of Kjeldahl nitrogen and phosphorus on the digestates.

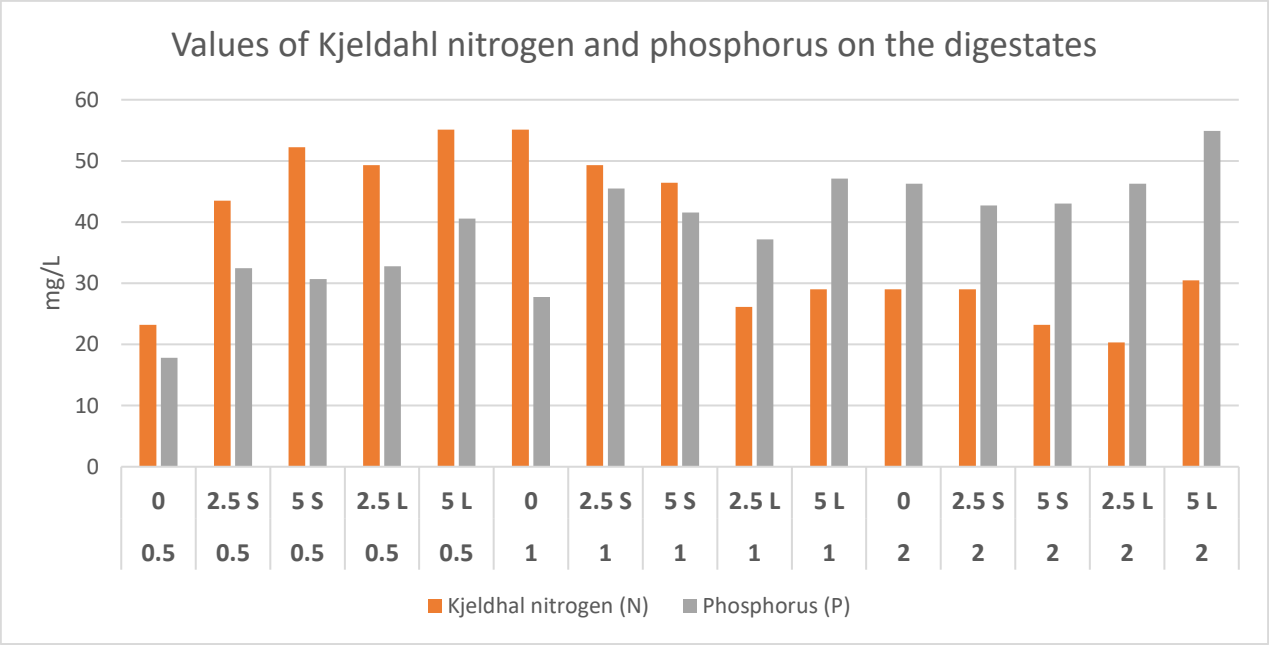

Figure 6 - Values of Kjeldahl nitrogen and phosphorus on the digestates. X-axis above: Biochar concentration and particle size (0, 2.5 and 5 g/L; S-smaller than 2 mm; L-larger than 2 mm). X-axis bellow: F/M ratio (0.5, 1 and 2 g/g)

The results obtained were within the expected. Song et al. (2021) reported total ammonia nitrogen and phosphorus values in the digestate from food waste were 4.70 mg N/L and 171.90 mg P/L respectively. However, the concentrations of Kjeldahl nitrogen cannot be compared to the concentration value of total ammonia nitrogen it represents only a part of Kjeldahl nitrogen. Regarding the P, the measured

concentrations are lower those reported by Song et. al, however, several factors may be involved in this difference, such as the characteristics of the inoculum used.

F/M ratio comparison

When comparing the results through the F/M ratio on Figure 6, it can be observed that Kjeldahl nitrogen concentrations decrease as F/M ratio increase and the P concentrations increase as the F/M ratio increases. Regarding the phosphorus concentrations, it was expected that higher F/M ratios would lead to higher concentrations of phosphorus on the digestate since anaerobic inoculum (sewage sludge) usually is considered among the most abundant sources of phosphorus as stated by Di Costanzo et al. (2021).

Biochar concentration and particle size comparison

When comparing the values from the biochar concentration and its particle size point of view, these factors did not have noticeable influence in the Kjeldahl nitrogen and phosphorus concentrations. The values do not show much variation when compared to each other in terms of biochar concentration and particle size, as the F/M ratio was the main factor affecting these nutrient contents in the digestate.

5.3.6. Biogas production

Being the biogas one of the most important aspect of this study, its production by the different reactors throughout the tests was studied. Figures 7 and 8 present the biogas produced in both absolute and specific terms.

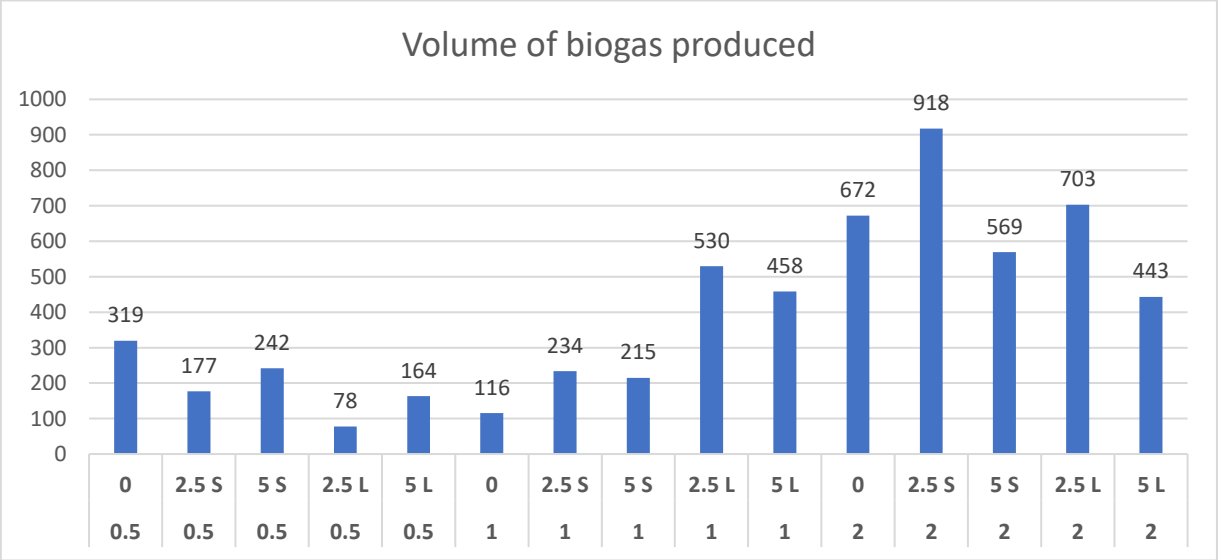

Figure 7 - Volume of biogas produced (mL). X-axis above: Biochar concentration and particle size (0, 2.5 and 5 g/L; S-smaller than 2 mm; L-larger than 2 mm). X-axis bellow: F/M ratio (0.5, 1 and 2 g/g)

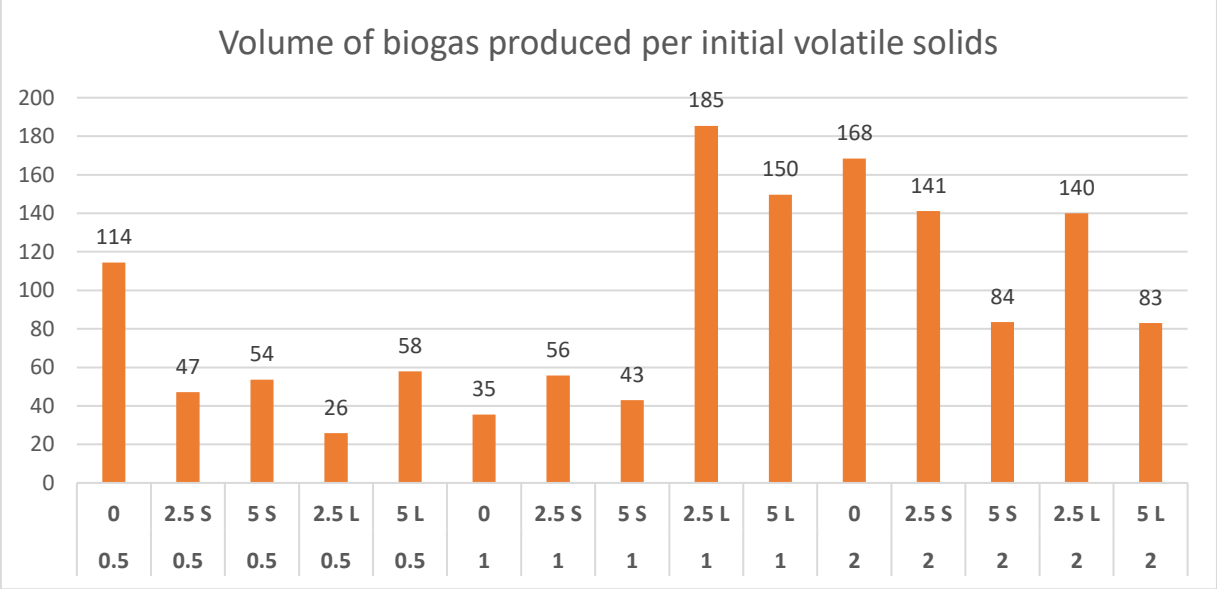

Figure 8 - Volume of biogas produced per initial volatile solids (mL/g VS). X-axis above: Biochar concentration and particle size (0, 2.5 and 5 g/L; S-smaller than 2 mm; L-larger than 2 mm). X-axis bellow: F/M ratio (0.5, 1 and 2 g/g)

The values shown above are the accumulated volume of biogas produced over the course of the tests.

In Figure 8 it can be seen that the assay F/M = 1 g/g ratio, reactor with biochar concentration 2.5 g/L of particle L yielded the highest biogas production (185 mL/g VS). In turn, the reactor that yielded the lowest biogas production is at the F/M = 0.5 g/g ratio, reactor with biochar concentration 2.5 g/L of particle L (26 mL/gVS).

F/M ratio comparison

As can be seen from the Figure 7, in general, the volume of biogas produced increased with the F/M ratio. As expected, the reactors that produced the highest volumes of biogas were found in the F/M = 2 g/g ratio, since these reactors contained more substrate they were able to convert more matter into biogas. This is corroborated by Figure 8 where the biogas production results are presented considering the initial volatile solids. When comparting both Figures 7 and 8, it can be observed that biogas production are similar.

Biochar concentration and particle size comparison

From Figure 8, it can be observed that the control reactors from F/M = 0.5 g/g ratio and F/M = 2 g/g ratio achieved high volumes of biogas produced.

In the F/M = 0.5 g/g ratio, it can be seen that the reactors that received a biochar concentration of 2.5 g/L had lower biogas production than those that received a biochar concentration of 5 g/L. It can also be seen that reactors with biochar particle size S had a higher biogas volume production when compared to the reactors with biochar particle size L.

At the F/M = 1 g/g ratio, the reactor that produced the lowest amount of biogas was the control reactor. Contrary to what happened with the F/M = 0.5 g/g ratio, the reactors with biochar of L particle size yielded higher biogas productions than the reactors with biochar of S particle size. As for the biochar concentrations, the opposite was also true for the F/M = 0.5 g/g ratio, with the reactors of biochar 2.5 g/L concentration producing the most when compared to the reactors of biochar 5 g/L concentration.

In the F/M = 2 g/g ratio, Figure 8 shows that the control reactor produced the most biogas. As was the case with the F/M = 1 g/g ratio, the reactors with the highest biochar concentration (5 g/L) were the ones that obtained the lowest biogas results when compared to the reactors with biochar of 2.5 g/L concentration. As for the biochar particle size, there was no great difference between the biogas volume obtained from the two reactors with biochar S particle size and the two reactors with biochar L particle size, in fact, the results were close (141 mL biogas/g VS for biochar concentration 2.5 g/L of particle S reactor and 140 mL biogas/g VS for biochar concentration 2.5 g/L of particle L reactor; 84 mL biogas/g VS for biochar concentration 5 g/L of particle S reactor and 83 mL biogas/g VS for biochar concentration 5 g/L of particle L reactor).

5.3.7. Biogas composition

The composition of the biogas in methane and carbon dioxide was obtained using a gas chromatographer. However, only the data obtained from the first test was taken into account, since the gas chromatographer broke down halfway through the second test and the few results obtained were not considered reliable.

Figure 9 shows the results of the biogas composition as a percentage of CH_4 and CO_2 on day 19 of the first test, that is, two days before the test ended and when the biogas production stabilized.

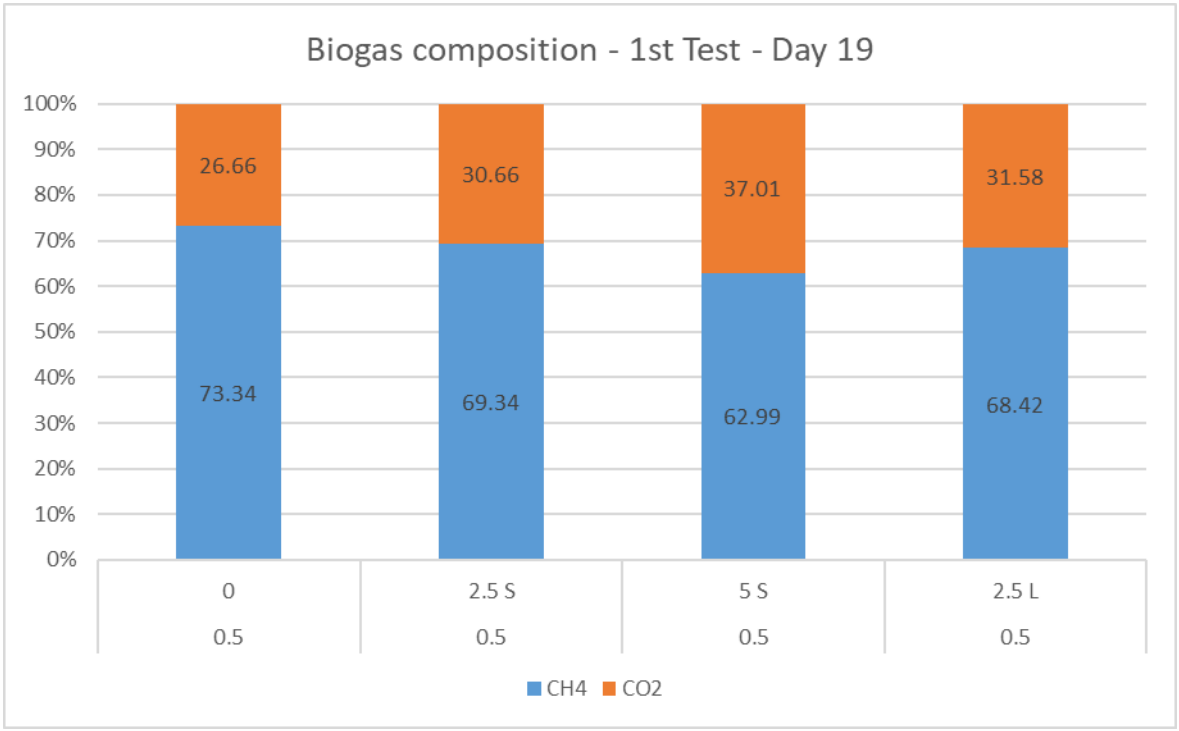

Figure 9 - Biogas composition on the 19th day of the first test. X-axis above: Biochar concentration and particle size (0, 2.5 and 5 g/L; S-smaller than 2 mm; L-larger than 2 mm). X-axis bellow: F/M ratio (0.5 g/g)

It can be noted from the graph that the results between the different reactors are similar, and it is believed that similar results would be obtained in the other tests with the other reactors, despite different parameters (specially the F/M ratio). It can be also noted that the two reactors with the same concentration of biochar had similar results, however,

this may be considered irrelevant since it could not be compared with the other F/M ratios due to the lack of results.

Ko et al. (2018) obtained a methane percentage from 65 to 68 % for the reactor that received activated carbon, a result close to the graph above. Mumme et al. (2014) and Sinervo (2017) have noted that the control reactor yielded a higher percentage of methane than the reactors that received biochar, indicating a lack of benefit in using biochar to increase the percentage of methane in the biogas composition. Despite these results are similar with the ones obtained in this study, other authors reported different results, this may happen because the biochar used in these studies is produced from other raw materials and in a variety of ways.

6. Conclusions and suggestions for further research

The main objective of this work consisted in the application of biochar in anaerobic digestion processes of food waste in batch mode, by varying particle size and concentrations, as well as the food-to-microorganism (F/M) ratio.

The proximate analysis of the biochar showed that biochar had a low moisture content (around 10 %) but a relatively high volatile matter content (around 25 %). Consequently, the fixed carbon content was low (around 55 %), thus suggesting high reactivity and low carbon stability possibly resulting from a short pyrolysis time.

Degradation of volatile solids was expected, which happened in practically all the reactors except 3, where their volatile solids removal efficiency was negative or very close to 0. The highest volatile solids degradation were obtained in the F/M = 2 g/g ratio and biochar was not considered to have had a major influence on volatile solids degradation.

The results of sCOD showed that, in general, there was sCOD degradation, except in 4 reactors at F/M = 2 g/g ratio. This may have been because the high organic load in these reactors may not have had enough time to degrade; also in this F/M ratio, the only reactor that obtained a positive sCOD removal efficiency (62.93 %) was the reactor with biochar concentration 5 g/L of particle S, which means that the addition of this biochar provided ideal conditions for degrading sCOD. If the test had been extended beyond 21 days, there could have been sCOD degradation in these 4 reactors (and consequently a greater volume of biogas produced).

The phosphorus and Kjeldahl nitrogen evaluated in the digestate showed that the Kjeldahl N values were higher in the ratios F/M = 0.5 g/g and F/M = 1 g/g (between 23 – 55 mg N/L), while the phosphorus values were lower in these ratios (between 17 – 47 mg P/L). This relationship was inverse in the F/M = 2 g/g ratio, with higher concentrations of phosphorus (between 42 - 54 mg P/L) and lower concentrations of Kjeldahl N (between 20 – 30 mg N/L). It is suggested that variation were not affected by the addition of biochar.

Regarding the biogas production, results showed that, in general, as the F/M ratio increases, so does the volume of biogas produced and, as expected, the highest volumes of biogas were found in the highest F/M ratio (2 g/g). It was also possible to observe that, in general, lower concentrations of biochar (2.5 g/L) obtained better biogas production results, with the particle size factor being of little relevance.

The main conclusions of this study are:

- the addition of biochar in the anaerobic digestion showed benefits in the biogas production, but at low concentration (2.5 g/L) and small particle size (< 2 mm);
- testing of high F/M ratios (i.e. 2 g/g) must be extended beyond 21 days to achieve higher sCOD degradation and higher volumes of biogas produced;
- overall the F/M ratio presented the main influence in the anaerobic digestion rather than the biochar addition.

Following the conclusion of this work, it is important to highlight the following topics for further study on the application of biochar to anaerobic digestion:

- Study other process conditions (temperature, continuous and semi-continuous processes, different F/M ratios and duration, etc);
- Carry out studies with other types of biochar, both from different raw materials and from different pyrolysis temperatures;
- Evaluate the ability of biochar to adsorb contaminants from AD;
- Evaluate the behavior of biochar in AD on a pilot scale, prior to industrial scale;
- Carry out a technoeconomic analysis on implementing the use of biochar in AD at an industrial scale;
- Carry out a life-cycle assessment to understand the environmental impacts and fate of biochar on AD processes.

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Appendix

Appendix A - Table with the components of each anaerobic digestion batch

BATCH 1	F/M	Biochar particle size	Biochar concentration	m sludge	V sludge	m OFMSW	V OFMSW	m biochar	V biochar	VS OFSMW	VS inoculum	VS biochar	V macronutrients solution	V micronutrients solution	V water to add
	gVS/gVS		(g/L)	(g)	(mL)	(g)	(mL)	(g)	(mL)	(g)	(g)	(g)	(mL)	(mL)	(mL)
1	0.5	0	0	151.52	149.27	4.86	4.23	0	0	1	2	0	1	1	844.50
2	0.5	S	2.5	151.52	149.27	4.86	4.23	2.5	10.70	1	2	1.96	1	1	833.80
3	0.5	S	5	151.52	149.27	4.86	4.23	5	21.41	1	2	3.93	1	1	823.09
4	0.5	L	2.5	151.52	149.27	4.86	4.23	2.5	15.90	1	2	2.16	1	1	828.60

BATCH 3	F/M	Biochar particle size	Biochar concentration	m sludge	V sludge	m OFMSW	V OFMSW	m biochar	V biochar	VS OFSMW	VS inoculum	VS biochar	V macronutrients solution	V micronutrients solution	V water to add
	gVS/gVS		(g/L)	(g)	(mL)	(g)	(mL)	(g)	(mL)	(g)	(g)	(g)	(mL)	(mL)	(mL)
1	0.5	L	5	192.31	189.46	4.86	4.23	5	31.79	1	2	4.32	1	1	772.52
2	1	0	0	192.31	189.46	9.72	8.45	0	0	2	2	0	1	1	800.09
3	1	S	2.5	192.31	189.46	9.72	8.45	2.5	10.70	2	2	1.96	1	1	789.38
4	1	S	5	192.31	189.46	9.72	8.45	5	21.41	2	2	3.93	1	1	778.68

BATCH 4	F/M	Biochar particle size	Biochar concentration	m sludge	V sludge	m OFMSW	V OFMSW	m biochar	V biochar	VS OFSMW	VS inoculum	VS biochar	V macronutrients solution	V micronutrients solution	V water to add
	gVS/gVS		(g/L)	(g)	(mL)	(g)	(mL)	(g)	(mL)	(g)	(g)	(g)	(mL)	(mL)	(mL)
1	1	L	2.5	157.48	155.15	9.72	8.45	2.5	15.90	2	2	2.16	1	1	818.50
2	1	L	5	157.48	155.15	9.72	8.45	5	31.79	2	2	4.32	1	1	802.60
3	2	0	0	157.48	155.15	19.44	16.90	0	0	4	2	0	1	1	825.95

BATCH 2	F/M	Biochar particle size	Biochar concentration	m sludge	V sludge	m OFMSW	V OFMSW	m biochar	V biochar	VS OFSMW	VS inoculum	VS biochar	V macronutrients solution	V micronutrients solution	V water to add
	gVS/gVS		(g/L)	(g)	(mL)	(g)	(mL)	(g)	(mL)	(g)	(g)	(g)	(mL)	(mL)	(mL)
1	2	S	2.5	156.25	153.94	19.44	16.90	2.5	10.70	4	2	1.96	1	1	816.45
2	2	S	5	156.25	153.94	19.44	16.90	5	21.41	4	2	3.93	1	1	805.75
3	2	L	2.5	156.25	153.94	19.44	16.90	2.5	15.90	4	2	2.16	1	1	811.26
4	2	L	5	156.25	153.94	19.44	16.90	5	31.79	4	2	4.32	1	1	795.36