Master Thesis

Anaerobic Co-digestion study and characterisation of different digesters to maximise biogas generation

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Abstract: The EU has recently started the Energy Transition, with the goal to achieve carbon neutrality by the year 2050 and therefore comply with the Paris Agreement. To achieve this, basic economic sectors such as energy or transportation must be amongst the first to complete said process by developing new technologies. One of those to be employed due to its strong circularity is biogas and its upgraded form biomethane produced using anaerobic digestion processes. As such, this project focused on the study of anaerobic co-digestion with the use of C-Fe nanoparticles to determine their impact on said process. This was analysed in three different experiments. The first were standardised BMP tests in 1-litre bottles; followed by a larger scale 2-litre batch reactors. Lastly, a semi-continuous reactor was also used. The research has shown that the nanoparticles present new interactions with the different chemical species identified, while also increasing the system's resilience and reducing the variability between samples. The project has identified new research leads to be developed in the future such as the CH_4-H_2 interaction or a sudden peak in H_2S. Despite this, the overall conclusion of the project is that the nanoparticles present a viable opportunity to improve anaerobic co-digestion.

Keywords: anaerobic co-digestion; biogas; biomethane; nanoparticles; residues; BMP tests; digestion reactors.

1. Introduction

The main problem faced by our society today is the named Ecological Transition to a greener economy by reducing the impact humans have on the planet because of anthropogenic climate change. This part of the "Twin Transitions" *[1]* is considered by the EU and its member states a pillar to define the direction in which Europe must lead the world, which resulted in the creation and approval of the European Green Deal *[2]* to provide a response to ever increasing climatic anomalies & disasters caused by climate change.

To permit and direct the required effort and investment, both of public and private nature, the Climate Delegated Act was passed to direct and align the various key players and technologies. Among this key technologies, bioenergy, and particularly biogas and biomethane, were identified as "low-carbon gases" meant to replace natural gas, produced through anaerobic digestion (AD), in the energy and industrial sectors coupled with prospective applications heavy-duty transport *[3]*.

The recent COP 26 held in Glasgow *[4]* further emphasized the need for the global temperature increase to be limited to 1.5ºC above pre-industrial levels. Therefore, global leaders reached a compromise to remove coal from their electrical generation mixes *[5]*, widely considered to be the most polluting of fossil fuels. Furthermore, the EU, the US as well as a hundred countries announced their intentions to reduce their methane emissions, particularly from natural gas leaks *[6]*. Methane emissions have long been considered one of the 8 stabilization wedges required to prevent an increase in global temperatures *[7]* due to its high global warming potential of 21 CO_2-eq *[8]*.

This new advancements in climate action will require substantial investments in infrastructure and research in new technologies to transition to a greener economy. To facilitate said change, the EU included renewable fuels and bioenergy as part of the 2020 SET Plan revision *[9]*. However, most projects are set to start by the 2022–2025-time frame once laboratory research is finalised and results can be used to design industrial applications.

On a national scale, the Ministry for Ecological Transition (MITECO) identified biogas as a new renewable energy source with the capability of preventing up to 2.1 million tonnes CO_2-eq. per year according to the Biogas National Plan *[10]*. The Plan highlights the minimal use of AD reactors, which produced 2.74 TWh of energy in 2020. The 146 operating plants are in landfills or wastewater treatment plants, with only thirteen used in commercial applications. Moreover, there are only five biomethane plants in the country, with the largest located in Valdemingomez, Madrid *[11]*.

The last aspect of the context in which this research occurred pertains to the goal of achieving a circular economy in which waste is minimised and resources used to their full potential, extracting all the value contained within them before final disposal *[12]*. AD proposes the transformation of sludge from a residue into a co-product to be used not only for energy generation, but also the upcycling of the obtained digestate as fertilizer. By considering the full life cycle of the sludge, eco-design of waste treatment processes can be expanded until a full cradle-to-cradle approach can be implemented. This advancements would reduce the energy intensity of water treatment facilities or similar industrial processes which require wastewater treatment plants to comply with environmental regulations.

Another important aspect of AD is the capability of reducing environmental pollution resulting from mishandling of residues. These residues currently have become a major problem due to their impact in soil and water pollution as diffuse and punctual emission sources percolate into superficial and ground water *[13]*, altering their chemical balances and requiring additional treatment of raw water before it is deemed potable. On the other hand, if said sludges are not effectively managed the environmental damage resulting from the addition of external bacteria leads to the eutrophication of superficial waters and all the related consequences.

2. State of the Art

Anaerobic digestion (AD) is a naturally occurring process in which complex organic matter is broken down in an oxygen-free environment, producing a homogenous gas mixture, known as biogas, and a mixture of mineralized nutrients and undigested matter known as digestate [14].

The digestion of organic matter occurs in four distinct steps, performed by four different microbial families with different environmental needs. This results in AD processes being unstable, especially if employed in continuous reactors, traditionally used in waste treatment processes with high organic pollution in dry (solid) or wet (liquid) form [15].

It must also be added that the obtained products (biogas and digestate), will present different compositions and thus uses depending on the nature and source of the treated waste. As a result, both the biogas and specially the digestate require strict control when determining their final uses, as they may require processing and cleaning techniques.

2.1. Usable Substrates in Anaerobic Digestion Processes

AD only requires the use of a substrate containing organic matter; however, each type of substrate may have different characteristics which may result in variations in biogas production. To that end, the main substrates used are listed in this section [16].

Crop Residue: Also known as agricultural residue, this refers to leftover crop or parts of the plant which were harvested by later discarded such as grain husks and dried crop such as hay. It is characterized by its low humidity content, paired with a 10% lignin, which inhibits AD. However, winter crops present a high C/N ratio between 80 and 100.

Animal Manure & Slurry: Manure content and composition varies based on the animal which produced it. Animal waste requires treatment because of its high nitrogen content and biological contamination which may be harmful to the environment. Similarly, slurries also represent an environmental hazard if they percolate into aquifers, due to their basic pH and high organic and nitrogen loading. Manure is rich in solid matter as well as organic loading. As a result, they are usually mixed and treated as a liquid mixture.

Wastewater sludge: Wastewater sludge is a prime substrate, due to its biological composition, which contains all the necessary bacterial families necessary to perform the AD process. It must also be mentioned that the sludge is itself poor when used on its own, as it is produced from previously treated waste which results in a low C/N ration [17] [18]. This in turn causes AD processes in wastewater treatment plants to not be self-sustaining energy wise, as they require more methane than they produce to maintain their operating conditions.

Organic Fraction of Municipal Solid Waste: Used as AD substrate in landfills, the OFMSW presents a high organic content as well as a high biodegradability but lacks any compound which may be used as a buffer for acidification. Another problem it faces is the presence of inorganic or inert waste which cannot be digestated, requiring separation and screening prior to digestion [15].

2.2. Influence of Substrates in Biogas Production and Composition

Due to the nature of the process in which organic waste is broken into simpler chemical compounds, the composition of said intake will influence the composition as well as the production rate of the biogas, with simpler and shorter organic compounds being digested at a faster rate, especially sugars [17].

On the other hand, lignin (a complex polymer which characterizes woody plants) which reduces biodegradability of organic compounds, considered to be a more representative indicator than the Volatile Solids in the waste being processed. As a result, the biodegradability can be estimated using equation 1 below [19]. It must also be mentioned that biodegradability in AD tests can be measured using BMP tests [20].

$$FB\ [p.u.] = 0{,}83 - 0{,}028 * lignin\ [\%_{db}] \quad (1)$$

Furthermore, the composition of said biogas can be estimated using de NMOC method, which employs the COD/TOC ratio (chemical oxygen demand/Total Organic Carbon), which can also be used to measure biodegradability, to estimate the methane composition in the produced biogas. The formula provided can be seen below in equation 2 and the related graph in **Figure 1** [14] [21] .

$$CH4\ [\%] = 18.75 * COD/TOC \quad (2)$$

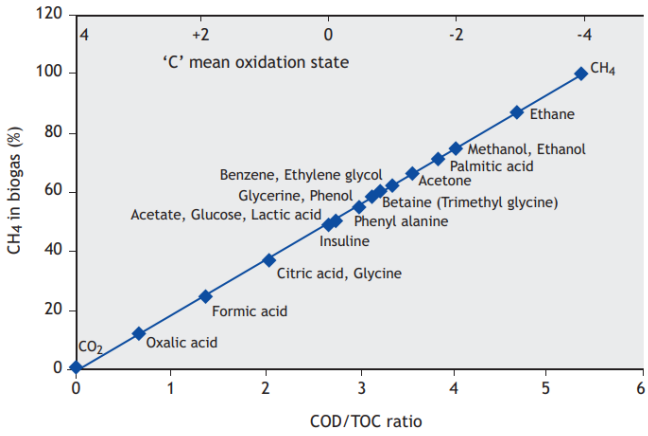

Figure 1. NMOC Methane Percentage Composition. Source: [14] [21].

Similarly, several observations on the decomposition of lipids, proteins and carbohydrates were made in [17] and summarized in [14].Regarding lipids, these present a high carbon content, which will yield a larger biogas generation, with a higher content of methane. However, as it will be explained later, these long-chained compounds present a slower degradation and risk the acidification of the reaction medium.

Proteins, contain nitrogen and sulphur, which reduce the methane yield during degradation and may produce hydrogen sulphide and ammonia. These unwanted products require monitorization as they may inhibit methanogenesis.

The final compound to be analysed were carbohydrates, which produce low quantities of biogas, with a low methane content. Despite this, they present the highest degradation within

the reactor and thus simplify the AD process. However, the fast decay may result in the acidification of the vessel if the simple sugars decompose into Volatile Fatty Acids and accumulate.

As previously stated, AD products will depend on the composition of the substrate, which can be a singular type (known as monodigestion) or a mixture of two or more different substrates, being thus referred to as co-digestion.

The biogas potential of each compound was also calculated in [17], whose results are compounded below in **Table 1**.

Table 1. Theoretical Composition and Production of Biogas using the Buswell-Mueller and Boyle Formulae. Source: [17] [22].

	Theoretical biogas production [L_N/kg-ST]	Theoretical Composition of biogas [% CH4 vol.]	[% CO2 vol.]	Reference
Lipids	1390	72	28	
Proteins	800	60	40	[22]
Carbohydrates	750	50	50	

Using the obtained data displayed throughout this section, the biogas production of various compounds can be estimated based on their lipids, protein, and carbohydrate content, although lignin and other inhibitors should also be considered when performing a detailed analysis of each substrate.

2.2. Anaerobic Digestion Stages.

As previously stated, the digestion process consists of four distinct stages. These stages occur simultaneously, although the dominant phase varies during batch processes. In continuous processes however, the four processes occur in equilibrium during operation. These stages, along with the products are shown below in **Figure 2.** In addition, detailed reviews of the stages are provided below.

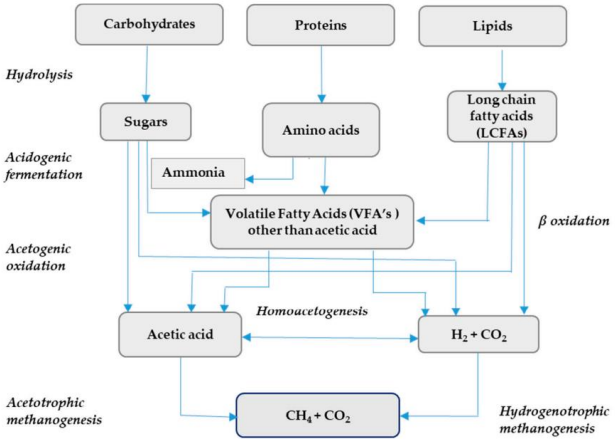

Figure 2. Anaerobic Digestion Stages. Source: [23].

2.2.1. Hydrolysis

The first step in the AD process. This represents the critical stage of the digestion as well as the only extra-cellular phase using enzymes released into the digestion medium [24]. The latter three occur inside the various microbes. During hydrolysis, long chain organic compounds, such as carbohydrates and proteins are broken down into simpler compounds such as acetate carbon dioxide and hydrogen gas.

These simpler compounds can then be absorbed by acidogenic bacteria, responsible for the following step of the process. It must also be mentioned that different compounds will present a smaller decay rate during hydrolysis based on their complexity and the elements found in the monomers, with sugar and carbohydrates being processes faster than lipids and proteins [25]. Lastly, the ideal pH at which the phase occurs is also of importance, with [26] identifying said range between 5 and 6. A sample equation for the decomposition of a glucan polymer was provided by [27] and is shown in equation 3 below.

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_6 + nH_2 \quad (1)$$

2.2.2. Acidogenesis

Once the complex organic compounds have been broken down, acidogenic bacteria absorb said compounds through their cell membranes and release Volatile Fatty Acids (VFA). These intermediate acids include compounds such as the acetate, the main compound from which methane is eventually produced after further digestion. It must also be mentioned that VFA can cause the sludge to acidify, reducing the pH drastically and inhibiting further digestion by killing the bacteria responsible for acidogenesis and methanogenesis [28]. The pH range at which acidogenesis occurs is between 5 and 8 [29].

This has led to developments in reactor design, which now propose the use of separate vessels for AD processes [14], connected through digitally controlled valve so that the first 2 stages occur in an acidic vessel, in their ideal conditions, while the remaining steps, which required a basic pH, may take place in the second vessel. However, this is currently being researched to assess viability and the complexity required to design the control system for the flow valve. Lastly, the acidogenic decomposition process is shown below in equations 4 through 6, compiled in [30], which can occur through various reversible reactions.

$$C_6H_{12}O_6 \leftrightarrow 2CH_3CH_2OH + 2CO_2 \quad (4)$$

$$C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3CH_2COOH + 2H_2O \quad (5)$$

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \quad (6)$$

Lastly, it must also be mentioned that the decomposition of proteins releases nitrogen in the form of ammonia, which presents a buffer to prevent acidification of the reaction medium. However, a large ammonia concentration may also result in the suppression of methanogenesis, requiring a balance with the carbon content to maximize production [31].

2.2.3. Acetogenesis

Acetic acid is the main compound from which methane is produced. The mechanism used depends on the temperature at which de reaction takes place. At a thermophilic temperature (65-70ºC), the dominant mechanism is hydrogenotrophic methanogenesis, which skips the stage currently being detailed and produces methane directly from acetate [32].

However, most processes used occur at mesophilic (30-40ºC) or psychrophilic (2-25ºC) [14]. At these temperature ranges, the dominant methanogenesis process is acetoclastic methanogenesis, which requires the production of acetic acid during the acetogenesis phase, with an ideal like that of acidogenesis, considering the production of acetic acid as a two-step process [33].

During said phase, the VFA as well as lighter compounds such as alcohols, previously produced are converted into acetic acid by the dehydrogenation of the acetate. Another reaction which produces acetic acid is the homoacetogenesis of H_2 and CH_4 previously released [23]. As with the previous processes, the decomposition of acetate into acetic acid is shown below in equations 7 to 9 [34].

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$$CH_3CH_2COH + H_2O \rightarrow CH_3COOH + 2H_2 \quad (7)$$

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO2 + 3H_2 \quad (8)$$

$$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2 \quad (9)$$

2.2.4. Methanogenesis

Once the acetic acid has been produced, it can then be converted into methane and carbon dioxide through acetoclastic methanogenesis as previously explained. Temperature has such a profound effect on the dominant mechanism that in thermophilic conditions, 90% methane is produced through the hydrogenotrophic process, while in mesophilic conditions around 66% of methane is produced via acetoclastic methanogenesis.

These bacteria, unlike the previous digesters, require a higher pH, between 6.5 and 7.6 [35]. In addition, these bacteria are overly sensitive to pH changes, which can inhibit the process when it reaches de 5.5 to 6.25 range [36].

Lastly, the equations for hydrogenotrophic methanogenesis are displayed below in equations 10 to 13. The equation for acetoclastic methanogenesis is shown in equation 14.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (10)$$

$$4HCOOH \rightarrow CH_4 + 3CO_2 \quad (11)$$

$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O \quad (12)$$

$$CH_3NH_2 + 2H_2O \rightarrow 3CH_4 + 4NH_3 + CO_2 \quad (13)$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (14)$$

2.3. Anaerobic Digestion Control Parameters

Certain parameters have been lightly mentioned throughout the previous subsections, such as the C/N ratio of the Volatile Fatty Acids. Due to their significance, they will be discussed in this subsection, as they represent the key metrics used to control and determine the state of the digester.

2.3.1. Temperature

As with all biological processes, temperature plays a key role in AD reactions, promoting or inhibiting the growth of certain microbial families which result in different compound decay rates and therefore methane production rates by changing the predominant methanogenesis mechanism from acetoclastic methanogenesis to hydrogenotrophic methanogenesis, as reviewed in subsection 2.2.4.

Temperature control is also a key element when performing AD experiments, as it was shown in [37] that a variation of 1ºC/day may result in a process failure [28]. Lastly, the temperature ranges for AD: psychrophilic (2-25ºC), mesophilic (20-40ºC) and thermophilic (65-70ºC) are displayed below along with their relative methane production in **Figure 3** [14] [38].

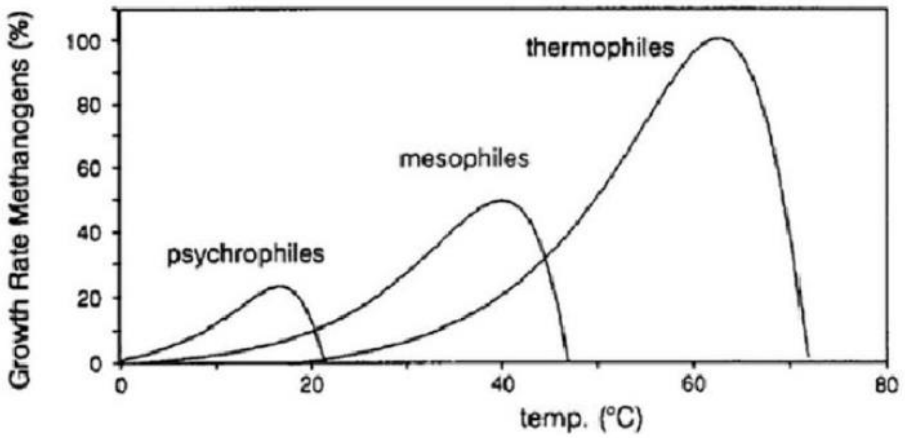

Figure 3. The dynamics of biogas production at different temperatures. Source: [38].

2.3.2. pH and Volatile Fatty Acids

pH is an essential parameter which can have severe impacts on the AD process due to the acidity or alkalinity levels required for each phase of the digestion. Various studies such as [39] found that the ideal pH level is an intermediate value between the optimum for each phase, resulting in a final value of 7.

Within pH control, Volatile Fatty Acids (VFAs) represent the most significant contributing factor. These are simple intermediate compounds found in the AD process such as acetic, butyric, propionic and valeric acid [28] [14].

To determine the VFA concentration within the reaction vessel, various methods have been developed, being [40] among the most widespread as it does not require the use of a gas or liquid chromatographer. This procedure was adapted in [17]. The proposed method employs a 2-stage titration to measure the intermediate, partial, and total alkalinity of a liquid sample. Of these, intermediate alkalinity is used to measure VFAs within the sample.

The performed titration requires the use of hydrochloric acid or sulphuric acid, preferably with standard normalities. The first titration stops when a pH of 5,75 is reached, while the second stage ends at a target value of 4.3. In both cases, the volume of titrant used (V_i) must be

recorded. Lastly, the formulas used for total, partial and intermediate alkalinity are presented below in equations 15 to 17, respectively.

$$AT\ [mgCaCO_3/L_{sample}] = V_3 * N_3 * 5000 / V \quad (15)$$

$$AP\ [mgCaCO_3/L_{sample}] = V_1 * N_1 * 5000 / V \quad (16)$$

$$AI\ [mgCaCO_3/L_{sample}] = V_2 * N_2 * 5000 / V \quad (17)$$

2.3.3. Chemical Oxygen Demand

Two main parameters are used to measure the pollution in wastewater, as well as the biodegradability of said pollution. 5-day Biochemical Oxygen Demand (BOD_5) is used to measure the oxygen demand required by bacteria to digest and decompose biodegradable pollutants.

Similarly, Chemical Oxygen Demand (COD) measures the total demand of oxygen required to fully oxidise all organic contaminants found in the sample. In addition, the BOD_5/COD quotient is used to determine the biodegradability, with the qualitative categories shown below in **Figure 4** [41].

DBO5/DQO	Biodegradabilidad del agua residual
0,4	Alta
0,2-0,4	Normal
0,2	Baja

Figure 4. Biodegradability Table of Wastewater. Source: [41].

Due to the anaerobic nature of AD processes, BOD5 cannot be used to measure the pollutant loading of the various liquid samples, being an essential parameter in water treatment. However, COD is used to characterise said samples and as a proxy to estimate Total Organic Carbon through the NMOC method [21] if the methane content of the biogas is known.

2.3.4. Total Organic Carbon

Total Organic Carbon (TOC) represents the most widely used method to determine the total carbon found in organic compounds in the sample [42]. As such, it represents the carbon susceptible of being digested in the AD process, permitting the calculation of the C/N ratio.

It is also considered a more direct measurement to determine the organic content of wastewater with smaller environmental impacts, as it does not require the use of chrome compounds, unlike COD. As a result, it is currently under review to use as a substitute in industrial water treatment processes [43].

2.3.5. Total Nitrogen

As previously mentioned, nitrogen plays a key role in AD, specifically nitrogen found as ammonia [44]. Ammonia dissolved in the digestion medium can have a positive or negative effect based on the concentration levels. At low concentrations, ammonia presents a buffer against acidification caused by VFA and promotes bacterial growth [28].

However, higher concentrations of ammonia (AN) may result in the inhibition of the acetoclastic methanogenesis due to their higher sensitivity.

The remaining nitrogen can be found in various organic compounds such as proteins and amino acids, being thus referred to as organic nitrogen (ON). To facilitate measuring, the total nitrogen (NTK) and used as part of the C/N ratio which governs AD [17].

2.3.6. C/N Ratio

Proven to be the governing parameter in anaerobic digestion, it is the quotient between TOC and NTK. This ratio represents the balance required to promote bacterial growth and thus biogas generation. The optimal value varies depends on the substrate and the feed used, although the consensus is that the optimal range is [20-30] [28] or [25-35] [45].

It must also be mentioned that the C and N are obtained from different elements, as substrates (particularly sludges) are typically rich in organic matter but poor in nitrogen content because of water treatment processes, which presents a restrictive limit on nitrogen emissions.

On the contrary, feeds used in co-digestion such as purines and food waste present high N content.

2.4. Anaerobic Co-digestion

AD processes can be used to treat different substrates individually, in which case it is known as anaerobic monodigestion. These can also be treated in a substrate mixture of two or more components in a process known as anaerobic co-digestion. This is the AD process studied in this project.

During co-digestion processes, a substrate-feed mixture is used to generate biogas. These must complement each other, depending on the composition of each. In the case of this study, the mixture used was Municipal Solid Waste (MSW) and wastewater treatment sludge.

Contrary to mono digestion processes, co-digestion presents several advantages which make it a viable alternative as well as the designated process to be used in biomethane generation according to the National Energy and Climate Plan [46] [10]. These official documents also include an illustration displaying the viability of different AD substrate as well as that of a mixture, shown below in **Figure 5**.

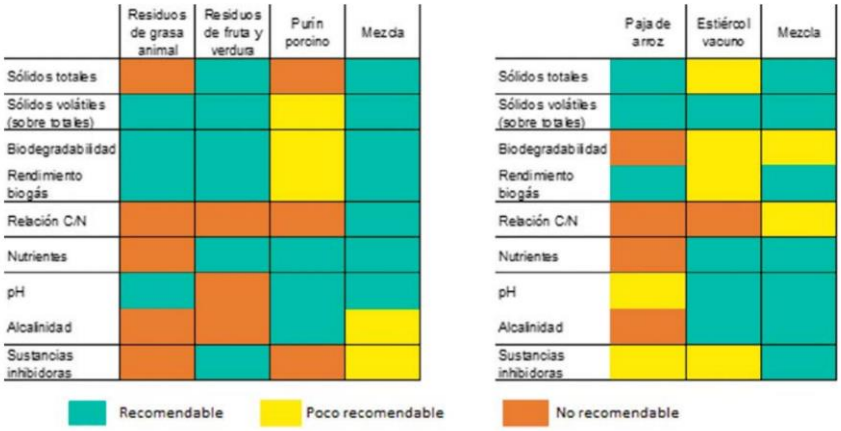

Figure 5. Substrate and Technical Parameters Relation. Source: [10].

A mixture of the various MSW, rural waste (animal fat and animal slurry) and wastewater sludge is shown to be the best substrate to use in AD processes, having the preferred characteristics for all technical criteria save for alkalinity due to organic MSW and rural waste and inhibitors from rural waste (both sources).

Another mixture utilizing animal slurry and agricultural waste (rice crop residue) would not be preferable based solely on biodegradability from the lignin content of found in the rice crop and the C/N ratio as animal slurry has a remarkably high nitrogen content.

By mixing different residues, their compositions may be used to complement each other by increasing the carbon content in the final mixture to a preferable level or by diluting inhibitors such as the volatile fatty acids. In addition, this allows for the centralization of the treatment facilities and processes.

Also, utilizing a mixture compensates for the seasonal variations found in different residues and their compositions, especially in MSW, whose organic content varies significantly between summer and winter [19]. The possibility to increase biogas generation by using otherwise spent substrate is still being researched.

3. Objectives

To achieve valid results & determine real world applications, the project will be divided into two distinct areas, pertaining the co-digestion study & application designs, respectively. Therefore, the following objectives were determined:

Sludge characterization: Sludge composition varies between digesters from different reactors, depending on the nature of the treated residual water. Furthermore, this will also affect the doses of nanoparticles used, as certain components may inhibit bacterial growth & negatively affect the digester.

Characterization of nanoparticles: The nanoparticles must be analysed to determine their elemental composition. Approximate estimates provided by the manufacturer suggest a 55% C content & 45% Fe content. However, an analysis must be performed to determine the exact Fe content.

BMP tests: The first set of tests required to assess the effects of the nanoparticles is the standardized BMP tests. These will be used to determine the initial biomethane & hydrogen generation in small batches. Due to their nature, the generated biogas will be sampled with a syringe through a septum and the volume produced will be measured indirectly.

Batch reactor tests: Once the sludge & feed mixture has been characterized through the previous tests, the reactor tests can be performed. These will maintain the same ratios used in the BMP tests. However, the volume measurement will be continuous using gasometers.

Continuous reactor tests: The last set of tests to be performed will take place in a custom built five litre reactor to determine the sludge's behaviour & viability in continuous operation aimed at resembling industrial anaerobic digestors. As a side requirement, the appropriate equipment not found in the Chemistry Lab must be procured.

4. Materials and Methods

This research Project aimed to determine the effects of C-Fe nanoparticles on anaerobic co-digestion reactors. Therefore, a standardized methodology was followed where stablished [20] [17], and a new one developed if none was available, to ensure results are consistent.

4.1. Materials Used

The study was performed in the Chemistry & Environment Laboratory located in Universidad Pontificia Comillas-ICAI [47]. As a result, the equipment found in the lab was be used.

Firstly, all experiments were be performed in sealed reactors or bottles, depending on the research stage. BMP experiments required the use of 1L-10bar bottles used in [17] with their respective caps and septum. Said bottles can be seen below in **Figure 6** and **Figure 7**.

Figure 6. Depressurization of BMP Batch Bottle. Source: Own.

Figure 7. BMP Batch Bottle Resealed with Silicone Paste. Source: Own.

To replicate semi-continuous testing at a bottle scale, 2 BMP bottles were modified utilizing a cap with valves to permit the replicate the sampling port and the feeding port. To that end, the feeding port's inner adapter was fitted with a tube to facilitate feeding and sample extraction. The final bottles along with the intended design are shown in **Figure 8** and **Figure 9**.

Figure 8. Semi-Continuous Bottle Final Construction. Source: Own.

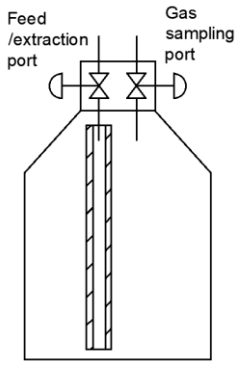

Figure 9. Semi-Continuous Bottle Design. Source: Own.

To incubate the samples during testing, the Optic Ivymen System's Orbital Shaker Incubator. This incubator can be used to agitate & maintain at a constant temperature up to nine bottles, limiting batches to said number. The pressure inside the BMP bottles was measured using an in-house custom-built barometer with a range of 0-2 bar with a needle attached to pierce the septum. The incubator can be seen in **Figure 10** and the barometer in **Figure 11**.

Figure 10: Ivymen Incubator. Source: Own.

Figure 11: Barometer. Source: Own.

The remaining experiments were performed in a 2-litre Scharlab 2000ml jacketed reactor with mechanical stirring [48]. In addition, the reactor used for semi-continuous testing was also connected to a Heidolph Hei-FLOW Precision 01 [49] peristaltic pump to inject the feed mixture daily. In addition, the reactor's bottom port received a spheric valve to extract the daily amount required. The design diagram appears in **Figure 12**.

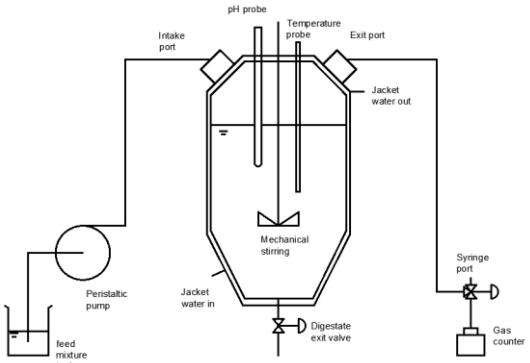

Figure 12. Semi-Continuous Reactor Design Diagram. Source: Own.

Furthermore, both the batch & semi-continuous reactors required auxiliary equipment for operation & sampling. Temperature inside the reactors was maintained using Selecta Digiterm-TFT-200 immersion thermostats [50] along with their thermal probe inside the reactor. The reactors were equipped with paddle stir rods to agitate the sludge-feed mixture.

To record direct biogas production, 2 Ritter MilliGascounters [51] were be used, attached to the sampling port through a three-way valve which was also used to obtain biogas samples. Reactor pH was measured using the XS pH70 pHmeter [52] using a 130mm probe in the batch reactor and a 250mm probe in the semi-continuous reactor. Final set up is shown in **Figure 13**.

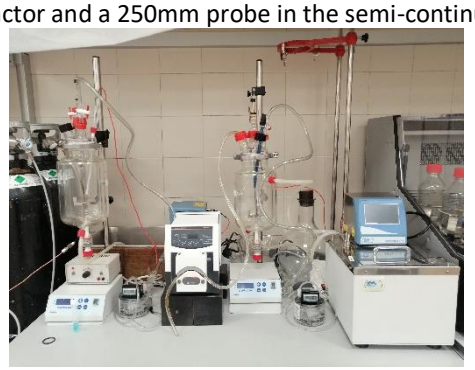

Figure 13. Final Reactor Set-Up. Source: Own.

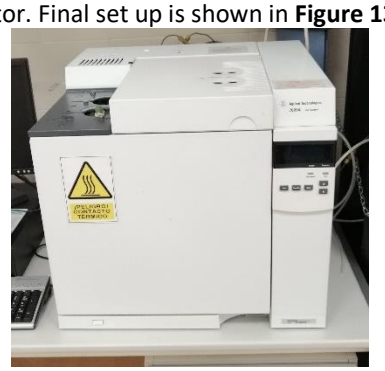

Figure 14. Aglient GC 7820A. Source: Own.

Gas sample analysis was performed using the Aglient 7820A gas chromatographer [53] employing nitrogen gas (N_2) as the carrier. Samples will be obtained using the syringe provided with the chromatographer.

Lastly, liquid sludge samples will be analysed using the HANNA HI 902 Potentiometric Titrator [54] to determine their VFA content. Similarly, other properties such as the NTK and the COD will be obtained using the Spectroquant Prove 100 spectrometer [55] along with specific NTK and COD kits. Regarding unused sludge samples, these will be kept in the ovens within extraction chambers in the lab. Lastly, the C-Fe nanoparticles' elemental analysis was outsourced, as there is no SEM capable equipment within the University. The chromatographer, titrator and spectrometer can be seen in **Figure 14**, **Figure 15**, and **Figure 16**, respectively.

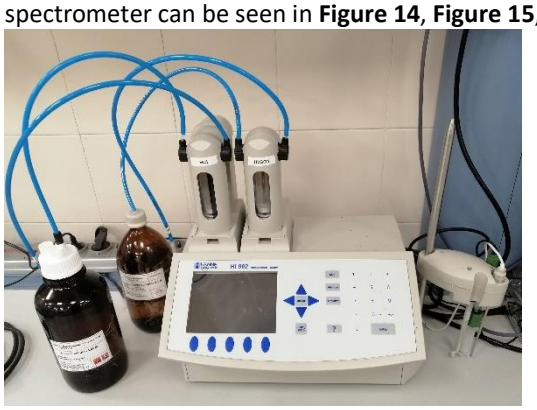

Figure 15. Hannah HI 902 Potentiometric Titrator. Source: Own.

Figure 16. Spectroquant Prove 100. Source: Own.

4.2. Experimental Procedure

4.2.1. Bottle Procedure

The first set of tests were BMP tests. These were performed as per the guidelines set in the norm ISO 11734:1999 [20], employed in previous research [17] which this project was based on. This Norm required the use of sealed 1L bottles in which 300ml of a sludge-feed mixture was sampled for 21 days, collecting daily pressure readings and releasing said pressure after the sampling process had finished. To ensure a hermetic seal, the bottles were closed using a septum and silicone paste.

Prior to sealing each bottle, the air contained inside was replaced with N_2. In keeping with previous research, the sludge-feed proportion was set at a 3:1 ratio [17]. Also, a 50% humidity was required [11], which set another constraint on the vessel composition. As such, the final proportions were collected into equations 18 to 20.

$$V_{water}\ [ml] = 0{,}5 * V_{vessel}\ [ml] \quad (18)$$

$$V_{sludge}\ [ml] = 0{,}375 * V_{vessel}\ [ml] \quad (19)$$

$$V_{feed}\ [ml] = 0{,}125 * V_{vessel}\ [ml] \quad (20)$$

It must be pointed out that the feed used will be pumpkin prepared by removing the seeds but maintaining the skin. This will in turn be processed with water to obtain the feed mixture at 50% by volume.

Pressure readings were used to determine the gas production through indirect measurements through the ideal gas law and the temperature. The conversion formula used can be seen below in equation 21.

$$n_{biogas}\ [Nm^3] = 0{,}9269 * P_{bottle}\ [bar] \quad (21)$$

The daily extracted gas samples were analysed to determine the composition of the biogas produced. The method used within the chromatographer was designed in [17] for this purpose. The temperature and flow graphs which determine the method are shown in **Figure 17**.

Lastly, it must be mentioned that the semi-continuous bottles require refinement for future tests as all samples were inhibited early in the run. As a result, no useful data could be extracted from said bottles.

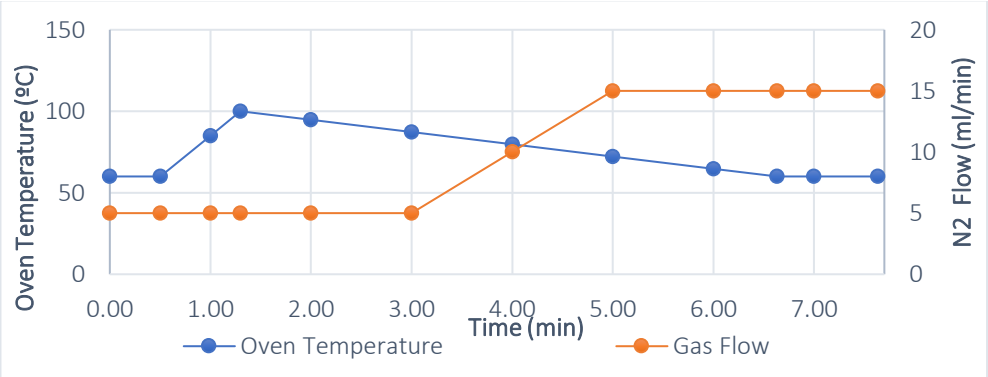

Figure 17. Gas Chromatography Method. Source: Own.

4.2.2. Reactor Procedure

Regarding gas sample collection, these were collected through the three-way valve and analysed daily in the chromatographer. Furthermore, biogas production was be measured continuously through milli gas counters connected permanently to the second exit port of said valve. However, it must be pointed out that due to the high hydraulic head loss caused by the various valves and exit ports, the devices did not record biogas production except for the initial 3 days. Therefore, no biogas production analysis could be made in the results section. As these reactors must also be in anaerobic conditions, the contained air will be removed using N_2.

Semi-continuous test required a 21-day residence time, maintaining the original experiment duration. This required the daily extraction and later injection of 95,24 ml of digestate and feed mixture, respectively.

Daily digestate samples were collected through the bottom port fitted with the spherical valve. In addition, the 25ml of the supernatant obtained from the digestate was diluted in 25ml of distilled water and used to determine VFA content in a two-stage HCl & H_2SO_4 titration, as per the [56] method. Lastly, COD and NTK tests were performed every 2 days using the supernatant.

Regarding the use of nanoparticles, the dosage used was 2gr per kg of total volatile solids (TVS). This was calculated by determining the sludge's density empirically, as the nanoparticle's TVS content was determined through an outsourced analysis.

5. Results and Discussion

In this section the results from the various experiments performed were presented and analysed, as such, it will be divided into sections pertaining to the three tests. Before proceeding, it must be mentioned that during the second round of experiments, day four coincided with a bank holiday and therefore no data could be collected that day. To facilitate data treatment and clarity, data was grouped with that obtained during the next sampling.

It must also be added that the reactor results were affected by the volatilization of the nanoparticles, which deposited on the stir rod and its seal.

5.1. BMP Bottle Results

Firstly, it must be mentioned that only the data obtained from BMP bottles will be analysed, as the semi-continuous bottles presented difficulties and were inhibited early into the study, as previously mentioned.

5.1. BMP Bottle Results

Raw pressure data was obtained daily using the barometer, as previously stated. In addition, the gaseous samples were also analysed in the chromatographer, producing chromatograms which could be later graphically analysed using a MATLAB [57] code developed to graphically obtain the composition of each analysed sample. A sample of an obtained chromatogram is shown **Figure 18**. In addition, pressure readings were recorded in **Table 2**.

From the obtained data, the normalised production by volume of sludge as shown in equation 22, was calculated an aggregated to obtain the average accumulated biogas production. This graph is displayed in **Figure 19**. In said graph, F stands for sludge, F+NP sludge with nanoparticles, B: BMP bottle and B+NP signifies BMP bottle with nanoparticles

$$n_{biogas}\ [Nm^3] = 0{,}9269 * P_{bottle}\ [bar] \quad (22)$$

Table 2. BMP Bottle Pressure Readings. Source: Own.

Day	1	2	3	7	8	9	10	11	14	15	16	17	18	21
F1	0.469	0.309	0.282	0.489	0.143	0.101	0.091	0.078	0.18	0.062	0.052	0.065	0.051	0.109
FN	0.423	0.27	0.205	0.342	0.092	0.061	0.072	0.042	0.056	0.028	0.031	0.04	0.03	0.034
B1	0.979	0.259	0.284	0.187	0.147	0.018	0.015	0.006	0.002	0	0	0	0	0
B2	0.947	0.606	0.21	0.122	0.097	0.085	0.109	0.077	0.056	0.045	0.039	0.039	0.054	0.109
B3	0.979	0.044	0.045	0.006	0.023	0.006	0.012	0.045	0.111	0.039	0.024	0.033	0.03	0.05
B4	1	0.524	0.512	0.408	0.17	0.093	0.101	0.085	0	0	0	0	0.026	0
B5	0.979	0.087	0.269	0.045	0.019	0.015	0.01	0.043	0.038	0.038	0	0.018	0	0
B6	0.979	0.452	0.388	0.108	0.046	0.045	0.057	0.055	0.07	0.025	0.027	0.043	0.041	0.082
B7	0.979	0.597	0.344	0.262	0.158	0.105	0.126	0.01	0.008	0	0	0.016	0.024	0.024
B8	0.979	0.618	0.001	0	0	0	0	0	0	0	0	0.003	0	0
B9	1	0.728	0.41	0.373	0.127	0.043	0.062	0.037	0.006	0.024	0.015	0.006	0.015	0.027
BNP1	0.975	0.464	0.366	0.295	0.129	0.086	0.064	0.035	0.04	0.029	0.026	0.046	0.048	0.046
BNP2	0.979	0.526	0.37	0.543	0.132	0.076	0.077	0.067	0.149	0.056	0.033	0.045	0.031	0.037
BNP3	0.979	0.417	0.421	0.234	0.093	0.056	0.059	0.05	0.101	0.049	0.026	0.032	0.023	0.058
BNP4	0.979	0.102	0.111	0.13	0.102	0.122	0.07	0.068	0.127	0.051	0.031	0.043	0.03	0.095
BNP5	0.979	0.436	0.325	0.371	0.097	0.005	0.009	0.03	0	0.017	0.026	0.037	0.035	0.024

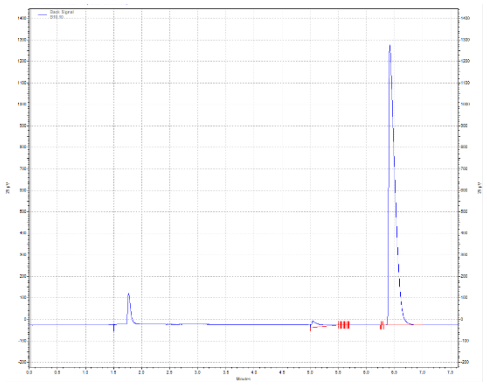

Figure 18. Chromatogram Obtained during Testing. Source:Own.

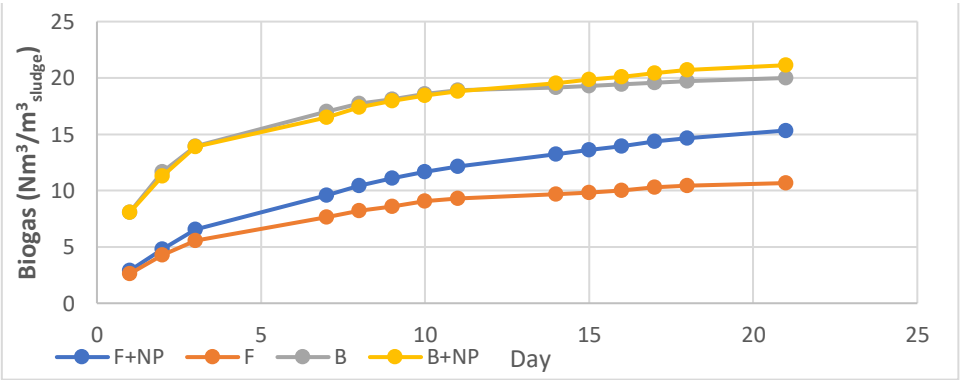

Figure 19. Average Accumulated Biogas Production for each BMP test. Source: Own.

As evidenced in the above figure, normalised biogas production was far superior in co-digestion tests when compared to the sludge and sludge & nanoparticle baselines. However, no further analysis could be made until a statistical analysis was performed on the data.

It must also be mentioned that the addition of nanoparticles did not increase biogas production significantly, which suggests that the main benefit of adding these into AD co-digestion

reactors would be added process stability, based on the data dispersion that can be seen in Table 2. To obtain a better analysis, B and B+NP data series were compared in **Figure 20**.

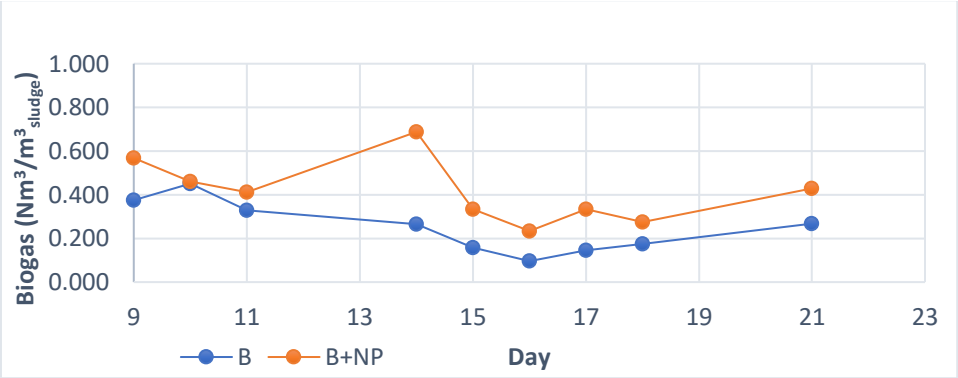

Figure 20. Daily BMP Co-Digestion Biogas Production. Source: Own.

As opposed to **Figure 19**, it is apparent in the above graph that the nanoparticles do improve production by a small margin after day nine. Also, both curve follow a similar trend during the final days, although B+NP presents a sudden increase in production which could indicate that said nanoparticles accelerate the AD process.

To determine the effects of nano particles, both in the average production and in the variability of said data points, a Student's t-test [58] was used to determine if both daily sample groups could have the same mean (assuming a confidence of 95% and that both populations follow a normal distributions). The null hypothesis used was that both means were equal.

Similarly, Snedecor's f-test was used to determine if the population's variance (estimated using the sample variance corrected for skewing) . In this test, the null hypothesis was that the variance of both populations was the same Lastly, the significance was set at 5%. Due to the various tests performed, the results for were combined into **Table 3** and **Table 4**, containing t-tests and f-test, respectively. If the null hypothesis were accepted, the displayed result was zero, if it could not be accepted, the result shown was zero. It must also be added that the results displayed for the f-test are the standard deviation.

As it can be appreciated in **Table 3**, the null hypothesis can only re rejected sparingly, primarily during the final days of methanogenesis in hydrogen composition. Regarding methane, the nanoparticles do not have a significant effect in the mean, as the null hypothesis is no rejected. This could be improved if a larger sample is obtained by performing more BMP tests.

Pertaining to the hydrogen sulphide, the null hypothesis must be accepted always, save for the central days of methanogenesis, like the hydrogen. In addition, the H2S displays a sudden increase in both production and composition in day eleven, being the only day where it presents a relevant change. Further study would be advised to analyse the effects of the nanoparticles on this chemical compound.

Lastly, the residuals behave statistically identical in both cases, as the hypothesis is always accepted in composition and production. Focusing on the composition, the nanoparticles do cause a small change, although not significant enough. Regarding production, the effects are more significant, as in certain cases the production doubles. Also, residue production is always higher in samples containing the nanoparticles except for three distinct days.

Table 3. Student's t-test on Mean Results. Source: Own.

Day	1	2	3	7	8	9	10	11	14	15	16	17	18	21
Methane Production														
Prod B (p.u.)	7.297	3.297	2.088	1.292	0.670	0.349	0.418	0.303	0.248	0.145	0.089	0.135	0.162	0.254
Prod. BN (p.u.)	7.318	2.932	2.408	2.392	0.782	0.529	0.429	0.381	0.639	0.310	0.218	0.312	0.256	0.400
H	0	0	1	1	0	0	0	0	0	0	0	0	0	1
Methane Composition														
Comp. B (p.u.)	0.904	0.921	0.927	0.931	0.927	0.927	0.929	0.926	0.929	0.927	0.929	0.930	0.935	0.947
Comp. BN (p.u.)	0.908	0.917	0.918	0.922	0.848	0.924	0.929	0.923	0.930	0.929	0.932	0.932	0.930	0.934
H	0	0	1	1	0	0	0	0	0	0	0	0	0	1
Hydrogen Production														
Prod B (p.u.)	0.005	0.002	0.001	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Prod. BN (p.u.)	0.003	0.002	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen Composition														
Comp. B (p.u.)	0.001	0.000	0.000	0.001	0.001	0.002	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.000
Comp. BN (p.u.)	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
H	0	0	0	0	0	0	0	0	0	1	1	1	0	0
Hydrogen Sulphide Production														
Prod B (p.u.)	0.008	0.002	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Prod. BN (p.u.)	0.006	0.003	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen Sulphide Composition														
Comp. B (p.u.)	0.001	0.001	0.000	0.001	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Comp. BN (p.u.)	0.001	0.001	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
H	0	0	0	0	0	0	0	0	0	1	1	1	0	0
Residue Production														
Prod B (p.u.)	0.765	0.282	0.166	0.091	0.049	0.026	0.032	0.024	0.018	0.011	0.007	0.010	0.011	0.013
Prod. BN (p.u.)	0.734	0.268	0.215	0.198	0.128	0.040	0.031	0.031	0.048	0.023	0.016	0.023	0.019	0.028
H	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Residue Composition														
Comp. B (p.u.)	0.095	0.078	0.072	0.068	0.071	0.071	0.070	0.073	0.069	0.072	0.070	0.069	0.064	0.053
Comp. BN (p.u.)	0.091	0.081	0.082	0.078	0.150	0.075	0.070	0.076	0.070	0.070	0.068	0.068	0.070	0.066
H	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4. Snedecor's f-test on Variance Results. Source: Own.

Day	1	2	3	7	8	9	10	11	14	15	16	17	18	21
Methane Production														
Prod B (p.u.)	0.131	1.878	1.279	1.171	0.511	0.307	0.370	0.231	0.303	0.147	0.116	0.130	0.147	0.316
Prod. BN (p.u.)	0.148	1.247	0.914	1.188	0.237	0.333	0.209	0.136	0.474	0.129	0.026	0.046	0.071	0.208
H	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Methane Composition														
Comp. B (p.u.)	0.008	0.008	0.008	0.005	0.007	0.009	0.008	0.007	0.008	0.005	0.004	0.005	0.008	0.008
Comp. BN (p.u.)	0.019	0.013	0.006	0.003	0.177	0.011	0.011	0.006	0.004	0.005	0.003	0.002	0.003	0.005
H	1	0	0	0	1	0	0	0	0	0	0	0	0	0
Hydrogen Production														
Prod B (p.u.)	0.007	0.004	0.002	0.001	0.001	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Prod. BN (p.u.)	0.003	0.003	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0	0	0	0	0	1	0	0	0	1	0	1	1	1
Hydrogen Composition														
Comp. B (p.u.)	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.002	0.002	0.001	0.001	0.001	0.001	0.000
Comp. BN (p.u.)	0.000	0.001	0.000	0.001	0.000	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000
H	0	0	0	0	1	0	0	0	1	1	1	1	1	1
Hydrogen Sulphide Production														
Prod B (p.u.)	0.008	0.003	0.001	0.001	0.001	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Prod. BN (p.u.)	0.007	0.003	0.002	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0	0	0	0	0	0	1	0	0	0	1	1	0	0
Hydrogen Sulphide Composition														
Comp. B (p.u.)	0.001	0.001	0.001	0.001	0.001	0.000	0.001	0.001	0.000	0.001	0.001	0.001	0.001	0.001
Comp. BN (p.u.)	0.001	0.001	0.001	0.000	0.002	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000
H	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Residue Production														
Prod B (p.u.)	0.057	0.156	0.101	0.079	0.036	0.021	0.028	0.018	0.022	0.011	0.009	0.010	0.011	0.015
Prod. BN (p.u.)	0.142	0.120	0.084	0.091	0.136	0.022	0.014	0.009	0.036	0.009	0.001	0.003	0.005	0.015
H	1	0	0	0	1	0	0	0	0	0	1	1	0	0
Residue Composition														
Comp. B (p.u.)	0.007	0.007	0.007	0.004	0.006	0.008	0.007	0.005	0.007	0.004	0.003	0.004	0.008	0.008
Comp. BN (p.u.)	0.018	0.012	0.006	0.003	0.175	0.010	0.010	0.005	0.003	0.005	0.003	0.002	0.003	0.005
H	1	0	0	0	1	0	0	0	0	0	0	0	0	0

Lastly, the data collected was used to model the binormal distribution of various species as well as the overall biogas production. This modelling was performed separately for the samples with and without nanoparticles. The only models with significant results, with no structured residuals and an acceptable R2 were those for biogas production, which are presented below. In both cases an exponential decay function was obtained using linear regression models.

The model for biogas without nanoparticles is shown below in equation 23.

$$n_{biogas}\ [Nm^3 / Nm^3_{sludge}] = N(\exp(1{,}425 - 0{,}192 * t),\ 1.2177 - 0{,}012 * t^2 + 0{,}001 * t^3) \quad (23)$$

In addition, the residual model parameters and the residual analysis are shown in **Figure 21**, the residuals in **Figure 22** and the distribution in **Figure 23**.

```
Number of observations: 14, Error degrees of freedom: 12
Root Mean Squared Error: 0.579
R-squared: 0.824,  Adjusted R-Squared: 0.809
F-statistic vs. constant model: 56.1, p-value = 7.33e-06
```

Figure 21. B Model Stats. Source: Own.

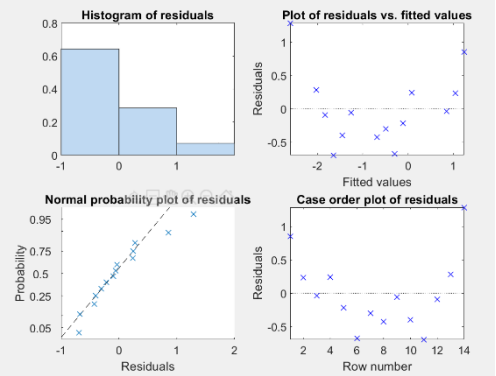

Figure 22. B Model Residual Analysis. Source: Own.

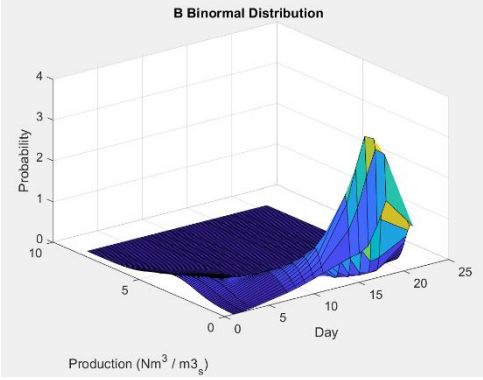

Figure 23. B Model Distribution. Source: Own.

Similarly, the model for biogas with nanoparticles is shown below in equation 24.

$$n_{biogas}\ [Nm^3 / Nm^3_{sludge}] = N(\exp(1{,}426 - 0{,}192 * t),\ 1.2177 - 0{,}012 * t^2 + 0{,}001 * t^3) \quad (24)$$

In addition, the residual model parameters and the residual analysis are shown in **Figure 24**, the residuals in **Figure 25** and the distribution in **Figure 26**.

```
Number of observations: 14, Error degrees of freedom: 12
Root Mean Squared Error: 0.546
R-squared: 0.769,  Adjusted R-Squared: 0.75
F-statistic vs. constant model: 40, p-value = 3.78e-05
```

Figure 24. B NP Model Stats. Source: Own.

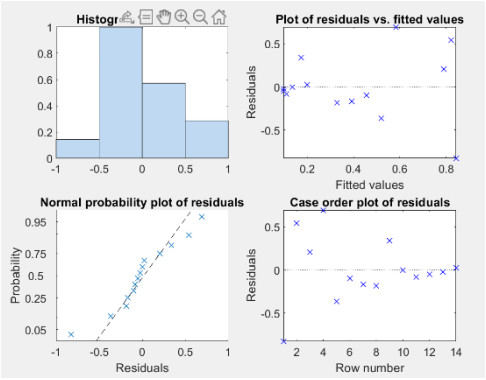

Figure 25. B NP Model Residual Analysis. Source: Own.

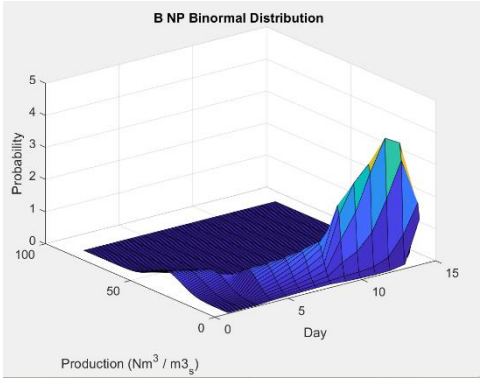

Figure 26. B NP Model Distribution. Source: Own.

5.2. Batch Reactor Results

As previously stated, both reactors presented problems when measuring production after day three of the experiment. Therefore, only the composition could be analysed in this section. It must also be added that, due to the low number of runs, no statistical analysis or modelling could by obtained.

Firstly, the composition of both reactors was determined using the MATLAB code previously mentioned. The results were divided by analysed species in the following order: methane, hydrogen, hydrogen sulphide and residues. These results were presented in **Table 5**, **Table 6**, **Table 7** and **Table 8**, respectively.

It is apparent that the nanoparticles had a positive contribution between days 9 and 14, whose effects were larger than those observed in the bottles, which suggests a magnifying effect caused by the vessel volume.

However, methane production, and therefore methanogenesis, stops by day fifteen, which indicates that the reactor is spent or the inhibition of the reactions. Production measurements would be required for a detailed analysis, although this behaviour is in line with the lower decay rate observed in the BMP bottles.

Moving on to hydrogen sulphide, the larger reaction vessel magnifies the observed effects on the BMP bottles, namely the sudden peak by day eleven before becoming a residual species.

Table 5. Effects of Nanoparticles on Batch Reactor Methane Composition. Source: Own.

Day	1	2	3	7	8	9	10	11	14	15	16	17	18	21
Comp R (p.u.)	0.379	0.821	0.044	0.174	0.727	0.099	0.109	0.571	0.341	0.397	0.432	0.053	0.387	0.508
Comp RN (p.u.)	0.827	0.681	0.734	0.642	0.516	0.616	0.586	0.346	0.620	0.012	0.000	0.000	0.000	0.000
NP Effect (-)	+	-	+	+	-	+	+	-	+	-	-	-	-	-
Specific Effect ($p.u./gr_{NP}$)	3.9976	-1.247	6.157	4.181	-1.886	4.610	4.265	-2.007	2.487	-3.442	-3.856	-0.474	-3.456	-4.537

It is apparent that the nanoparticles had a positive contribution between days 9 and 14, whose effects were larger than those observed in the bottles, which suggests a magnifying effect caused by the vessel volume.

However, methane production, and therefore methanogenesis, stops by day fifteen, which indicates that the reactor is spent or the inhibition of the reactions. Production measurements would be required for a detailed analysis, although this behaviour is in line with the lower decay rate observed in the BMP bottles.

Table 6. Effects of Nanoparticles on Batch Reactor Hydrogen Composition. Source: Own.

Day	1	2	3	7	8	9	10	11	14	15	16	17	18	21
Comp R (p.u.)	0.253	0.042	0.436	0.271	0.065	0.290	0.262	0.112	0.249	0.161	0.171	0.279	0.167	0.168
Comp RN (p.u.)	0.050	0.116	0.074	0.110	0.127	0.100	0.115	0.169	0.092	0.368	0.293	0.280	0.262	0.227
NP Effect (-)	-	+	-	-	+	-	-	+	-	+	+	+	+	+
Specific Effect ($p.u./gr_{NP}$)	-1.81	0.662	-3.231	-1.431	0.560	-1.693	-1.314	0.514	-1.398	1.852	1.095	0.013	0.846	0.529

Pertaining to hydrogen composition, this species behaves inversely to methane, having opposing trends on most days, as shown in **Figure 27**. This persistent inversion suggests the existence of an overall observable and impactful interaction between them. This could be caused by the increased methane production, which causes the population of methane producing bacteria to oscillate.

It must also be pointed out that the hydrogen is positively impacted by the nanoparticles, which drastically increases production during the early stages of the AD process.

Table 7: Effects of Nanoparticles on Batch Reactor Hydrogen Sulphide Composition. Source: Own.

Day	1	2	3	7	8	9	10	11	14	15	16	17	18	21
Comp R (p.u.)	0.000	0.002	0.001	0.000	0.002	0.003	0.000	0.004	0.000	0.005	0.000	0.003	0.002	0.004
Comp RN (p.u.)	0.001	0.000	0.001	0.000	0.003	0.001	0.001	0.000	0.002	0.002	0.003	0.000	0.002	0.002
NP Effect (-)	+	-	-	-	+	-	+	-	+	-	+	-	-	-
Specific Effect (p.u./gr_{NP})	0.010	-0.020	-0.010	0.000	0.014	-0.027	0.005	-0.037	0.017	-0.026	0.029	-0.024	-0.004	-0.024

Moving on to hydrogen sulphide, the larger reaction vessel magnifies the observed effects on the BMP bottles, namely the sudden peak by day eleven before becoming a residual species.

Table 8. Effects of Nanoparticles on Batch Reactor Residue Composition. Source: Own.

Day	1	2	3	7	8	9	10	11	14	15	16	17	18	21
Comp R (p.u.)	0.368	0.135	0.519	0.556	0.206	0.607	0.630	0.313	0.410	0.437	0.398	0.666	0.444	0.320
Comp RN (p.u.)	0.122	0.203	0.192	0.248	0.353	0.284	0.299	0.485	0.287	0.618	0.704	0.720	0.736	0.771
NP Effect (-)	-	+	-	-	+	-	-	+	-	+	+	+	+	+
Specific Effect (p.u./gr_{NP})	-2.202	0.605	-2.915	4.181	-2.750	1.312	-2.890	-2.956	1.529	-1.105	1.616	2.732	0.485	2.614

Lastly, the weight of residues in the biogas composition displayed a similar behaviour as in the BMP bottles by reducing their appearance until day seven. They also present a sudden peak in day eleven which reached 48.5% of the composition, coinciding with the peak in H_2S. As the data varies significantly, the specific effect caused is displayed in **Figure 27** and **Figure 28**.

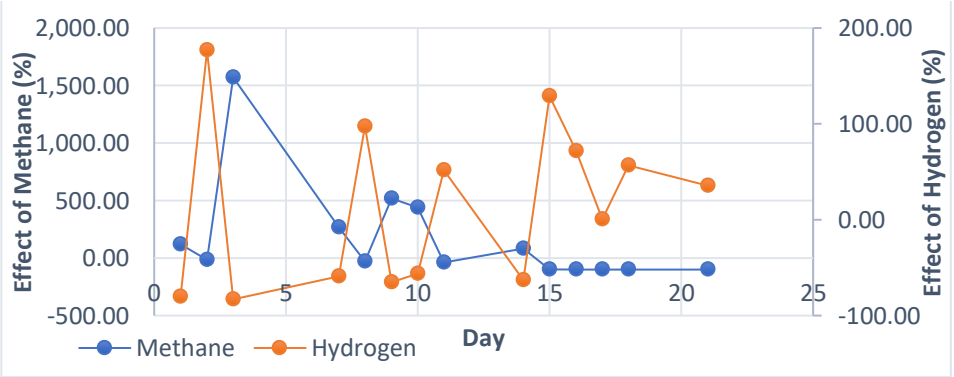

Figure 27. Effect of Nanoparticles on Methane and Hydrogen in Batch Reactor. Source: Own.

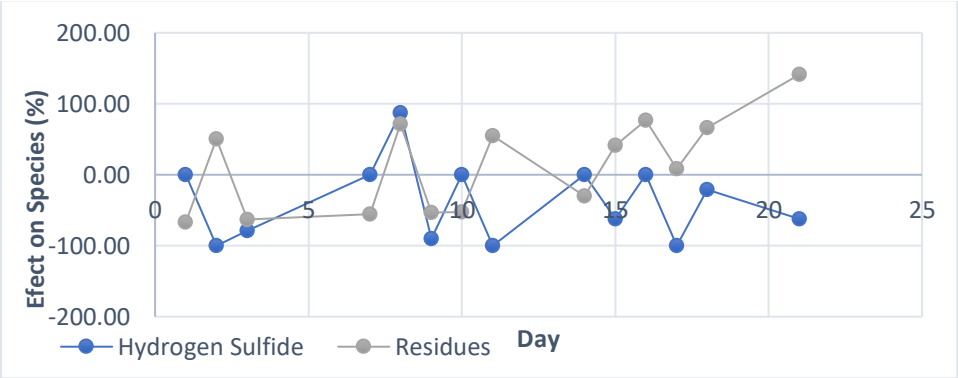

Figure 28: Effect of Nanoparticles on Hydrogen Sulphide and Residues in Batch Reactor. Source: Own.

As previously stated, these figures reinforce the notion of a CH_4-H_2 as well as an increase in reaction speed which suggests that the residence time could be reduced significantly. On the other hand, the sudden peak in H2S and residues suggests that the

5.3. Semi-Continuous Reactor Results

Due to the nature of the reactor, it operated continuously for 42 days, the length of the two sets of runs made. During this time, the reactor operated the first 21 days without nanoparticles, which were added the 22nd day of operation. This dosage included the daily injection as well as the initial dose required.

As a result, the results obtained are not comparable to any of the previously presented, as the continuous bottles failed. Therefore, the only possible analysis is that of the composition, which is shown split in **Table 9** for days 1 to 21 and **Table 10** for days 22 to 42. It must also be pointed out that the missing day in this case is not day four, but day twenty-five, as the bank holiday occurred during the second round of testing.

Table 9. Semi-Continuous Reactor Composition, Days 1-21. Source: Own.

Day	1	2	3	4	7	8	9	10	11	14	15	16	17	18	21
Methane (p.u.)	0.065	0.720	0.847	0.862	0.669	0.726	0.283	0.603	0.746	0.332	0.192	0.335	0.275	0.434	0.360
Hydrogen (p.u.)	0.311	0.075	0.048	0.031	0.106	0.078	0.216	0.110	0.062	0.227	0.254	0.216	0.196	0.137	0.266
Hydrogen Sulphide (p.u.)	0.006	0.002	0.003	0.002	0.002	0.000	0.000	0.002	0.000	0.007	0.005	0.004	0.006	0.005	0.000
Residues (p.u.)	0.617	0.203	0.102	0.105	0.223	0.195	0.501	0.285	0.191	0.435	0.548	0.446	0.523	0.425	0.374

Table 10. Semi-Continuous Reactor Composition, Days 22-42. Source: Own.

Day	22	23	24	28	29	30	31	32	35	36	37	38	39	42
Methane (p.u.)	0.065	0.720	0.847	0.862	0.669	0.726	0.283	0.603	0.746	0.332	0.192	0.335	0.275	0.434
Hydrogen (p.u.)	0.311	0.075	0.048	0.031	0.106	0.078	0.216	0.110	0.062	0.227	0.254	0.216	0.196	0.137
Hydrogen Sulphide (p.u.)	0.006	0.002	0.003	0.002	0.002	0.000	0.000	0.002	0.000	0.007	0.005	0.004	0.006	0.005
Residues (p.u.)	0.617	0.203	0.102	0.105	0.223	0.195	0.501	0.285	0.191	0.435	0.548	0.446	0.523	0.425

When performing a qualitative analysis of the presented data, it is apparent that the weight of methane in the composition varies from the first section of the study (pre day 21) to the second.

Regarding the first 21 days, similar behaviours to that of the BMP bottles can be seen, such as de peak in secondary species found on day nine. However, this peak is mitigated but the semi-continuous nature of the reactor.

This semi continuous nature also contributes to achieve a baseline in hydrogen production throughout the study, due to hydrolysis being the first stage of the AD process.

Focusing on the methane, the addition of the nanoparticles on day twenty-one has a visible impact on methane production, causing an increase from 0.36 p.u. to 0.72 in day twenty-three. Also, the reactor also an interaction between hydrogen and methane, as seen in the other sets of studied results.

Lastly, it can be determined that the hydrogen sulphide is not a significant species when in continuous operation, as it never exceeds a 0,7% weight. However, residues found in the gas samples do increase drastically, being the dominant compound in certain days, stablishing two operating ranges for the four analysed compounds: The higher range in which residues and methane can be found and the lower range which contains the hydrogen and hydrogen sulphide

are found. The cut off for these ranges appears to be around 30%. To facilitate the analysis, the results are also shown below in **Figure 29**.

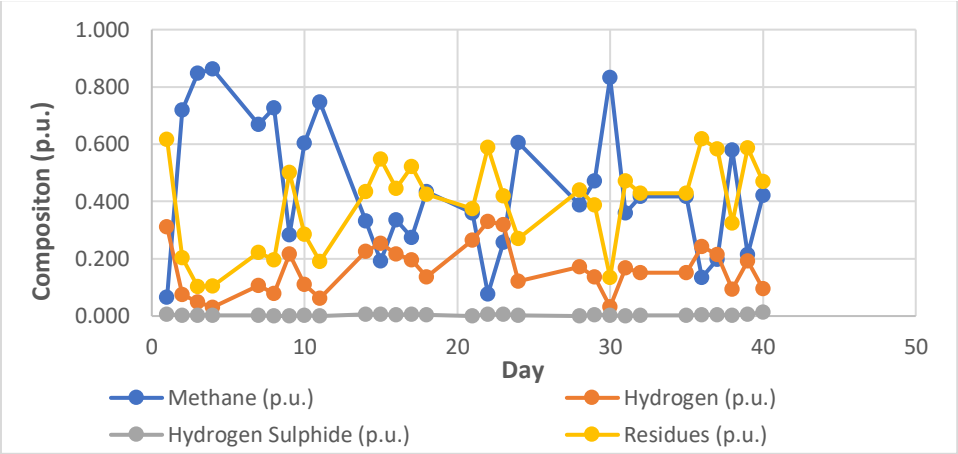

Figure 29. Semi-Continuous Reactor Composition. Source: Own.

7. Conclusions

Once the various experiments, modelling and analysis were completed, conclusions could be made based on the findings of the present study. Due to the varied nature of the experiments performed, as well as the complications of larger reactor tests, this section will focus on the BMP test results.

7.1. BMP Test Conclusions

The first conclusion drawn from the study is that further experimental runs are required to validate the findings of this study. The various BMP tests displayed promising results in biogas production and quality. However, this study could not statistically determine that the C-Fe nanoparticles affect the daily production's mean or variance, except during certain dates and periods regarding the standard deviation of hydrogen.

Despite the lack of statistical conclusions, all BMP samples in which the nanoparticles were used did achieve an increase biogas and biomethane production over the simple co-digestion samples, registering a peak of 23,2928 Nm^3/gr_{NP} and 25,0605 $Nm^3/gr_{NP,}$ respectively.

Another conclusion drawn from the data is the decrease in digestion speed of the sludge-feed mixture, reflected in the lower decay rate of the samples with nanoparticles. As was previously discussed. As a result of this, a new line of research resulting from this project could be the study, design, and operation of anaerobic digestion reactors with a short residence time.

It should also be mentioned that the nanoparticles reduce the percentage of biomethane while increasing that of the hydrogen sulphide and the various residues and unidentified components during the hydrolysis and acidogenesis stages of de AD process. To achieve a more detailed analysis by separating other compounds of significant interest such as CO_2, a new chromatography method should be developed in the future.

Regarding the effects of the nanoparticles on the hydrogen mole concentration, the most significant finding is the CH_4 and H_2 cyclical correlation observed, consistent with previous research in the use of hydrogen as an indicator of the digestion process.

Focusing on the H2S, its weight decreases during the second half of the experiment, from day nine onwards, as evidenced by the 78,92% peak registered during the eighth day, followed by a reduction by three orders of magnitude, becoming a residual compound. It must also be mentioned that this reduction also affects the standard deviation, which was reduced by around 50%. To fully determine the effects of the nanoparticles on the compound, a detailed analysis on the acidogenesis process would be required and result into a new research line pertaining the interactions between the particles with the various microorganisms predominant during each stage of the AD stages.

The residual compounds were positively impacted by the nanoparticles, reducing their appearance both in the composition and overall generation when comparing both sets of bottles. In addition, their sharp increase during the final days of the methanogenesis stage further reinforce the previously drawn conclusion on reaction speed. Finally, the nanoparticles also reduced their variability from day ten onwards, achieving a drop of 83,73% in the daily variance change.

Lastly, the binormal distribution modelling performed on the biogas, methane and hydrogen did provide well fitted models with no apparent residual structure and an acceptable R2 value for certain models. However, a finely tuned model would require further analysis of the data as well as more degrees of freedom, obtained from performing additional BMP experiments to obtain over forty mean data points and thus ensure that the central limit theorem is fully applicable. Moreover, increasing the degrees of freedom will ensure a more robust model and permit the identification of underlying data structures. The use of advanced analysis techniques based on machine learning should also be considered once enough samples have been collected.

7.2. Batch Reactor Conclusions

Firstly, batch reactors showed a similar behaviour to the BMP tests, as the experiment performed on these was identical, barring the volume change resulting from using a 2-litre vessel. Furthermore, the increase in volume magnified the tendencies previously mentioned such as the CH_4 and H_2 interaction as well as the faster reaction speed, displaying a clear decrease in methane generation during the sixteenth day, proposing this as the initial residence time for future research on this.

Another important conclusion obtained from the larger vessels was the need to further improve the set up and sealing techniques, as well as the stirring to prevent the volatilization and deposition of the nanoparticles, particularly on the stirring rod seal. This issue was addressed in a posterior run, which was not part of this project, by applying vacuum grease around the exterior side of the seal, although no clear improvement was made.

Further improvements required would require a new method to measure biogas production once biogas production decreases significantly once the vessels have stabilised after the start-up process, preventing the biogas from having sufficient energy to overcome the head loss resulting from the use of the Ritter milli gasometer. To address this problem, the use of a differential pressure water column has been suggested and its viability will be assessed in a future project.

7.3. Semi-Continuous Reactor Conclusion

Firstly, a new and refined sample feeding methodology is recommended to facilitate this daily procedure. As previously mentioned, it was observed that by preparing the water-pumpkin feed slurry in advance (1 to 2 days), the resulting mash was easier to inject into the bottle and suctioned by the peristaltic pump used for the reactor. Therefore, it is proposed that the feeding be prepared and allowed to soften before use; this will have the added benefit of obtaining a feed which more closely resembles the OFMSW.

The reactor displays a similar behaviour to the previous experiments, which persist through the change in reactor design and behaviours. However, there are certain aspects which differentiate this reactor from the batch experiments, namely the change in residue and methane generation. As this was the first time this type of reactor was used in the University, further research into continuous and semi-continuous reactors is required, which could represent yet another research lead.

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Data Availability Statement: Due to the nature of the research agreement, the data obtained in this project was not published in a public databank.

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Conflicts of Interest: The author declares no conflict of interest.

Appendix A: Sustainable Development Goals Alignment

Sustainability has been typically portrayed as the intersection of three distinct areas: Social; Economic & Environmental. Therefore, a sustainable project must seek to make substantial contributions either directly or indirectly, to all three areas. To achieve it, this Project will be

aligned with Sustainable Development Goals. It must be pointed out that due to the reduced nature of this descriptive memoir, only the three main SDGs tackled will be discussed.

As the Project focuses on the production of biogas to use as an alternative primary resource, the first and most evident SDG is number 13: Climate Action [59]. The main goal of the study is to further develop an existing carbon neutral alternative to natural gas [60]to be used in electricity or heat generation or in industrial processes which require methane or hydrogen, which can be obtained through reforming [61], which can further contribute to this Goal through the use of carbon-capture technology [62]. Specific applications of the biogas produced pertaining SDG 13 would focus on the substitution of natural gas in energy generation & in industrial applications, which represent 38% & 33% of US demand, respectively [63]. This applications will directly contribute towards Target 13.2, which focuses on climate change measures, which includes the energy generation applications of this research as previously stated. Similarly, if applied in developing countries & funded through the UN's Climate Change Framework, it will permit its inclusion in Target 13.a to reduce the impact of developing economies, promoting economic growth without damaging the planet.

The proposed study will also contribute to the fulfilment of SDG 9: Industry, Innovation, and Infrastructure [64] through the applications of the research as anaerobic digestion is used for sludge treatment in large water treatment plants. Retrofitting said plants with reactors and upgrading existing ones to incorporate the feed mechanisms, will enable industries to generate their own biogas to be used in house or have said gas upgraded through various methods and injected into the natural supply network as biomethane or green hydrogen, depending on future developments, directly contributing towards Target 9.4. Furthermore, biogas generation can be easily implemented in wastewater treatment plants, which can be used to create isolated electrical power systems in isolated regions with water treatment facilities around which small scale industrial businesses could originate. This will directly contribute to the fulfilment of Target 9.2, which focuses on promoting and creating an inclusive and sustainable industrialization. It must also be pointed out that the very nature of this project can be included within Target 9.5 as this is a research project aimed at developing new technologies.

Lastly, this research project is also aligned with SDG 11: Sustainable Cities and Communities [65]. This goal focuses on improving cities by making them more sustainable. This is of special importance in developing countries, whose economies are based on agriculture to achieve economic growth [66]. The increase in agriculture results in the increase of water, air & soil pollution if residues are not correctly managed. As previously explained, anaerobic digestion processes can operate with a variety of organic waste, utilizing residues to reduce waste, utilizing residues to produce both energy & fertilizer through the digestate, contributing towards Target 11.6 to reduce the environmental impact of cities. This will also improve the transition towards a circular economy. In addition, the use of this technology will also facilitate developing reliable infrastructure by minimizing and simplifying operating requirements in treatment plants. This will improve the implementation of Target 11.4 by helping safeguard the world's natural heritage through the reduction in resource intensity.

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