Università
Ca'Foscari
Venezia

Master's Degree Course in Environmental Sciences

DM 270/2004

Thesis

Anaerobic fermentation of cheese by-product and sewage sludge for the volatile fatty acids (VFA) production and hydrogen accumulation

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Academic year

2023/2024

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Abstract

The pursuit of sustainable waste management and renewable energy solutions has fueled an exploration of anaerobic fermentation as a pivotal process. In this study, mesophilic and thermophilic batch experiments were conducted to unravel the dynamics of anaerobic fermentation using cheese by-product (CbP) and sewage sludge (SS) substrates. Also, this study presents a comprehensive exploration of anaerobic fermentation dynamics in thermophilic condition through a reactor experiment, so that in this experiment was employed SS and SS+Zeolite as substrates. The introduction of Zeolite (Z) into each sample was examined for its impact on the function of the samples like increasing amount of volatile fatty acid (VFA) accumulation. Remarkably, the mesophilic experiments showcased superior functionality of CbP over SS, with Z augmentation yielding positive outcomes. Additionally, the co-fermentation of SS and CbP proved synergistic, establishing substrate composition as a critical determinant of efficiency. Transitioning to thermophilic conditions, similar trends were observed, albeit with amplified results. The heightened metabolic activity under elevated temperatures led to increased VFA quantities. These findings underscore the potential for enhanced anaerobic digestion in both temperature ranges, reaffirming the role of substrate composition and temperature in optimizing this eco-friendly waste-to-energy process.

1. Introduction

The increasing global population and the corresponding rise in urbanization have led to a significant increase in the generation of organic waste and sewage sludge (SS). These waste streams, if not effectively managed, pose environmental challenges and health risks. In recent years, there has been growing interest in exploring sustainable and environmentally friendly methods to treat and utilize these waste materials. (Jenice et al., 2020).

The sustainable growth is the main aspect of the ambitious plan adopted by the European Union (EU) 2030 strategy regarding the "Circular Economy" methods and their application. This plan allows take measures for the improvement of the life cycle of products, with benefits for environment, economy and society. Hence, in this context, the research lines need to adopt those approaches which foreseen a real conservative use of resources. Among others, carbon and nutrients recovery from organic wastes is still an actual and pivotal concept. In particular, the organic waste produced within an urban scenario (at the best case fully separated or source sorted collected) constitutes a valuable resource (Rodríguez-Antón et al., 2022). Its biodegradability is the main characteristic which allows to apply anaerobic processes in order to recover platform chemicals and/or bioenergy. The anaerobic digestion (AD) is one of the most widespread and well-known technologies in Europe and, in the last decade, it has been adapted for the treatment of a combination of different organic wastes in view of eco-design process for a better resources' utilization (Valentino et al., 2021). The consideration of a treatment plant as a real production process within a context of an integrated cycle is the background idea for the application of the circular economy concept(Gottardo et al., 2017) . For this purpose, the actual facilities need to be newly designed or integrated in order to develop a biorefinery platform, leading to several well-defined economic and environmental advantages deriving from: a) a differentiation of the obtainable products and, b) the possibility to adapt/use the existing facilities (Moretto et al., 2020).

In the perspective of the reduction of the greenhouse gas emissions, the generation of clean energy in a sustainable way represents a priority, in combination with a conscientious utilization of the bioresources (Rana et al., 2020). As one of the most established technologies at industrial scale, the anaerobic digestion (AD) allows to obtain energy, and in some cases fertilizers from organic waste feedstock (Vasco-Correa et.al 2018). Volatile fatty acids (VFA), the secondary metabolite of microbial fermentation, are used in a wide range of industries for production of commercially valuable chemicals (Bhatia et al., 2017). VFAs are water-soluble fatty acids frequently observed in nature, which can be distilled at atmospheric pressure (Aghapour et al., 2020). VFAs have also been referred to by other terms such as short-chain fatty acids, volatile organic acids, and low-weight carboxylic acids (Vijay et al., 2022). Examples of VFAs include acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and caproic acid which contain between 2 and 6 carbon atoms. These

VFAs have found application as platform chemicals in diverse industrial processes such as those in the manufacture of other chemicals, pharmaceuticals, food and agricultural products (Wainaina et al., 2019). Among the aforesaid VFAs, the market size for acetic acid is estimated to be the largest at 350,000 tons/year, followed by propionic acid at 180,000 tons/year (Jankowska et al., 2017). However, the average market price is highest for valeric acid at 4.63 USD/kg, followed by isobutyric acid at 2.75 USD/kg and butyric acid at 2.55 USD $kg{-}1$ (Ramos-Suarez et al., 2021).

An important source of VFAs is the leachate produced during the degradation of organic fraction of municipal solid waste (OFMSW) from landfills, where their concentration can reach up to 5000 mg/L (Siedlecka et al., 2008). The occurrence of VFAs in SS is attributed to other anthropogenic activities like rearing of pigs and production of food (food industry) (Worwag and Kwarciak- Kozłowska, 2019).

The tannery sector merits also remarkable consideration, as Italy is one of the leading countries in this industry, with a value of production of EUR 3.5 billion in 2020. In 2020 the production of finished leather products in Italy, was around 97 million square meters; also, according to the Italian tannery industry sustainability report of 2020, Italian tanneries generate an average of 1.65 kg of waste per square meter of leather produced, with 20.8% of it being SS. (UNIC Italian Tanneries., 2020).

In the pursuit of sustainable and renewable energy sources, anaerobic fermentation has emerged as a promising technology for the production of valuable compounds, particularly volatile fatty acids (VFAs) and hydrogen gas. Anaerobic fermentation, a biological process that occurs in the absence of oxygen, utilizes various organic feedstocks to generate energy-rich products through the activity of anaerobic microorganisms. VFAs are important chemicals that find applications in many industries like chemical, textile, food, and pharmaceutical. They can be chemically converted to form esters or polymerized to produce plastics and polymers like polyvinyl acetate or cellulose acetate propionate. Another use is in the biological conversion for the generation of medium-chain fatty acids, fertilizers, or biopolymers like polyhydroxyalkanoates (PHA) (Khardenavis et al., 2005; Khardenavis et al., 2009). Production of electricity via fuel cells and bioenergy such as hydrogen and biogas from VFAs is another option possible due to biological conversion (Ramos-Suarez et al.2021). Moreover, VFAs can be used as substrate for biological nitrogen removal and phosphorus removal from wastewater.(Vijay Sodhi et al., 2021).

The growing demand for VFAs is met mainly through chemical synthesis that relies on petroleum. These methods include the following; (i) ethylene oxidation, methanol carbonylation for acetic acid production, (ii) oxidation of propionaldehyde, ethylene hydrocarboxylation for propionic acid synthesis, (iii) butyraldehyde oxidation for butyric acid production (Zacharof and Lovitt, 2013). Nonetheless, because of issues related to the availability and cost of worldwide oil resources in addition to growing concern about their ecological impacts in terms of climate change and pollution, the focus has shifted towards alternative strategies for VFAs generation. A potential alternative is the microbial production which however is beset with shortcomings such as the low process yields and its expensive nature in comparison to the chemical production. A possible explanation could be the lesser attention microbial VFA production has received from the research community. (Aghapour Aktij et al., 2020).

1.1 Anaerobic fermdentation: routes and barriers

Anaerobic fermentation is a complex metabolic process that involves a consortium of microorganisms, including bacteria and archaea. These microorganisms break down complex organic compounds present in various feedstocks, such as agricultural residues, food waste, or wastewater, through a series of biochemical reactions. The primary byproducts of anaerobic fermentation are biogas, which typically consists of methane ($\mathrm{CH_4}$) and carbon dioxide ($\mathrm{CO_2}$). However, the production of VFAs and hydrogen gas can be enhanced by optimizing process parameters and feedstock composition. (Angelidaki et al., 2004).

Several factors influence the efficiency and product distribution during anaerobic fermentation. These include the choice of feedstock, substrate composition, pH, temperature, hydraulic retention time, and the microbial community present in the fermentation system. Each of these parameters can be adjusted and tailored to achieve specific fermentation goals, such as maximizing VFA production or hydrogen gas accumulation.(Wang et al., 2019). The advantages of anaerobic fermentation for VFA production and hydrogen accumulation are manifold. Firstly, it offers a sustainable and environmentally friendly approach to waste management by converting organic materials into valuable products. By utilizing waste streams such as agricultural residues, food waste, or industrial by-products, anaerobic fermentation reduces the burden on landfills and minimizes greenhouse gas emissions. Secondly, the process contributes to the transition to a circular economy by transforming organic waste into useful resources. Finally, the generation of VFAs and hydrogen gas provides opportunities for the production of renewable chemicals and clean energy.(Huang et al., 2021). Research efforts in this field aim to optimize anaerobic fermentation systems for enhanced VFA production and hydrogen gas accumulation. This involves exploring different feedstocks, process configurations, and microbial communities to achieve high yields and specific product profiles. Furthermore, the integration of anaerobic fermentation with other technologies, such as bioelectrochemical systems or microbial electrolysis cells, holds promise for further improving VFA and hydrogen production efficiency.

Anaerobic fermentation represents a promising avenue for the production of VFAs and hydrogen gas. By harnessing the metabolic potential of diverse microorganisms, this process offers a sustainable solution for waste management, resource recovery, and renewable energy production. Continued research and development in this field hold great potential for addressing environmental challenges and fostering a more sustainable future. (LUO et al.,2020)

The below picture (figure.1) shows the route of fermentative process. It is needless to say that our experiments did not include the last methanogenic steps, so the last products were acetic, hydrogen and CO2, as in all the anaerobic fermentation step.

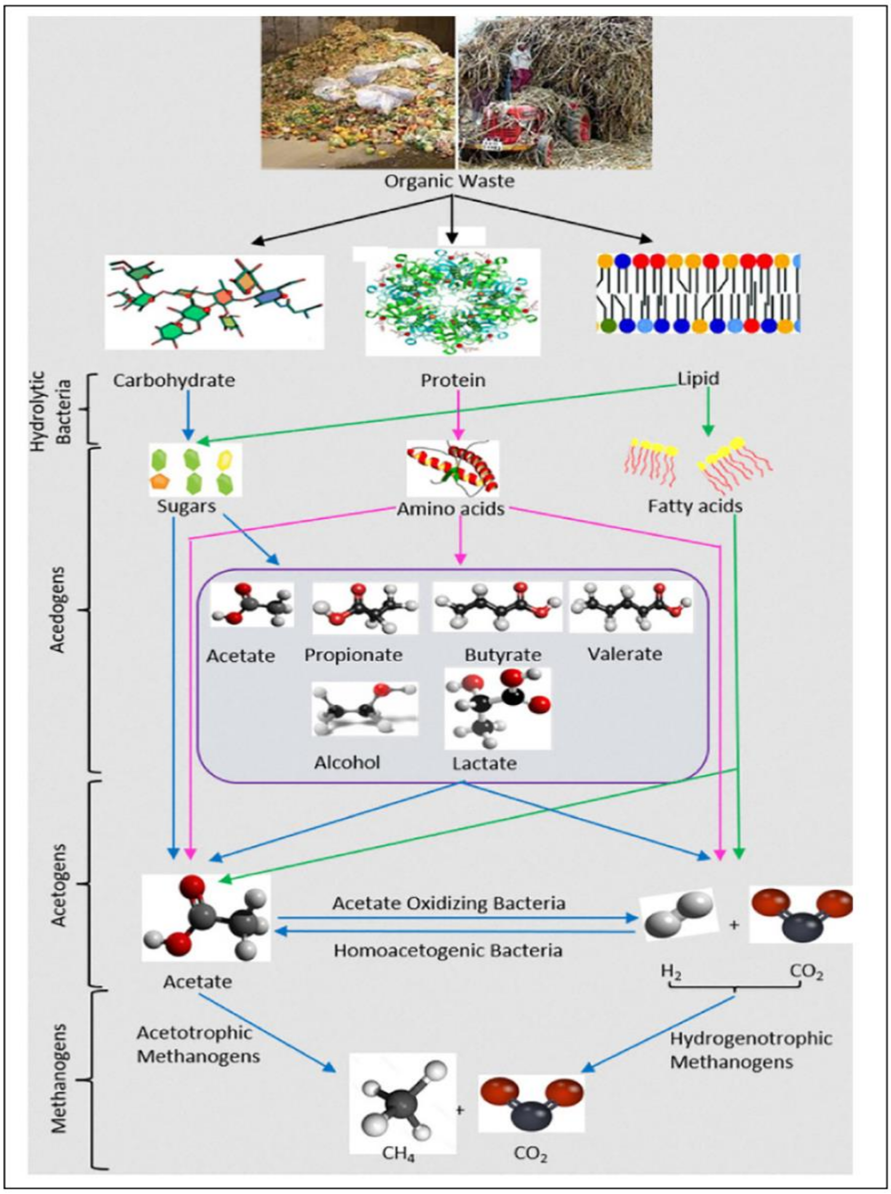

Figure.1. Route of fermentative process (Varghese et al., 2022)

1.1.1 Sewage Sludge (SS)

Anaerobic fermentation of SS has emerged as a promising approach for the production of volatile fatty acids (VFAs) and hydrogen gas, offering a sustainable solution for waste management and renewable energy production (Zhang & Angelidaki, 2015; Li et al., 2017). SS, a by-product of wastewater treatment processes, contains a significant amount of organic matter that can be efficiently utilized through anaerobic fermentation to generate valuable products. Anaerobic fermentation, a biological process that occurs in the absence of oxygen, relies on a diverse microbial community to break down complex organic compounds present in SS. Through various biochemical reactions, these microorganisms convert the organic matter into VFAs and hydrogen gas, which have numerous potential applications in various industries. (Anabela Leitão et al., 2022).

The production of volatile fatty acids (VFAs) from SS fermentation offers several advantages. VFAs, such as acetic acid, propionic acid, and butyric acid, have a wide range of uses, including as chemical precursors, biofuels, and platform chemicals (Li et al., 2017). Hydrogen gas, a clean and versatile energy carrier, can be utilized in fuel cells, power generation, and industrial processes.

The anaerobic fermentation of SS presents an environmentally friendly approach to waste management by converting organic waste into valuable resources. This strategy reduces the reliance on traditional disposal methods and contributes to the circular economy by promoting resource recovery and minimizing waste (Liu et al., 2020). Optimizing the anaerobic fermentation process for SS requires careful consideration of various factors such as temperature, pH, hydraulic retention time, and substrate composition. These parameters influence the microbial community dynamics and metabolic pathways, ultimately affecting the yield and quality of VFAs and hydrogen gas (Zhang et al., 2020).

Research studies have investigated the anaerobic fermentation of SS for VFA production and hydrogen accumulation, providing valuable insights into process optimization, microbial community analysis, and technological advancements (Liu et al., 2020; Zhou et al., 2021). These studies have highlighted the importance of controlling process conditions and substrate characteristics to enhance fermentation performance and product yields.

Thus, anaerobic fermentation of SS for VFA production and hydrogen accumulation offers a sustainable solution for waste management and renewable energy production. The utilization of SS as a feedstock in anaerobic fermentation contributes to resource recovery, reduces waste, and offers opportunities for the production of valuable chemicals and clean energy.

1.1.2. Cheese by-product (CbP)

The anaerobic fermentation of CbP has gained significant attention as a sustainable approach for the production of volatile fatty acids (VFAs) and hydrogen gas, offering a valuable solution for waste valorization and renewable energy generation (Patel et al., 2017; Arslan et al., 2020). CbP, a residual stream from CbP production, contain high organic content and can be effectively utilized through anaerobic fermentation to generate valuable products. The production of volatile fatty acids (VFAs) from CbP fermentation offers several advantages. VFAs, including acetic acid, propionic acid, and butyric acid, have multiple uses, such as in the production of chemicals, biofuels, and platform chemicals (Gadhe et al., 2021). Hydrogen gas, a clean and versatile energy carrier, can be utilized in fuel cells, power generation, and industrial processes.

The anaerobic fermentation of CbP presents an environmentally friendly approach for waste valorization by converting the organic waste into valuable resources. This strategy reduces the reliance on traditional waste disposal methods and contributes to the circular economy by promoting resource recovery and minimizing waste generation (Bharti et al., 2019). Optimizing the anaerobic fermentation process for CbP involves careful control of various factors, such as temperature, pH, substrate concentration, and microbial community composition. These parameters significantly influence the fermentation performance, product yields, and the dynamics of microbial populations involved in the process (Patel et al., 2017; Arslan et al., 2020).

Research studies have investigated the anaerobic fermentation of CbP for VFA production and hydrogen accumulation, providing valuable insights into process optimization, microbial community analysis, and technological advancements (Gadhe et al., 2021; Bharti et al., 2019). These studies have contributed to the understanding of the factors influencing fermentation performance and the potential applications of the produced VFAs and hydrogen gas.

2. Objectives

One of the primary objectives of this thesis is to evaluate the influence of the co-digestion of CbP and SS on the production and composition of volatile fatty acids. The unique composition of CbP and SS can provide a diverse range of substrates, contributing to the production of various volatile fatty acids. Understanding the dynamics of VFA production and their composition is crucial for optimizing the anaerobic fermentation process and maximizing the yield of valuable bioproducts.

Another important aspect of this research is the investigation of hydrogen accumulation during anaerobic fermentation. Hydrogen, a clean and high-energy fuel, holds great potential for various applications, including fuel cells and industrial processes. Exploring the factors influencing hydrogen accumulation and identifying strategies to enhance its production are essential for realizing the full potential of anaerobic fermentation as a sustainable energy generation technology.

Through this research, to address several key research questions it was searched:

- How does the anaerobic fermentation and co-fermentation of CbP and SS affects the production and composition of VFA?
- How can the process parameters be optimized to maximize VFA production?
- Which one has more production of VFA?
- How much hydrogen can be accumulated during anaerobic fermentation of SS?
- How much is the effect of adding Z in the samples?

By investigating these questions, this work is aimed to contribute to the knowledge and understanding of anaerobic fermentation processes and the potential for utilizing CbP and SS as valuable resources for sustainable biogas production, VFA generation, and hydrogen accumulation. The findings of this research have implications for waste management strategies, renewable energy production, and the development of a circular economy. The utilization of CbP and SS in anaerobic fermentation processes can not only help mitigate environmental pollution but also contribute to the production of bioenergy and valuable biochemicals.

In the subsequent sections of this thesis, there is the methodology employed, present and analyze the experimental results, and provide a critical discussion of the findings. The research outcomes will contribute to the scientific knowledge base and inform potential applications and future research directions in the field of anaerobic fermentation and waste valorization.

To achieve these objectives, a comprehensive experimental study was conducted. Various process parameters, such as substrate loading, hydraulic retention time, and temperature, were investigated to optimize VFA production and hydrogen accumulation. Analytical techniques were employed to analyze the composition and quantity of volatile fatty acids. Hydrogen production was measured using appropriate instrumentation and techniques.

By investigating these research questions, this thesis aims to contribute to the knowledge and understanding of anaerobic digestion processes and the potential for utilizing CbP and SS as valuable resources for sustainable biogas production, VFA generation, and hydrogen accumulation.

3. Materials and Methods

The method and materials used in an experiment play a crucial role in ensuring accurate data collection and reliable results. In this study, an experimental investigation was conducted in a laboratory located in Treviso to examine the anaerobic digestion process for the production of volatile fatty acids (VFA) and the analysis of key parameters such as soluble chemical oxygen demand ($\mathrm{COD_{Sol}}$), total solids (TS), volatile solids (VS), and pH. The experiment was carried out under two different temperature conditions: mesophilic and thermophilic, to evaluate the impact of temperature on the fermentation process.

The laboratory-based approach provided a controlled environment, allowing us to manipulate and monitor the experimental conditions effectively. The duration of each experimental condition spanned approximately 2 to 3 weeks, enabling sufficient time for the stabilization and steady-state operation of the anaerobic fermentation process.

The analysis of $SCOD$, VFA, TS, VS, and pH served as crucial indicators to assess the efficiency and performance of the fermentation process. COD_{sol} measurements provided insights into the organic matter content and its biodegradability, while VFA concentrations indicated the production of valuable intermediate products. TS and VS analysis assisted in determining the solid content and organic fraction of the substrates, respectively. Moreover, monitoring the pH allowed for the assessment of the fermentation process stability and the establishment of optimal operating conditions.

Through a comprehensive evaluation of these parameters, it is aimed to gain a deeper understanding of the anaerobic digestion process, its feasibility for volatile fatty acid production, and the influence of temperature conditions on process performance.

By conducting this study, it is contributed to the existing body of knowledge regarding the valorization of organic waste materials and the potential for generating renewable energy sources through anaerobic fermentation. The findings from this research have practical implications for waste management strategies, resource recovery, and the development of sustainable biotechnological processes.

The SS obtained from the wastewater treatment plant in Treviso was subjected to centrifugation to separate the solid and liquid fractions. Centrifugation was performed at a specified speed and duration to separate the liquid (supernatant) and solid (SS cake) fractions. After centrifugation, the SS cake was collected and used as the substrate for the designated batch reactors in the experiment.

According to our experimental methodology, the CbP samples used in this study were sourced from local companies located in the Veneto region. These samples were obtained after undergoing a centrifugation process to separate the solid and liquid fractions of the whey. Centrifugation is a common technique used to remove particulate matter and concentrate the liquid portion, which is rich in organic content. By utilizing centrifuged CbP samples, it was aimed to ensure a more consistent and homogeneous composition, facilitating accurate analysis and evaluation of the fermentation process.

3.1 Substrates and mineral characteristics

The SS came from the static thickener after the biological nutrient removal (BNR) process was applied in the Treviso WWTP water line. As it was mentioned, the CbP used was from local companies located in the Veneto region. Their characteristics are the following:

Parameters	Unit	Value
Total Solids (TS)	g/kg	30
Volatile Solids (VS)	g/kg	21.5
Total Kjeldahl nitrogen (TKN)	g N/kg TS	40
Phosphorous (P)	g P/ kg TS	15
Soluble COD ($\boldsymbol{COD_{sol}}$)	g COD/L	0.15

Table.1. Characteristics of SS

Parameter	Unit	Value
TS	g/kg	16.6%
VS	g/kg	16.2%
Total Kjeldahl nitrogen (TKN)	g N/kg TS	0.67
Soluble COD ($\boldsymbol{COD_{sol}}$)	g COD/L	3.6

Table.2. Characteristics of CbP

The mineral called Cabasite 200 mesh, consisting mostly of Cabasite (about 65%), which is presented as a white/yellowish solid (Figure 2), with a dry content equal to 94.6% and a degree of water retention equal to 37.9%.

Figure.2. Cabasite 200 mesh

The sieving analysis showed that approximately 98% of the mineral material tested was less than 0.15 mm, while the remaining 2% was between 0.15 mm and 0.5 mm (Figure 3).

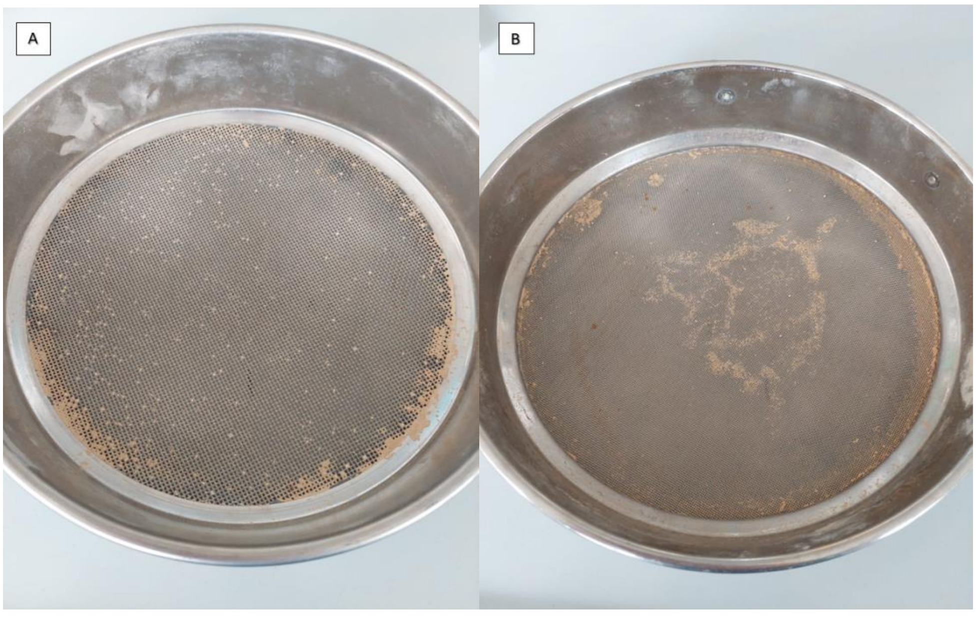

Figure 3. Cabasite 200 mesh retained in the sieve (A mesh 1mm; B mesh 0.5 mm; C mesh 0.15 mm).

The following figure summarizes the results of the grain size analysis performed on Cabasite 200 mesh (Figure 4).

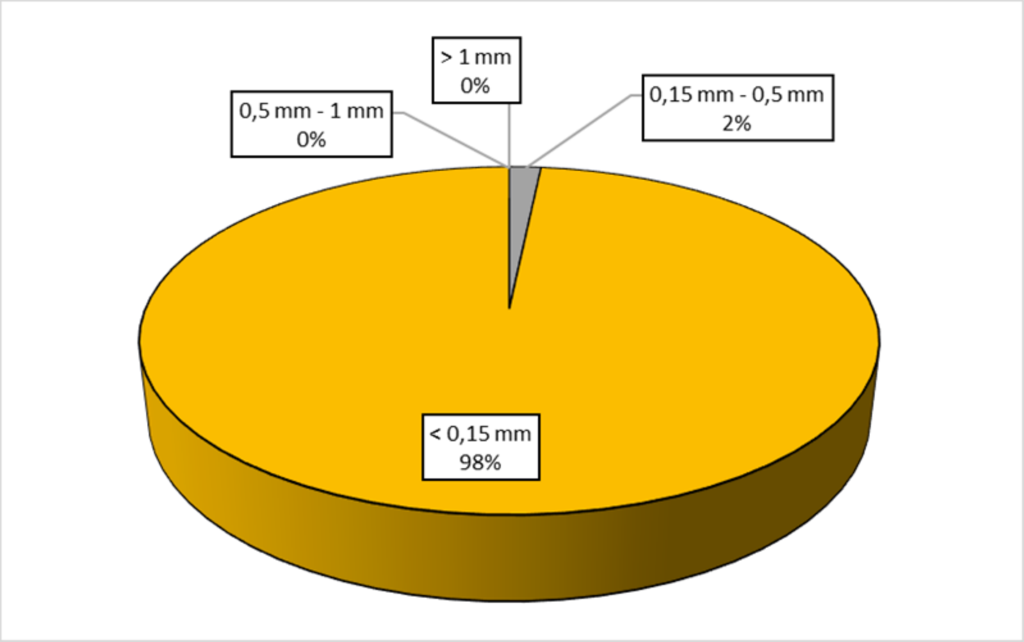

Figure.4. Grain size distribution of Cabasite 200 mesh

3.2 Running experiment: sample preparation

For the samples preparation in batch anaerobic fermentation tests, the following procedure has been followed:

Test A:

- Collect 200 g of humid SS from the full-scale WWTP after centrifugation;
- Dilute the SS with tap water to a total volume of 800 ml. Ensure thorough mixing to achieve homogeneity of 80 VS/L;
- Label this batch as "Sample A: SS."

Test B:

- Prepare a batch with CbP;
- Weigh 200 g of humid CbP and place it into a separate container after centrifugation;
- Dilute the CbP with tap water to a total volume of 800 ml. Ensure thorough mixing to achieve homogeneity of 80 VS/L;
- Label this batch as "Sample B: CbP."

Test C:

- Prepare a batch with SS and Z;
- Weigh 200 g of humid SS with the same previous process and 20 g of Z;
- Mix the SS and Z thoroughly;
- Dilute the SS+Z mixture with tap water to a total volume of 800 ml. Ensure thorough mixing to achieve homogeneity of 80 VS/L;
- Label this batch as "Sample C: SS+Z".

Test D:

- Prepare a batch with CbP and Z;
- Weigh 200 g of humid CbP with the same previous process and 20 g of Z;
- Mix the CbP and Z thoroughly;
- Dilute the CbP+Z mixture with tap water to a total volume of 800 ml to achieve homogeneity of 80 VS/L;
- Label this batch as "Sample D: CbP + Z."

Test E:

- Prepare a batch with a CbP+SS mixture;
- Weigh 130 g of humid CbP and 70 g of SS;
- Mix the CbP and SS thoroughly;
- Dilute the CbP-SS mixture with tap water to a total volume of 800 ml to achieve homogeneity of 80 VS/L;
- Label this batch as "Sample E: CbP+SS."

Test F:

- Prepare a batch with a CbP+SS and Z;
- Weigh 130 g of humid CbP, 70 grams of humid SS, and 20 g of Z;
- Mix the CbP, SS, and Z thoroughly;
- Dilute the CbP-SS-Z with tap water to a total volume of 800 ml to achieve homogeneity of 80 VS/L;
- Label this batch as "Sample G: CbP+SS + Z."

3.2.1 Experimental Procedure

- Transfer each prepared batch into separate bottles with appropriate working volumes in nautilus.
- Seal the containers to create anaerobic conditions.
- Incubate the containers at the desired temperature (e.g., mesophilic or thermophilic conditions) to initiate the anaerobic digestion process.
- Monitor the process parameters, including pH, temperature, $COD_{sol}$and VFA concentrations, at regular intervals as per the experimental design.
- Collect samples from each batch at specific time points for analysis

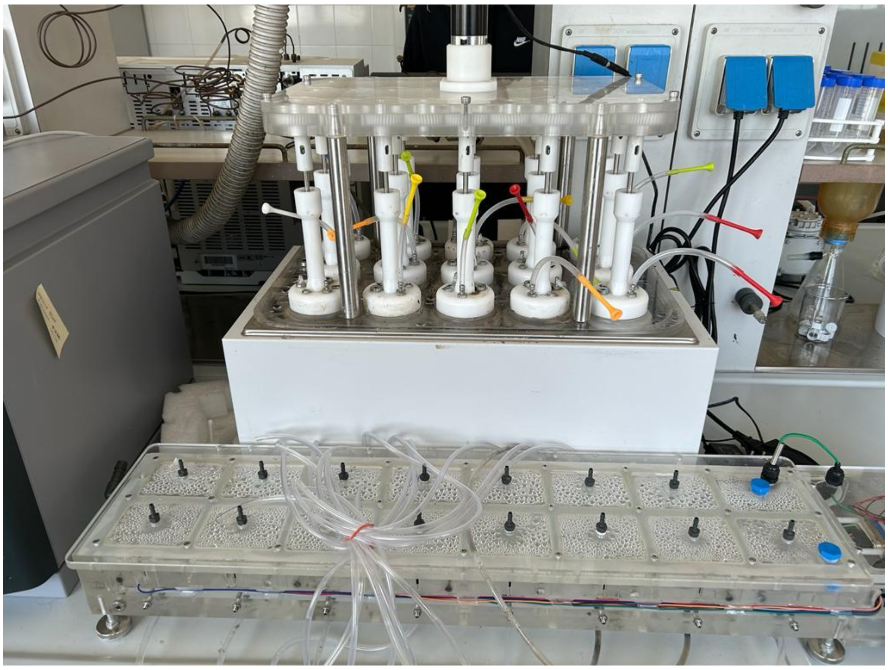

Figure.5. Nautilus device for the anaerobic fermentation tests in batch mode

The experiment was conducted in a laboratory located in Treviso, equipped with anaerobic reactors suitable for batch testing under controlled conditions. The experimental setup consisted of multiple batch reactors, each with a working volume appropriate for the desired substrate loading rates and analysis requirements. The reactor was equipped with temperature control systems to maintain the desired temperature conditions: mesophilic (around 37°C) and thermophilic (around 53°C).

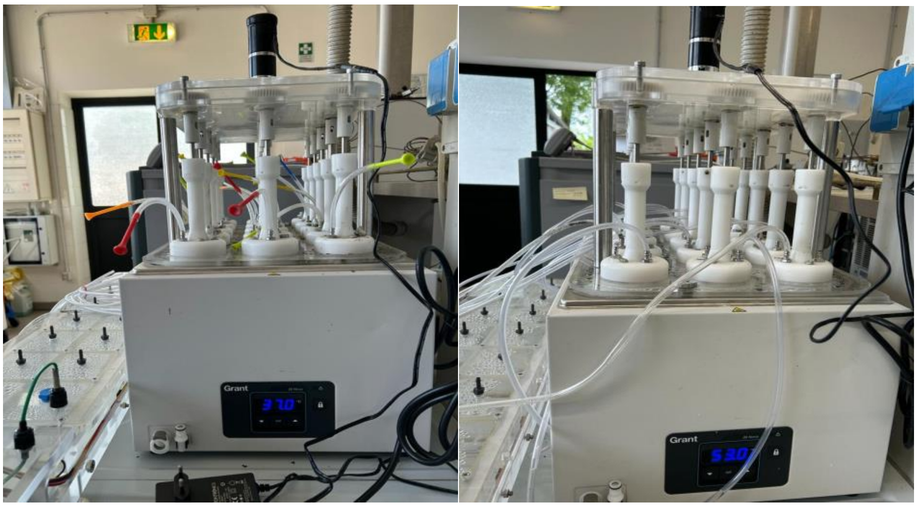

Figure.6. Thermophilic and mesophilic condition

3.2.2 Substrate Preparation

SS was collected from a local wastewater treatment plant. The SS was characterized for parameters such as total solids, volatile solids, and chemical oxygen demand (COD) to provide a baseline understanding of its composition.

CbP, were obtained and analyzed for parameters like pH, total solids, and COD to determine their suitability as a substrate for anaerobic fermentation.

Z, a natural adsorbent material, was obtained, washed, dried, and ground to a suitable particle size for further use in specific batches.

3.2.3 Data Analysis

The collected samples were analyzed for soluble chemical oxygen demand (COD_{sol}), VFA concentrations, total solids (TS), volatile solids (VS), and pH.

COD_{sol} measurements provided insights into the organic matter content and its biodegradability, indicating the substrate's potential for anaerobic fermentation.

VFA concentrations were determined using appropriate analytical method with GC device.

TS and VS analysis helped determine the solid content and organic fraction of the substrates, respectively, contributing to process characterization and efficiency evaluation.

pH measurements were performed using pH meters to monitor the fermentation process stability and optimize operating conditions.

The results were interpreted in the context of the research objectives, highlighting the influence of temperature conditions (mesophilic vs. thermophilic) and substrate composition on the anaerobic fermentation process. The findings were compared to existing literature and previous studies to contribute to the understanding of volatile fatty acid production and the potential.

As a summery in the following table it is summarized the batch experiment that has been conducted.

Code	Batches	Mesophilic/ Thermophilic
A	Test A	SS
B	Test B	CbP
C	Test C	SS + z
D	Test D	Cbp + z
E	Test E	70g ss + 130g cBp
F	Test F	70g ss + 130g cbp + z

Table.3. Batch experiment summary

3.3 Semicontinous stirred tank reactor (sCSTR) at pilot scale

In addition to the laboratory-scale batch testing, a larger-scale experiment was conducted using a 200-liter fermenter to investigate the anaerobic fermentation process of SS. The fermenter was equipped with a handy feeding system, mixing mechanism, temperature control, and gas collection and measurement facilities .Adequate insulation and sealing were ensured to maintain anaerobic conditions throughout the experiment. This experiment aimed to assess biogas production, hydrogen production, and volatile fatty acid (VFA) generation, as well as to analyze parameters such as pH, total solids (TS), volatile solids (VS), and soluble chemical oxygen demand (COD_{sol}). The experiment spanned 50 days with a hydraulic retention time (HRT) of four days, and involved the daily addition of 50 liters of SS to the fermenter. After conducting the month-long experiment using a large-scale reactor, the Z was introduced into the system to observe its impact on the fermentation process. By adding Z to the reactor, it was aimed to investigate its potential role in enhancing the generation of volatile fatty acids (VFA) and other fermentation byproducts.

The large-scale setup allowed for a more realistic representation of anaerobic fermentation under continuous feeding conditions, simulating a scenario closer to practical applications. By monitoring various parameters, the study aimed to gain insights into the performance, efficiency, and stability of the fermentation process at an extended duration and higher volume, enabling a better understanding of the system's potential for biogas and hydrogen production from SS.

Only SS was used as the substrate for this experiment. The SS was sourced from a wastewater treatment plant and characterized for parameters such as TS, VS, and $COD_{sol}$to determine its composition. In the second step of experiment, in day 25, 160 g of Z were added every day, to have a final Z concentration in the sCSTR of 20 g/L, according to the results of Silva et al. (2021), where Z was used to favor the fermentation routes and the hydrogen production under this Z dosage. The experiment operated under a hydraulic retention time (HRT) of four days, which represents the average time a particle of substrate spends inside the fermenter. Every day, 50 liters of SS were added to the fermentator to maintain a continuous feeding schedule. The SS was mixed thoroughly with the existing contents in the fermenter to ensure homogeneity.

3.3.1 sCSTR process monitoring and data analysis

Biogas production: The volume and composition of the biogas generated in the fermenter was measured using GC device.

Hydrogen production: The hydrogen content in the biogas was specifically measured using GC.

VFA analysis: Samples were collected periodically to measure the concentration of VFAs, such as acetic acid, propionic acid, and butyric acid, using GC.

pH measurement: The pH level inside the fermenter was monitored regularly using pH meters.

TS, VS, and $\mathbf{COD_{sol}}$analysis: Periodic sampling was performed to measure the total solids (TS), volatile solids (VS), and soluble chemical oxygen demand ($\mathrm{COD_{sol}}$,) of the SS using standard methods.

The collected data, including, biogas production, hydrogen production, VFA concentrations, pH values, and TS, VS, and COD_{sol}measurements, were compiled and analyzed.

The results were interpreted to evaluate the performance, stability, and efficiency of the anaerobic fermentation process and its potential for biogas and hydrogen production from SS.

By conducting this large-scale experiment, this study aimed to gain valuable insights into the anaerobic fermentation process using SS as the substrate. The monitoring and analysis of various parameters provided a comprehensive understanding of the system's performance, and biogas and hydrogen production potential, contributing to the development of sustainable waste management practices and renewable energy generation from organic waste materials.

3.4 Analytical Methods

Analytical methods for monitoring and characterizing biogas production in a reactor experiment can be critical for understanding the process and assessing its efficiency. Here are analytical methods used in reactor experiments:

Gas Chromatography (GC): GC is often employed to analyze the composition of biogas. It separates the various gases (e.g., methane, carbon dioxide, hydrogen, and trace gases) in the biogas sample, allowing for quantitative measurement of each component. This method was done with GC device in the lab. Also, in liquid sample analytical process, a representative sample from the main samples were carefully collected and then filtered through a 22-micrometer filter to remove any solid impurities. Next, precisely 1 ml of the filtered sample was injected into a Gas Chromatography (GC) device. More precisely, the quantification of the VFA was conducted using AGILENT 6890N gas chromatograph equipped with a flame ionization detector (with T of 200 ◦C), a fused silica capillary column, DB-FFAP (15 m x 0.53 mm × 0.5 μm thickness of the film), and hydrogen was the gas carrier. The chromatographic run was conducted by increasing the temperature from 80 °C to 200 °C at 10 °C/min. The samples were analysed before being centrifuged and then filtered with a 0.20 μm filter.

Regarding the gas analysis, and H_2 in particular, the latter was determined by a gas chromatograph GC Agilent Technology 6890 NTM equipped with a column HP-PLOT MOLESIEVETM (30 m x 0.53 m ID x 25 μm thickness of the film), using a thermal conductivity detector (TCD) at 250 °C. The injector T was 120 °C and a constant pressure of 70 kPa in the injection port was maintained. Samples were taken using a gas-type syringe in 200 μL biogas amounts. The analyses were conducted at a constant T of 40 °C for 8 min, by using Argon as gas carrier.

Figure.7. GC device

VFA analysis: this analysis can be used to analyze the concentration of volatile fatty acids (VFAs) in the reactor effluent. It provides accurate quantification of individual VFAs, which are essential intermediates in anaerobic digestion.

pH Measurement: Monitoring the pH of the reactor contents is crucial because it can affect the microbial activity and the overall efficiency of anaerobic digestion.

Total Solids (TS) and Volatile Solids (VS) Analysis: Determining the TS and VS content in the influent and effluent samples helps assess the degradation of organic matter during anaerobic digestion. TS and VS are typically measured using standard gravimetric methods.

Hydraulic Retention Time (HRT) Calculation: HRT is calculated based on the influent flow rate and reactor volume. It's a critical parameter for assessing the efficiency of anaerobic digestion and can be used to optimize reactor operation.

Soulable Chemical Oxygen Demand (COD_{sol}) Analysis: $COD_{sol}$is a measure of the organic content in a sample. It's often used to assess the extent of organic matter degradation in the reactor.

3.5 Calculations

3.5.1 Total solids

Total Solids In order to determine the solids content of the substrate, the fermented product, and the digestate, the standard technique described in the literature for determining total solids (TS) and volatile solids (VS) was used. The former is measured by weighing the empty crucible first (tare weight), followed by the crucible containing the sample (wet weight); the latter is then dried in an oven for two days before the dry weight is calculated. The following equation gives the content of TS expressed as gTS * Kg-1.

TS= [(W2-W0)/ (W1-W0)]* 1000

3.5.2 Volatile Solids

The VS content is measured by incinerating the same sample that was used to estimate the TS concentration in a muffle furnace for one night (550 ° C). As W3 is the weight in g of the crucible containing the ashes of the sample after ashing in the muffle, the sample content in TVS is derived from the following equation:

TVS= [(W2-W3)/ (W1-W0)]* 1000

Where,

W0 – weight of the empty crucible

W1 – weight of the crucible + wet sample

W2 – weight of the crucible + oven dried sample

W3 – Weight of the crucible + sample dried in the muffle furnace

Figure.8. Oven and Muffle Furance

3.5.3 pH Determination

The pH of digestate, fermentate, or sample was always determined using a pH meter equipped with a magnetic mixing mechanism and capable of determining values in both continuous and steady readings.

Figure.9. pH meter

4. Results

The results obtained from the experimental investigation provide valuable insights into the anaerobic fermentation process, specifically focusing on the production of volatile fatty acids (VFA) and the influence of Z addition. The analysis of various parameters and performance indicators sheds light on the efficiency, stability, and potential applications of the fermentation system.

This section presents the key findings and outcomes derived from the experimental data, highlighting the trends, variations, and significant observations. The results encompass the characterization of VFA production, biogas and hydrogen accumulation, pH dynamics, microbial analysis, and other relevant parameters.

By examining the obtained results, a comprehensive understanding of the impact of different substrates, reactor conditions, and Z addition on the anaerobic fermentation process is achieved. These findings contribute to advancing the knowledge in the field of waste valorization, renewable energy generation, and resource recovery.

4.1 Mesophilic acidogenic fermentation batch tests

The experimental investigation conducted in the laboratory involved operating the anaerobic fermentation process under mesophilic conditions, with a specific temperature of 37 °C. This section presents the accumulated data and observations obtained from the experiment, focusing on the production of volatile fatty acids (VFA).

4.1.1 SS

Figure 10a shows that after about 8 days the production of VFA started to be constant. Inoculum addition was not necessary since the available microbial fermentative consortia was sufficient to have a remarkable VFA production (between 10-12 g COD/L) after 8 days of fermentation. On the contrary, there was a low production of short-chain alcohols (mainly methanol and ethanol) which was less than 1.0g COD/L and lactic acid which was around 1g COD/L. This is due to the type of feedstock; in fact the high protein content of the SS is usually not ideal to achieve high level of lactic acid and alcohols, according to the most known metabolic fermentation routes. The figure 10b shows the evolution of the VFA composition (in terms of COD) from day 0 to day 23. Acetic acid was the most present, with a final content above 40% COD/COD, followed by propionic acid (close to 30% COD/COD). The other VFA and lactic acid were below 10% COD/COD. It is noteworthy that butyric acid decreased from 20% (day 2) to 1% (day 23) in favor of the increase of acetic and propionic acid.

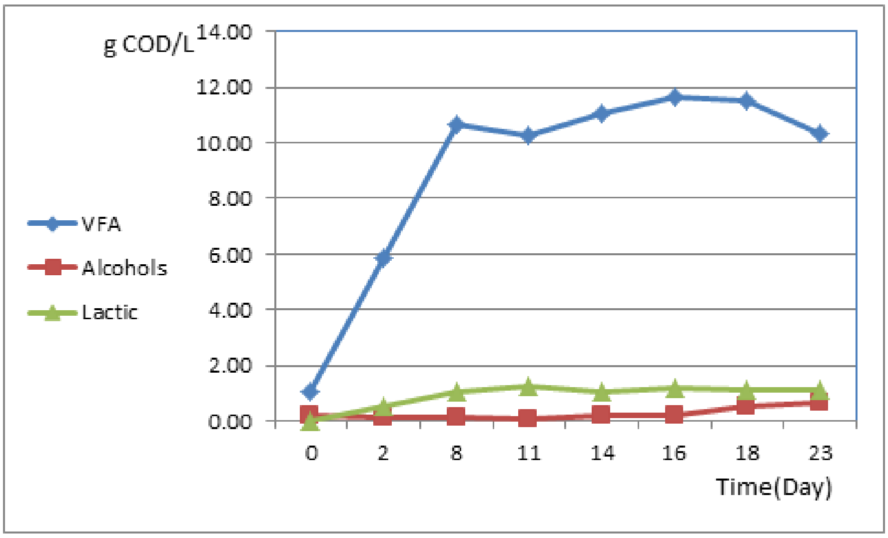

Figure.10a. Trends of VFA, alcohols and lactic acids;

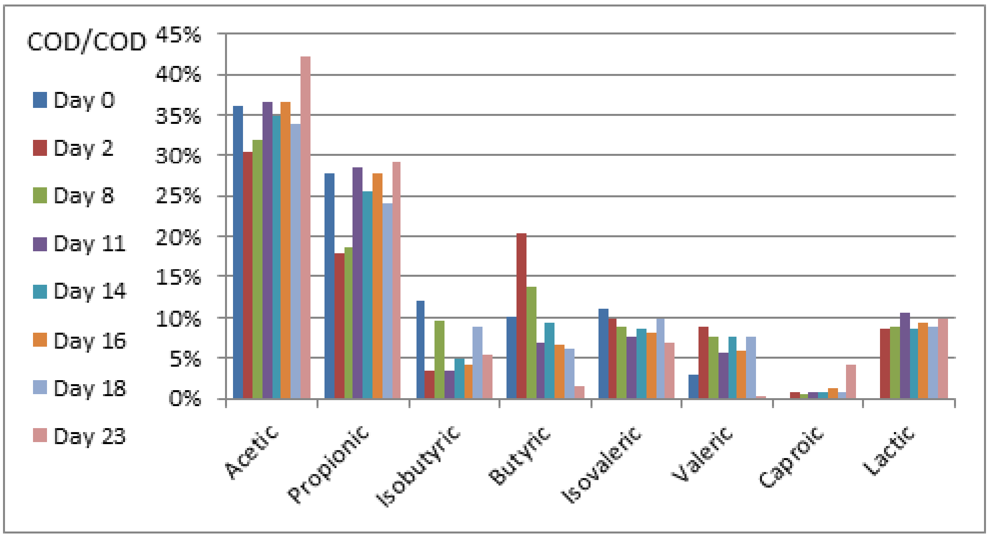

Figure.10b. VFA composition over the whole tests

4.1.2 CbP

The figure 11a shows more amounts of VFA, Lactic and alcohols in comparison with SS fermentation. In this case, the plateau of VFA production was achieved. Also, after about 16 days (roughly) it was equal to 22-23 g COD/L (doubled compared to the SS). High level of butyric acid was obtained and, in particular, the increasing trend of butyric acid (higher than 40% COD/COD at the end of the experiment) was due to the progressive consumption of lactic acid, which decreased from 10 g COD/L (50% COD/COD; day 8) to 2 g COD/L (7% COD/COD; day 23) (figure 11a and 11b).

Here, also, alcohols production was higher than that one observed with SS, but always below 5 g COD/L. The trend VFA-lactic acid observed here can be explained by some information available in the literature. Bella et al. (2022) showed that lactic acid production can be rapidly converted to VFAs by microbes such as Megasphaera, Caproicproducens and Solobacteria. Also, Detman et al. (2019) showed that Mmicrobial communities from dark fermentation bioreactors or pure culture of Clostridium butyricum are able to convert lactate and acetate to butyrate. Moreover, Butyribacterium methylotrophicum and Clostridium diolis are also able to utilize lactate and acetate, converting them to butyrate, CO_2 and H_2. The results of a number of studies indicate that the presence of lactic acids within fermentation substrates can stimulate the production bio-H_2, probably accumulated in our experiments (even though the gas composition was not analyzed).

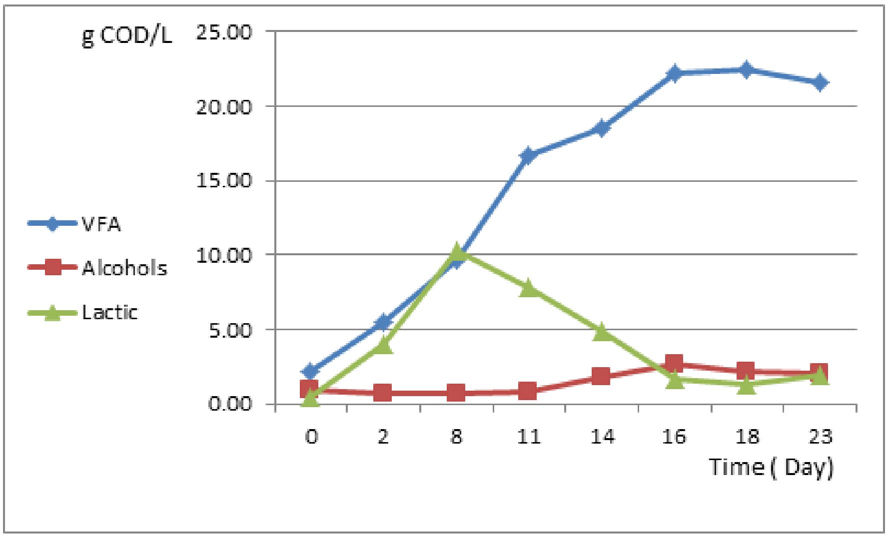

Figure 11a. Trends of VFA, alcohols and lactic acids

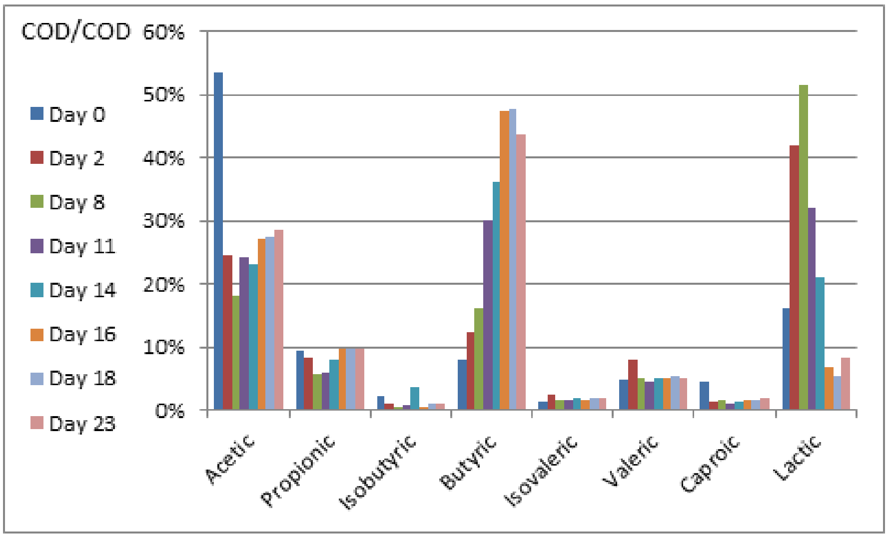

Figure.11b. VFA composition over the whole tests

4.1.3 SS + Z

In figure 12a it can be seen that after 8 days, still there are some fluctuations and production of VFA compared with SS. Also, the amount of VFA was a little more (between 10 to 13 COD/L) after day 8. Lactic acid and alcohols were a little more due to being Z in the substrate so that the average of lactic was a little bit more than 1 COD/L. In figure 12b, the acetic acid was the dominant one in the value of production with around 50% COD/COD. After that propionic which was around 19% COD/COD and the rest were less than 20%. Here, the amount of butyric acid did not decrease which can be due to having Z in the substrate.

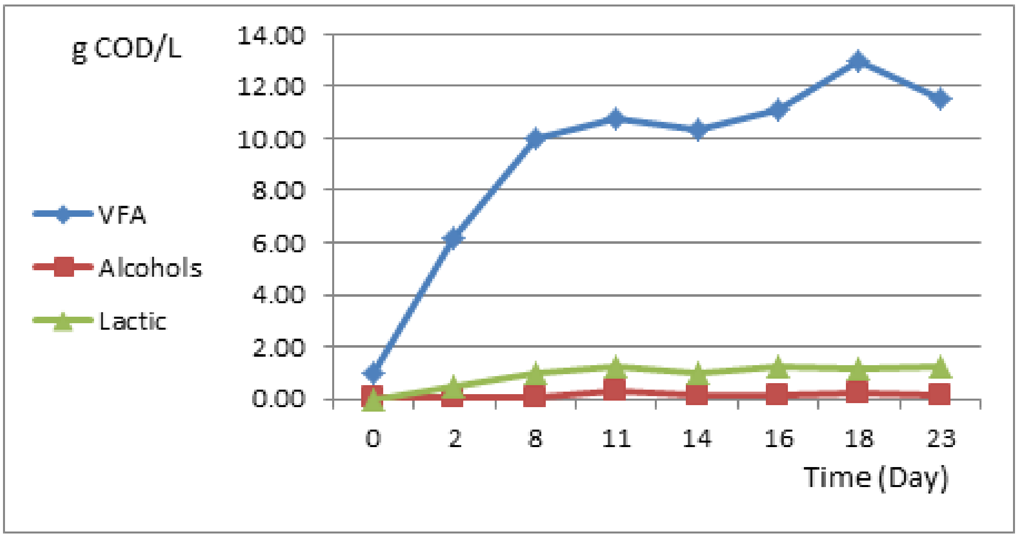

Figure.12a. Trends of VFA, alcohols and lactic acids

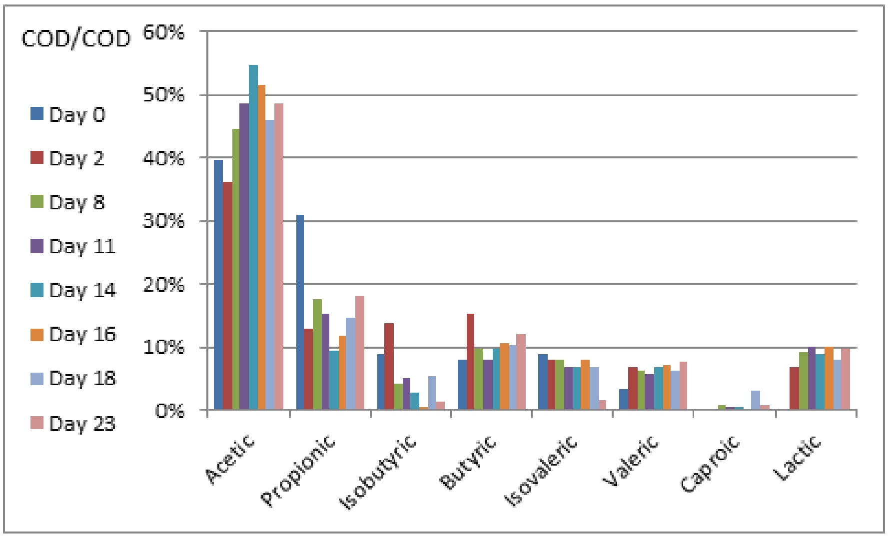

Figure.12b. VFA composition over the whole tests

4.1.4 CbP + Z

Here, it is shown that after 18 days also In this case, the plateau of VFA production was achieved which can be due to being Z in the sample which is resulted in 2 days delay to reach the maximum level and also more amount of VFA. Moreover, it is shown that the amount of VFA (25 COD/L) and lactic (Average of 5 COD/L) were more. Over time there is a decrease of the lactic acid and increasing butyric which can be interpreted like previous condition. Also, there was not much amount of production of alcohol. Thus, the behavior of VFA production in this condition is like CbP, just with higher amounts which is due to being Z. In figure 13b the concentration of acetic acid with the average of 30% COD/COD and then butyric and lactic acids with the average of 20% COD/COD were the most ones.

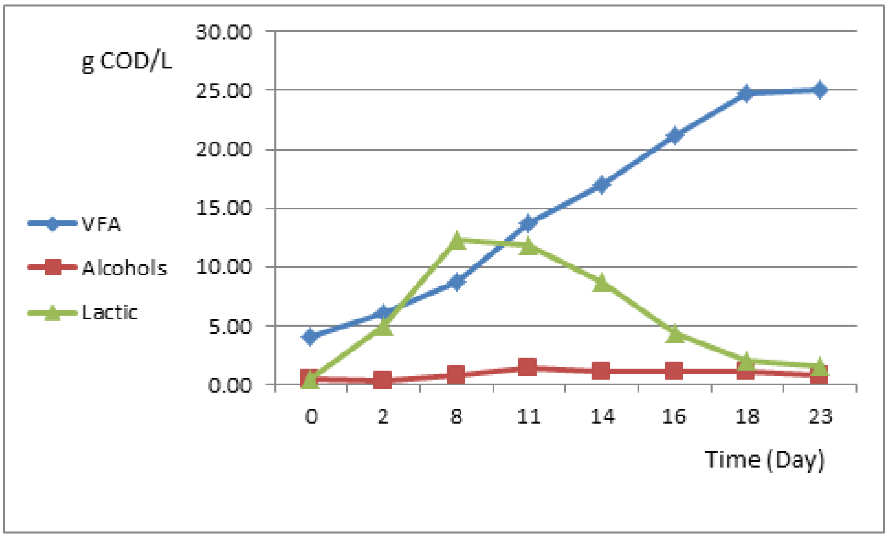

Figure.13a. Trends of VFA, alcohols and lactic acids

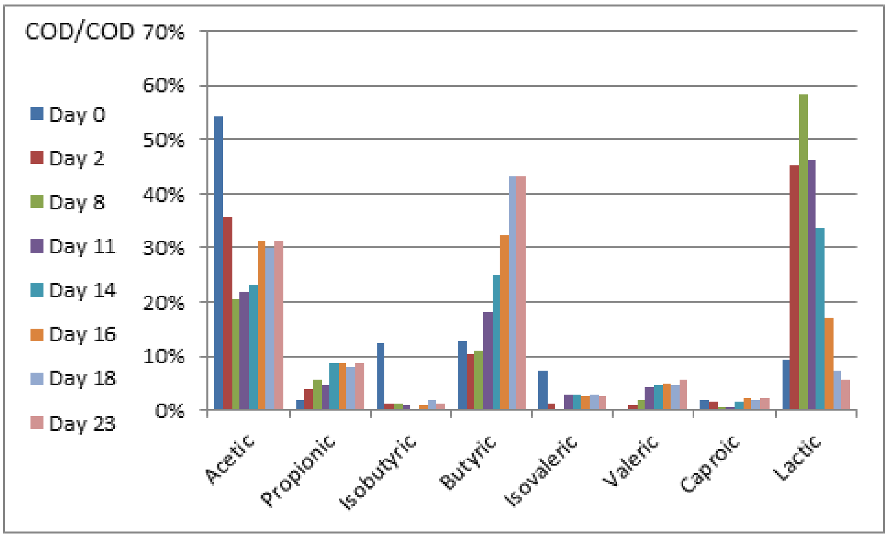

Figure.13b. VFA composition over the whole tests

4.1.5. SS + CbP

In the mixed one (Figure 14a) where it was added 130g CbP and 70g SS, the plateau after 16 days was reached. As it can be seen the value of VFA in co-digestion has been improved which reached around 23g COD/L. Also, again there were consumption of lactic acid and converting to the butyric acid in this sample. Amounts of alcohols are not high and the average amount of lactic acid was around 4g COD/L. In the end of experiment the amount of butyric acid is the highest one with the 30% COD/COD which can be a good result. After that acetic and propionic had high values.

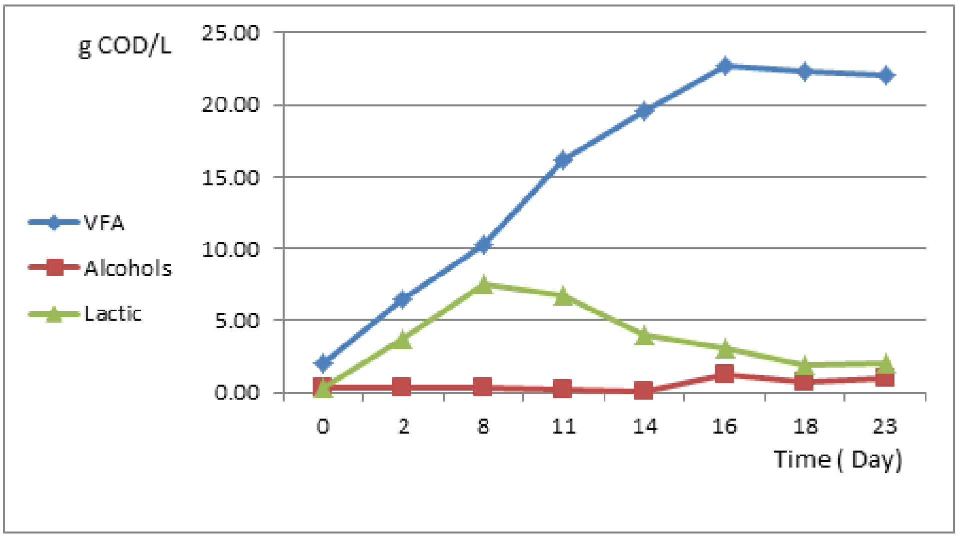

Figure.14a. Trends of VFA, alcohols and lactic acids

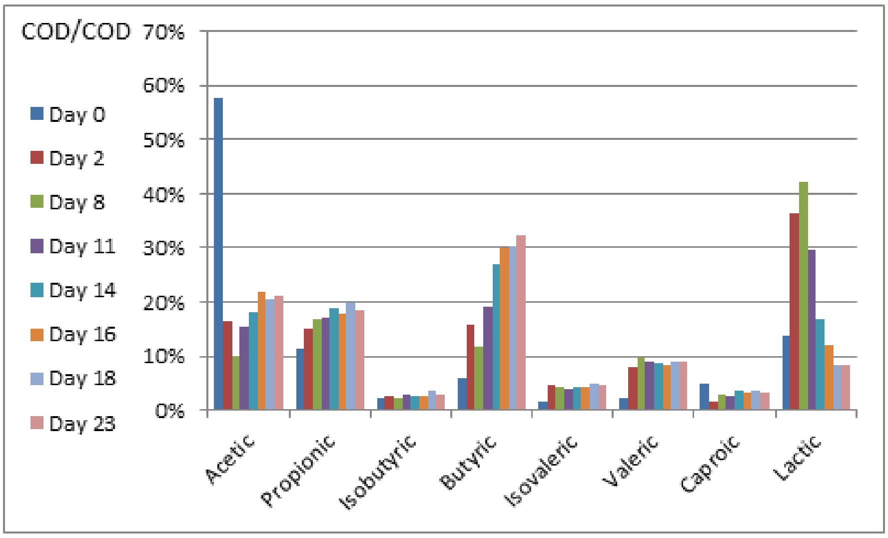

Figure.14b. VFA composition over the whole tests

4.1.6 SS + CbP + Z

After 16 days the production of VFA was stoped but the amount of VFA was more than without Z. Also, the consumption of lactic acid and converting to the butyric acid was like the previous condition. Acetic acid butyric acid and propionic acid were highest values respectively. It can be resulted that adding Z firstly, made the amount of VFA (25g COD/L) and secondly, percentage of the acetic acid higher compared to without Z. In the last day the amount of butyric and acetic were the highest.

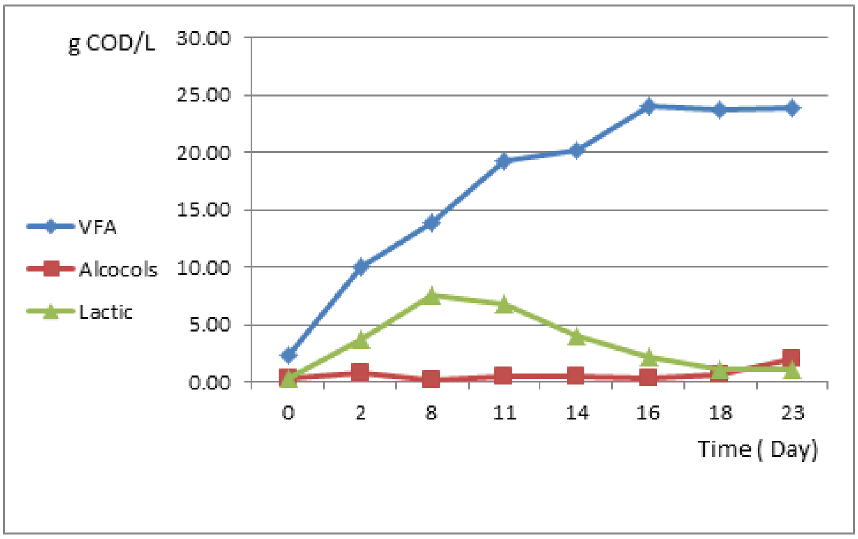

Figure.15a. Trends of VFA, alcohols and lactic acids

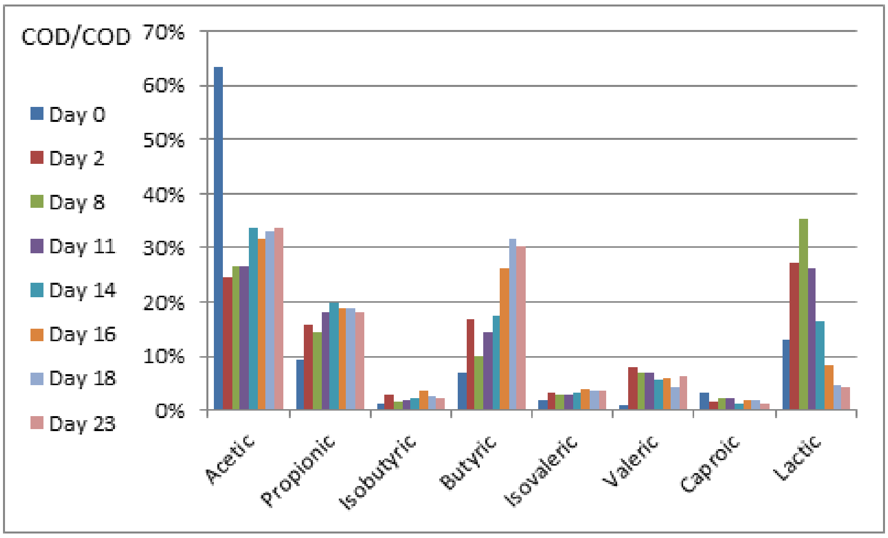

Figure.15b. VFA composition over the whole tests

In total it is resultd that adding Z has the positive effect on producing VFA. Indeed, the first application of Z in aerobic processes was assessed by Carrondo et al. (1980), evaluating the effect of Z on the performance of an activated SS. Lin et al. (2020), investigated the batch anaerobic digestion of swine manure under 60 g/L Z and found that Z increases biogas yield by 20%. All of these results can prove our accurate results.

4.2 Thermophilic condition

In the experimental investigation of anaerobic fermentation, the thermophilic condition played a pivotal role in influencing the performance and outcomes of the digestion process. The thermophilic condition involved maintaining the experimental temperature at elevated levels, typically around 50 to 55 degrees Celsius. This section presents the accumulated data and observations obtained from the experiment conducted under thermophilic conditions, with a focus on the production of volatile fatty acids (VFA) and hydrogen gas.

The thermophilic condition creates an environment that fosters the growth and metabolic activity of a distinct microbial community, well-adapted to higher temperatures. This adaptation potentially accelerates the degradation of organic matter and promotes the generation of biogas, including hydrogen gas and methane. By analyzing the data collected during the experiment, a comprehensive understanding of the impact of thermophilic conditions on SS and CbP waste digestion is achieved.

4.2.1 SS

In figure 16a, it is seen that after 9 days the production of VFA was stoped then a little bit decreased which means the VFAs were being consumed. Also, the amount of alcohol and lactic acid were low (less than 1 COD/L), however there was high amounts of acetic acid and propionic acid which is similar to mesophilic condition but higher values in this condition. Indeed, the advantages of thermophilic condition, such as a larger degree of pathogen deactivation, increased destruction rates of organic solids and higher biogas production yields, have been observed (Ahring et al, 2003). Here also it was observed the higher values than mesophilic condition. Moreover, there is considerable amount of butyric acid (15% COD/COD) which was almost constant until the end of experiment and did not have increasing trend like mesophilic condition.

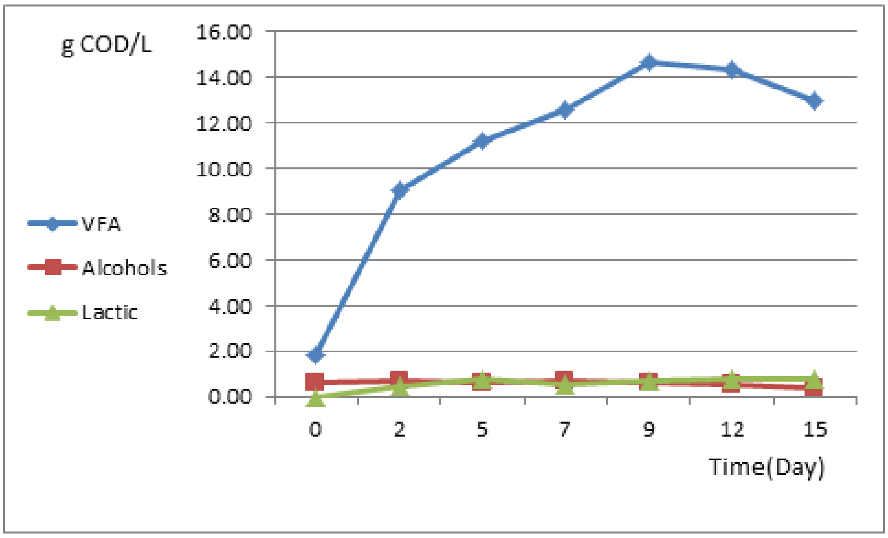

Figure.16a. Trends of VFA, alcohols and lactic acids

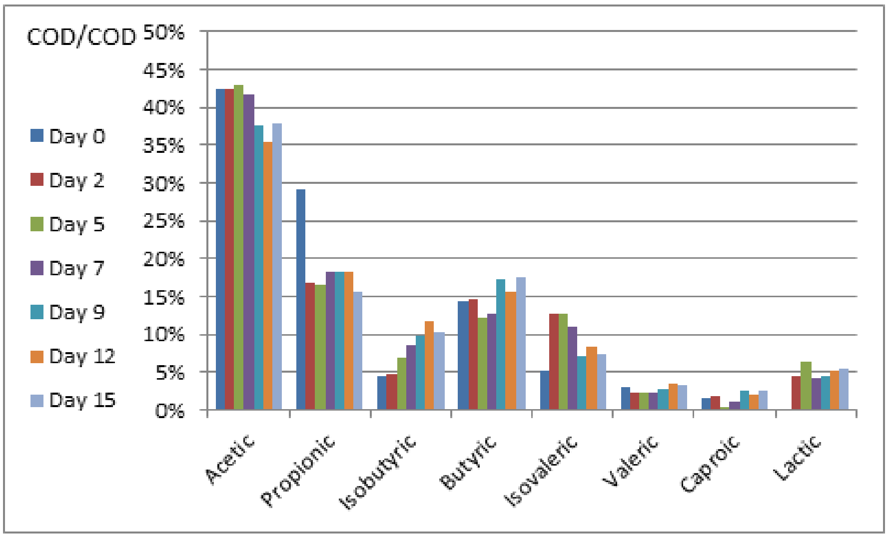

Figure.16b. VFA composition over the whole tests

4.2.2 CbP

In this condition as it was expected, there are more production of VFA, lactic and alcohols due to more temperature in the experiment. Also, in this condition it is reached to the plateau in day 12 which is sooner that of mesophilic condition. Also, it can be seen that again the consumption of lactic acid and converting to the butyric acid. Moreover, in this condition there were more amount of alcohol (around 10g COD/L in average) production and higher amount of VFA which reached to 24g COD/L.

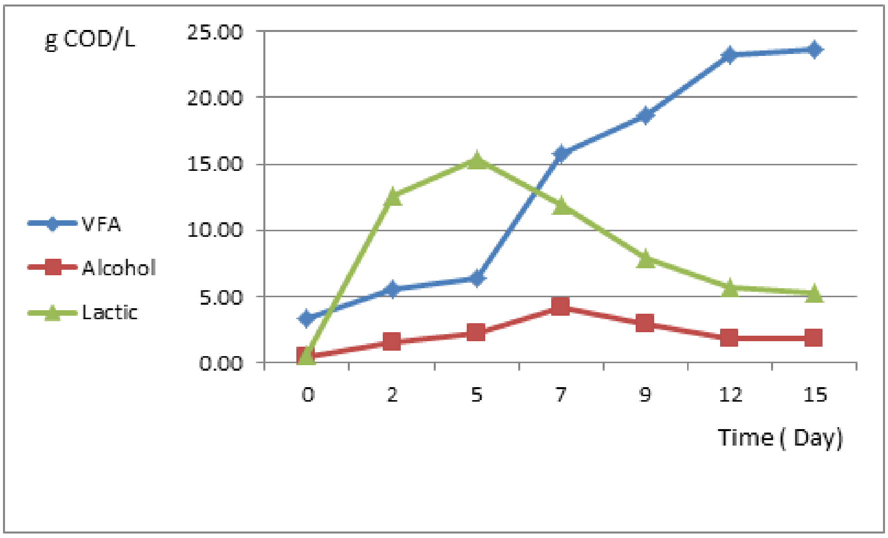

Figure.17a. Trends of VFA, alcohols and lactic acids

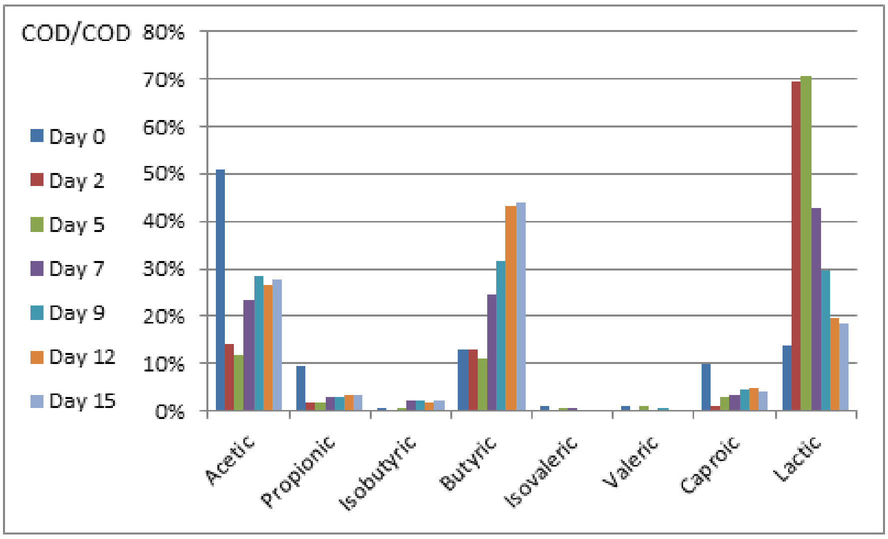

Figure.17b. VFA composition over the whole tests

4.2.3 SS + Z

In this condition by adding Z, the production of VFA reached to a constant level after 7 days. To the compare with SS sample it is reached sooner to the plateau and also the amount of VFA is more than that which is due to adding Z. Also acetic acid had the most amount among other acids which was about 40% COD/COD. After that the propionic acid and butyric acid with 20% had the highest ones. Moreover, the amount of butyric was increasing during the time which was constant in the sample without Z.

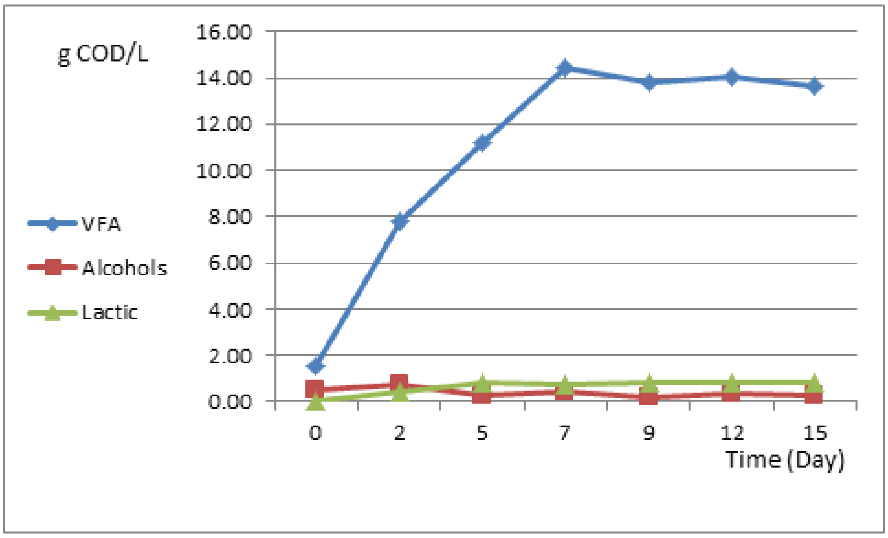

Figure.18a. Trends of VFA, alcohols and lactic acids

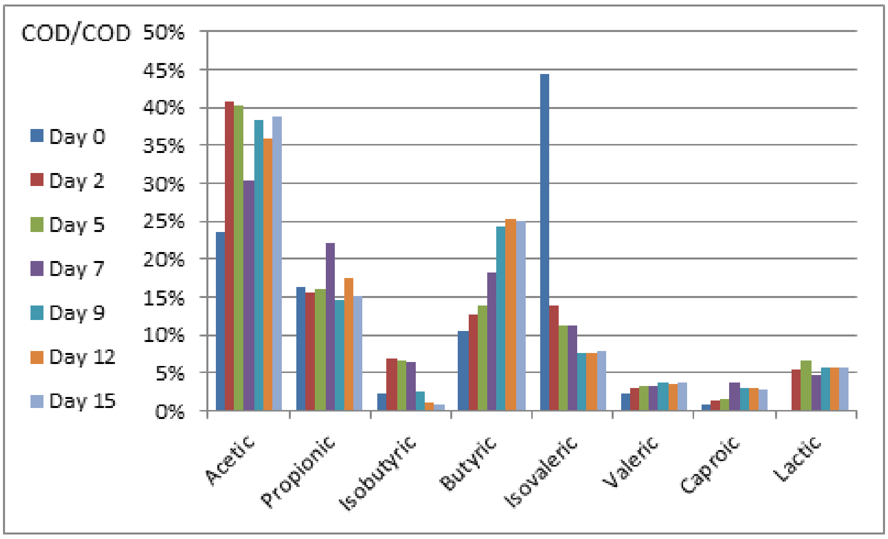

Figure.18b. VFA composition over the whole tests

4.2.4 Cbp + Z

In figure 19a after 15 days the chart did not reach to the plateau which means the production of VFA still I running however in a decreasing trend. After 15 days it is reached to the 31g COD/L amount of VFA which is highest value until now. The amount of alcohol is around 2g COD/L and the average of lactic acid is 7g COD/L. Figure 19b shows high amount of VFA specially in Butyric, acetic and lactic acids. In this condition butyric acid had the highest amount of production which can express good substrate and condition for producing butyric acid. However, it is important to say that there is a little lower amount of alcohol compared with CbP substrate while much higher value of butyric. Again here the amount of lactic acid was decreasing after day 7 and amounts of butyric was increasing in the same day.

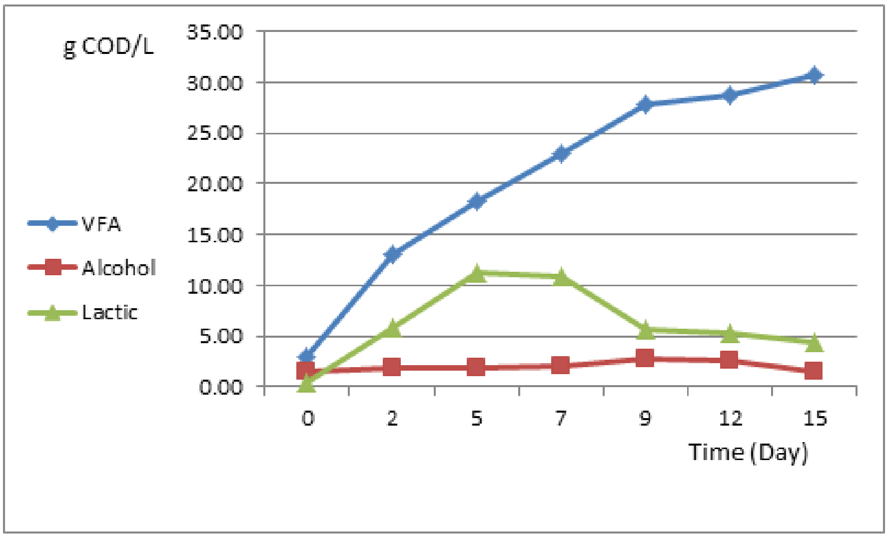

Figure.19a. Trends of VFA, alcohols and lactic acids

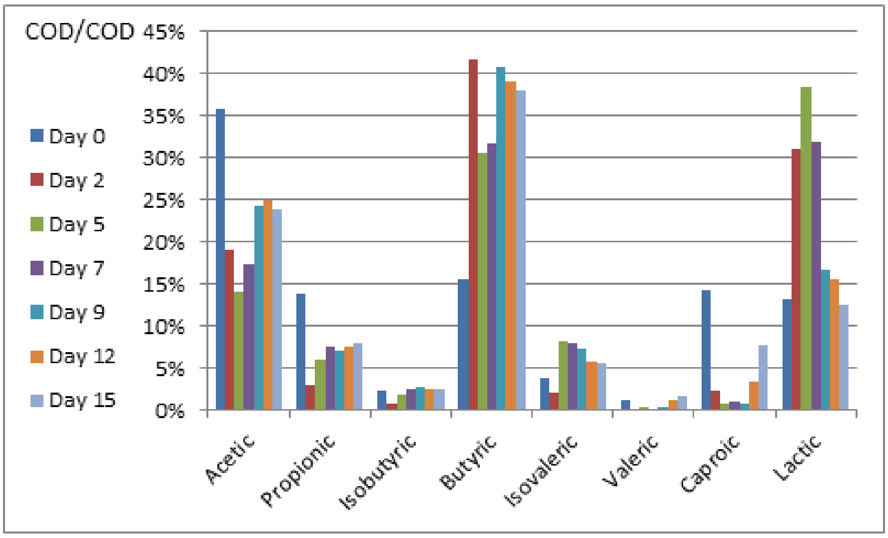

Figure.19b. VFA composition over the whole tests

4.2.5 SS + CbP

After 9 days it reached to the plateau with the amount of 23g COD/L. the amount of alcohol and lactic acid were 2.5g and 4g COD/L respectively. Here, acetic acid had the highest amount and then butyric acid. Iglesias (2020) showed that a decrease of the SS proportion in the co-digestion with CbP waste led to a higher degree of acidification, which this result can confirm our results. The consumption of lactic acid and converting to the butyric acid also is considerable in the figure 20b.

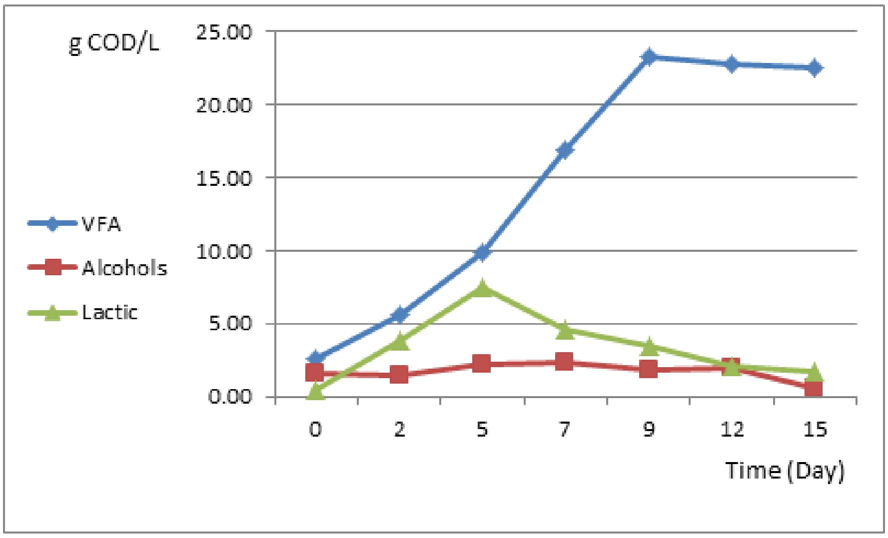

Figure.20a. Trends of VFA, alcohols and lactic acids

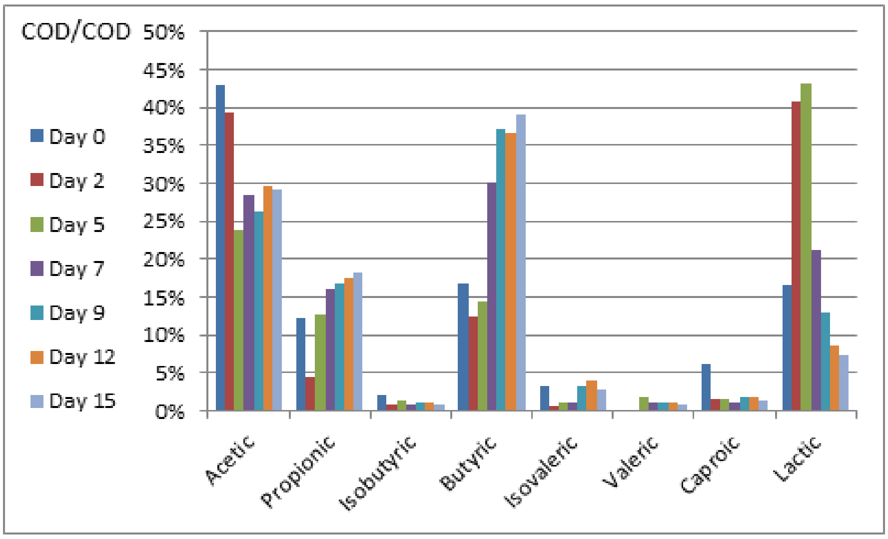

Figure.20b. VFA composition over the whole tests

4.2.6 SS + CbP + Z

In figure 21a after 12 days it is reached to the plateau. It is shown that adding Z made the VFA higher than previous condition. Alcohol and lactic acid had a downward trend during the experiment, and again butyric and acetic acid had the highest amount with 35 and 30% COD/COD respectively.

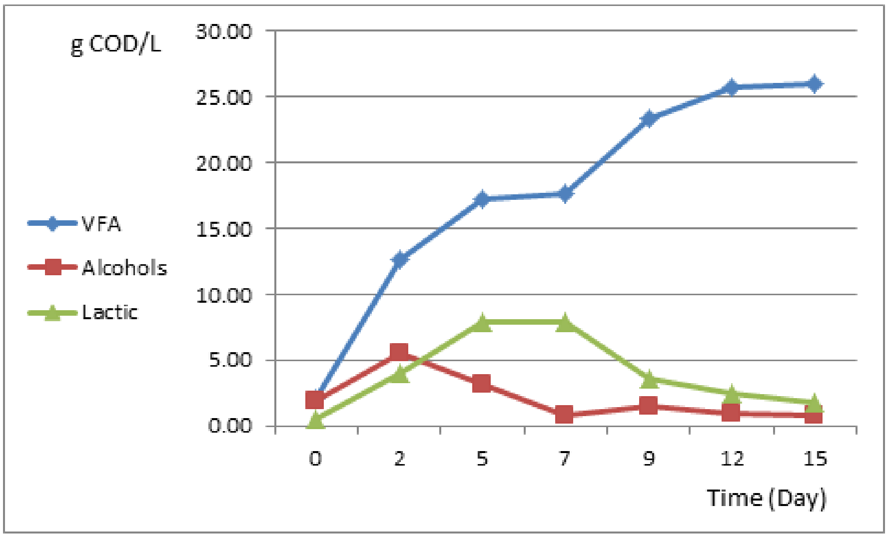

Figure.21a. Trends of VFA, alcohols and lactic acids

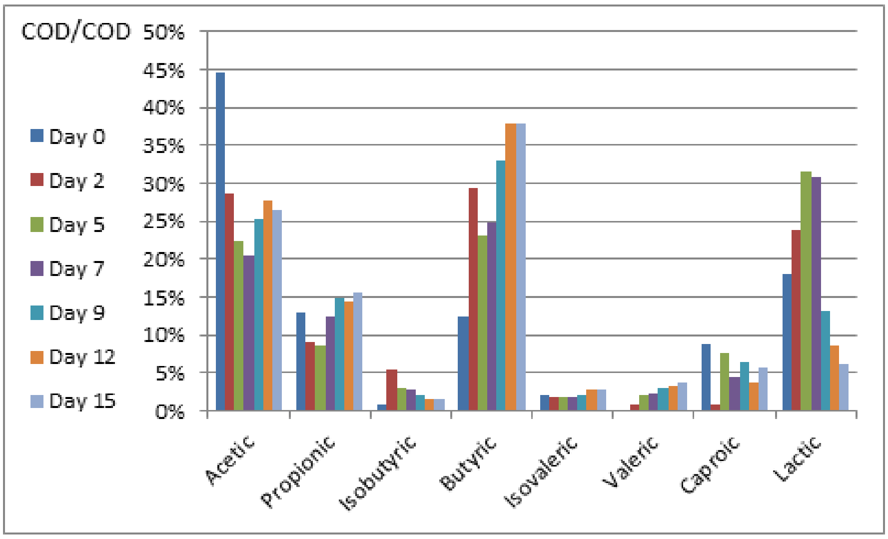

Figure.21b. VFA composition over the whole tests

Overall, similar to mesophilic condition, here by adding Z in this condition there were more amounts of VFA production. CbP with Z had the highest VFA production.

3.3 Summary of results in mesophilic conditions

The maximum values of COD_{sol}, total alcohols, Lactic acid, total VFA, VFA/COD_{sol}, Yield and pH range for each sample during experiment are shown (Table.4). Firstly, it is shown that the values of COD_{sol}, which the maximum one is for Cbp+Z with 44.6 gO_2/L and minimum value is for SS+Z with 23.7 gO_2/L among our samples. Also, it is clear that after fermentation the value of $\mathrm{COD_{sol}}$ has been increased. In the next one, we see the pH range which the most change in the pH is for CbP+Z and the minimum for SS which means the acidity feature of cheese which should be controlled during the experiment to produce maximum VFA, because the accumulation of VFAs reduces the pH, and then inhibit the anaerobic process. CbP had the most alcohols production among others while SS+Z was the least one but the values are not considerable. For Lactic acid and total VFA, CbP+Z had the maximum value with 12.32 and 25.11 gCOD/L and the minimum was for SS with 1.22 and 11.65 gCOD/L. For VFA/COD_{sol}, the S+Cbp+Z was the highest one with 0.73, which means that mixture of the SS and CbP increase the VFA/COD_{sol} or in other words make the quality of the sample much better compared with using them separately. And finally, SS+CbP+Z and CbP+Z had the maximum values in yield. In total, it can be resulted that SS had the least function in terms of profitability in the experiment and CbP+Z, CbP and SS+CbP+Z were better respectively. However, it should be considered the high value of the CbP+SS and CbP+SS+Z in yield and VFA/$\mathrm{COD_{SOL}}$. In these 2 samples in the large scale can be more profitable considering abundance of SS which can be mixed by CbP to have more profitable production. Moreover, there was a positive effect of zeolite on SS+Z and also CbP+Z and finally CbP+SS+Z which increased the production of VFA.

Test Name	Substrate	Parameters						
		COD_{sol}, (gO_2/L)	pH	Total Alcohols (gCOD/L)	Lactic Acid (gCOD/L)	Total VFA (gCOD/L)	VFA/COD_{SOL} (gO2/gO2)	Yield (gCODVFA/gVS0)
A	SS	24.7	6.11-6.55	0.64	1.22	11.65	0.51	0.18
B	SS + z	23.7	5.98- 6.5	0.3	1.24	13	0.57	0.20
C	CbP	42.3	4.75- 6.5	2.65	10.32	22.22	0.54	0.35
D	CbP + Z	44.6	4.45- 6.5	1.48	12.32	25.11	0.59	0.39
E	SS+Cbp	38.6	5.61- 6.5	1.31	7.47	22.74	0.66	0.36
F	SS+CbP + Z	35.4	5.31- 6.5	1.97	7.63	24.08	0.73	0.38

Table.4. Summary of parameter of batch experiment in mesophilic condition

3.4 Summary of results in thermophilic conditions

First, In general it is clear that the values for this condition are higher than previous one. By starting with $\mathrm{COD_{sol}}$, it can be seen that CbP+Z had the most value with 50.6 (gO_2/L) which is the best one similar to mesophilic condition. For the pH range also CbP-Z had the maximum value which is similar to mesophilic condition. The maximum value for the total alcohol in this condition was for SS+CbP+Z with 4.11 gCOD/L which was different with mesophilic condition, however the amount of alcohol in total is not high. For the value of the Lactic acid, it is shown that CbP by 15.32 gCOD/L has the highest value which in previous it was for CbP+z. Similar to mesophilic the maximum value for total VFA was for Cbp+Z which can say high quality of CbP material for producing VFA among others. And highest VFA/COD_{sol}, was for SS+CbP sample and maximum yield was for CbP+Z which both are different from previous condition. In total, in thermophilic condition there were higher values but not the same increase in all of the samples, in other words increasing temperature does not affect the value of sample the same and in some samples increase the production more than other. Moreover, regarding table it is indicated that effect of adding zeolite in SS in thermophilic condition in producing VFA was not considerable and almost no effect of adding zeolite in thermophilic condition in SS which is not similar to mesophilic condition. However, in CbP and CbP+SS it is considerable and adding zeolite effected the production and all other value positively.

Test name	Substrate	Parameter						
		COD_{sol}, (gO_2/L)	pH	Total Alcohols (gCOD/L)	Lactic Acid (gCOD/L)	Total VFA (gCOD/L)	VFA/COD_{sol} , (gO2/gO2)	Yield (gCODVFA/gVS0)
A	SS	26.4	5.5- 6.5	0.72	0.78	14.67	0.6	0.23
B	SS + Z	31.78	5.65- 6.5	0.77	0.84	14.43	0.54	0.23
C	Cbp	45.4	4.77- 6.5	4.11	15.32	23.6	0.53	0.37
D	CbP + Z	50.6	4.43- 6.5	2.74	11.32	30.66	0.68	0.48
E	SS+CbP	37	5.56- 6.5	2.4	7.54	23.26	0.75	0.36
F	SS+CbP + Z	40.7	5.41- 6.5	5.51	7.93	26.02	0.65	0.41

Table.5. Summary of parameter of batch experiment in thermophilic condition

In total, in both condition if we want to choose the best options are CbP+SS+Z and CbP+Z which had the best values.

3.5 Pilot scale CSTR experiment

In the pursuit of advancing our understanding of anaerobic fermentation processes, a pivotal stage of our study involved a reactor experiment focused on the utilization of SS and SS+Z substrates. This reactor experiment spanned duration of 50 days, during which it was sought to comprehensively investigate the dynamic interplay of these substrates under thermophilic conditions. An intriguing aspect of this investigation involved the introduction of Z into the SS+Z reactor at the midpoint of the experiment, specifically on day 25. This strategic inclusion aimed to elucidate the potential impacts of Z on the digestion dynamics.

The extended duration of the reactor experiment allowed us to observe and analyze the complex interactions between the microbial consortia, organic matter degradation, and biogas production in a controlled environment. By assessing both the SS and SS+Z reactors, it was sought to unravel the multifaceted effects of Z addition on the anaerobic digestion process.

The decision to introduce Z into the reactor after 25 days was driven by the aim to capture the effects of Z in a context where the initial microbial activity had been established. This approach enabled us to investigate whether Z would exert discernible changes in the ongoing fermentation process and subsequent biogas production.

The results obtained from this reactor experiment are poised to provide valuable insights into the mechanisms underlying the influence of Z on anaerobic digestion dynamics. By comparing the performance of the SS reactor with that of the SS+Z reactor before and after Z addition, it was aimed to discern any notable shifts in biogas composition, volatile fatty acid production, or substrate utilization.

The data analysis presented in this section not only offer insights into the impact of Z addition but also contribute to the broader understanding of substrate interactions in thermophilic anaerobic fermentation. It is within this context that it is presented the results and observations garnered from the reactor experiment, shedding light on the potential benefits and implications of Z enhanced anaerobic digestion.

The effectiveness of anaerobic fermentation processes is profoundly influenced by various operational parameters, with hydraulic retention time (HRT) standing as a critical factor in determining substrate interaction and microbial activity. In our comprehensive investigation of the reactor experiment involving SS and SS+Z substrates, HRT played a pivotal role in shaping the dynamics of the anaerobic fermentation process

For the duration of the reactor experiment, a controlled HRT of 4 days was maintained. This HRT was achieved by adding a consistent volume of substrate to the reactor each day, ensuring a uniform and manageable input of organic matter. In a reactor with a working volume of 200 ml, the addition of 50 ml of substrate every day was employed to maintain this hydraulic retention time.

3.5.1 Biogas production

This section delves into the core of our experimental exploration, delving into the phenomenon of biogas production within the controlled environment of our reactor experiment. Figure 22 shows biogas production during the experiment. In the first half of the chart which substrate is SS, it is shown that a continuous increasing of the biogas production before adding Z. After day 25 when the Z was added, there was a sharp increase of biogas production after some days, then, it reached to almost constant level.

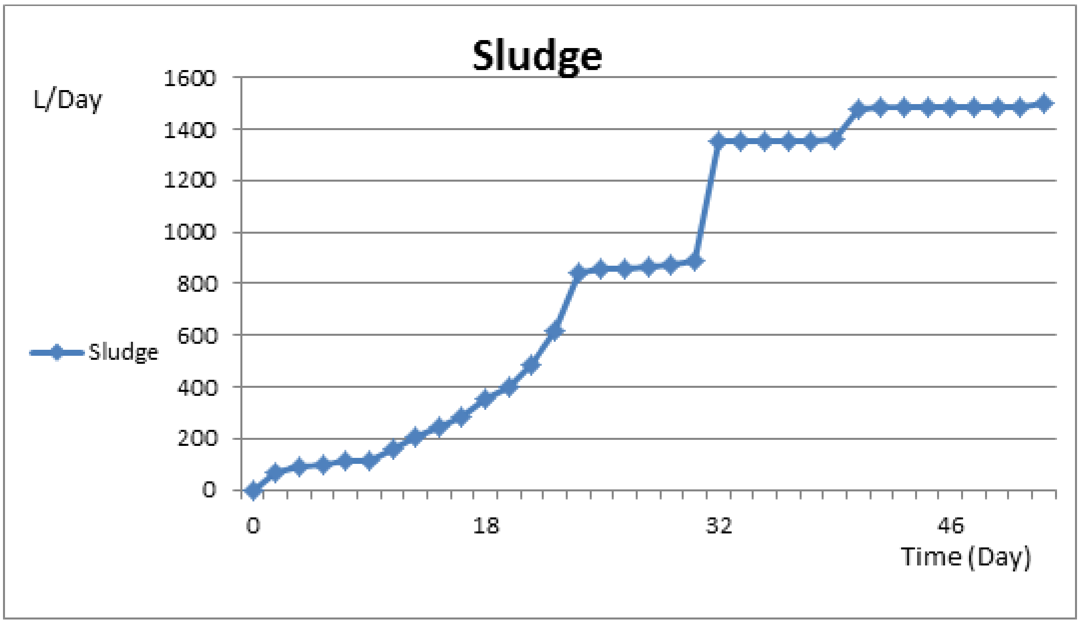

Figure.22. Biogas production during experiment

3.5.2 VFA production without Z

The pie chart (figure 23) shows the average quantity of production of the materials during experiment with SS substrate. It can be seen that acetic acid had the highest production and after that the butyric and propionic were higher respectively. These results can prove our last results in the batch experiment thermophilic condition where there were the highest values for acetic acid, butyric acid and propionic acid.

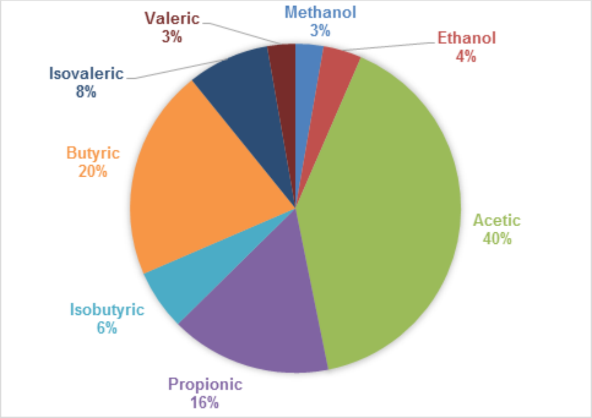

Figure.23. Average of VFA production without Z

3.5.3 VFA production in SS + Z

After adding Z, also it can be seen that again acetic acid propionic acid and butyric acid had the highest value among others. After acetic acid, butyric and propionic acids again were the next materials in terms of quantity like previous substrate which are the same materials in the batch experiment and prove our right and accurate results.

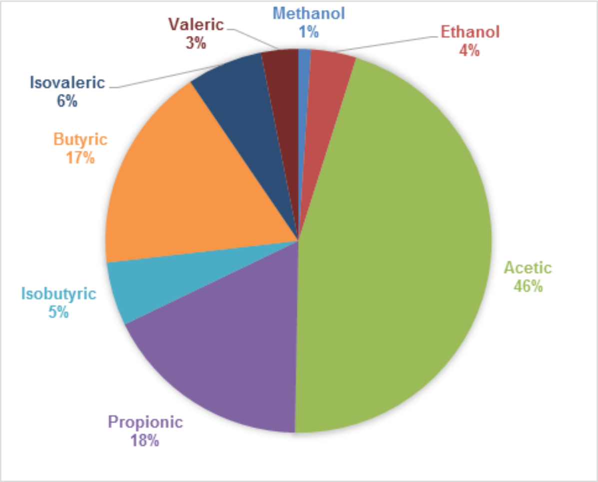

Figure.24. Average of VFA production with Z

3.5.4 VFA vs pH – H_2 production

Figure 25 shows the production of VFA and pH values during the experiment. The H_2 trend is also showed as gas percentage. VFA production shows an increasing trend during the experiment while after adding Z there is a sharp decrease in the pH and after some days, the pH is going to increase until reaches to a its previous level. The VFA shows the increasing trend which after 50 days I reached to the 10g COD/L. It is quite clear observing that zeolite addition was completely in favor of higher fermentative activity since the average VFA concentration was 9.1 ± 0.3 g COD/L vs 6.0 ± 0.1 g COD/L in the first part of the experiments conducted without zeolite addition. It was also observed how the increasing fermentative activity favored the H_2 production, which is another product of the fermentation process. The average H_2 content in the gas phase was 7.6 ± 0.8 vs 15 ± 1 v/v (%) respectively in the part of the experimentation without and with zeolite.

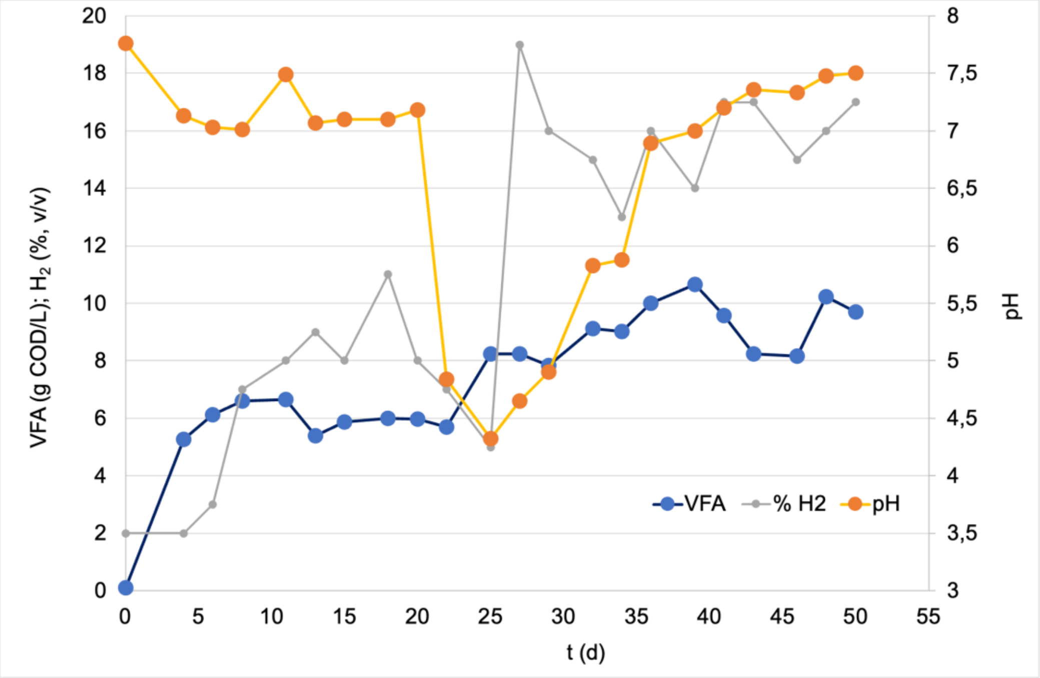

Figure.25. VFA production and pH during experiment

3.5.5 VFA/SOD vs Yield

Figure 26 shows the amount of VFA/COD_{sol}, and Yield during the experiment. The yield increased until 0.5 and VFA/COD_{sol}, also had increasing trend until 0.8. Silva et.al, (2021) showed that the addition of Z improved the yields production by dark fermentation. This result can improve our results so that after adding Z the Yield started to increase. The importance to quantify the fermentation yield is due to the following mass-balance assessment (not included in this thesis), which can give an idea on how much feedstock is necessary for a certain production of VFA. The choice is strictly related to the exploitable market potential of the VFA and the economic scenario that faces within the biorefinery context.

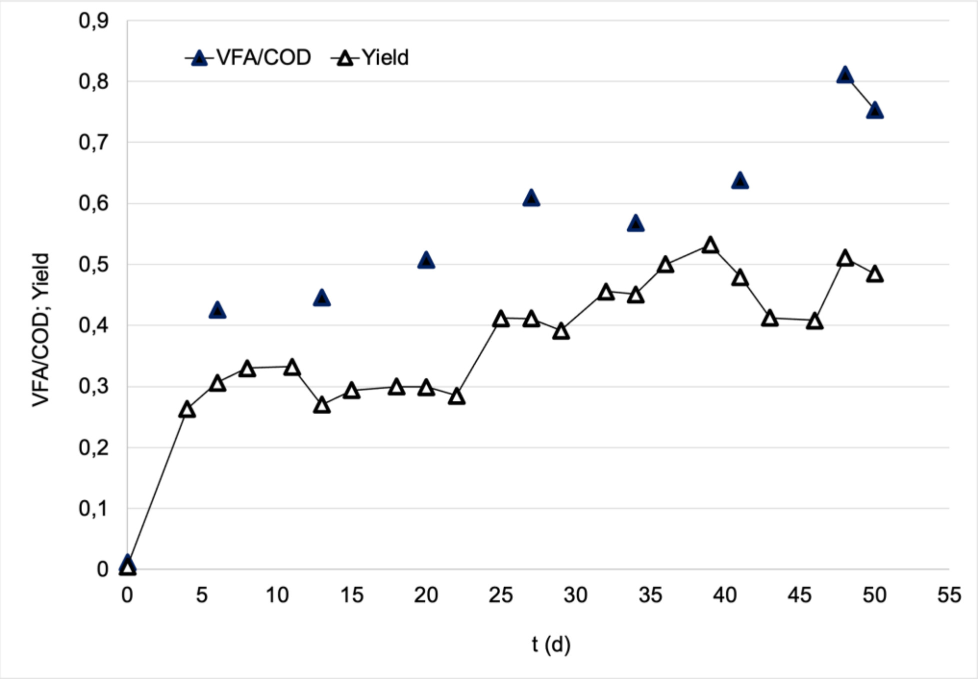

Figure.26. VFA/COD and fermentation Yield

5. Conclusion

In conclusion, the journey through this research endeavor has illuminated significant insights into the realm of anaerobic fermentation, waste valorization, and sustainable energy generation. The convergence of meticulous experimentation, comprehensive data analysis, and in-depth literature review has propelled us towards a deeper comprehension of the complex interplay of substrates, and operational parameters. The conclusions drawn from this study not only contribute to the academic discourse but also hold pragmatic implications for waste management strategies, resource recovery, and the pursuit of renewable energy solutions. As it is taken from the scientific exploration and real-world applications, the findings presented in the subsequent sections encapsulate the culmination of our efforts and set the stage for future advancements in this dynamic field.

1- In the mesophilic batch experiment, a comprehensive evaluation of the anaerobic fermentation process revealed notable distinctions between the performances of CbP and SS substrates. Remarkably, the CbP substrate exhibited enhanced functionality in comparison to the SS counterpart which was double in VFA and yield. The introduction of Z into each sample emerged as a pivotal parameter, yielding positive outcomes across the board which the maximum ones were for CbP+Z and CbP+SS+Z with 0.39 and 0.38 gCODVFA/gVS0 respectively. Furthermore, a significant finding emerged from the juxtaposition of SS and CbP, indicating a synergistic effect that led to superior performance when compared to sole utilization of SS.

2- The outcomes of the thermophilic batch experiment closely mirrored those observed in the mesophilic condition, albeit with notable distinctions. Due to the earlier findings, the thermophilic environment again demonstrated superior performance, such as volatile fatty acids (VFA) and Yielding which the maximum one was for CbP+Z with 30.66 gCOD/L and 0.48 gCODVFA/gVS0 respectively . The amplification of these outcomes in the thermophilic setting signifies the heightened metabolic activity within this elevated temperature range.

3- In the CSTR experiment it was seen that the amount of biogas production was low. Also, in the second step when the Z was added, the amount of VFA increased. And finally the amount of acetic acid was the highest one during the experiment. And in the end, it was concluded that it is better to use from the mix of SS with other substrates like CbP for the better production of hydrogen rather than using SS only.

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