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Acidogenic Fermentation at a Thermophilic Temperature from Municipal Sewage Sludge for the Production of VFAs

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Population growth has increased the generation of solid waste, especially conventional plastics, which are the precursors of major environmental problems. On the other hand, given the complexity of converting large quantities of organic matter contained in the sludge from wastewater treatment plants (WWTP) into value-added products, it is necessary to evaluate alternatives that enhance the production of bioplastics from polymers of polymeric origin. Furthermore, it is necessary to evaluate alternatives that enhance the production of bioplastics from polymers of biological origin. One of these alternatives is the production of Polyhydroxyalkanoates (PHAs) which result from taking VFAs as a carbon source. Therefore, this project aims to evaluate the anaerobic fermentation conditions that maximize the yield of VFA production through laboratory-scale tests. The experimental design was constructed for a working volume of 200 ml, using organic loadings, 4 gVS/L, and 6 gVS/L for digested sludge and 10 gVS/L, and 14 g VS/L for primary sludge, and controlled pH values of 9.0 ± 0.5, 10 ± 0.5 and 11 ± 0.5 at a constant thermophilic temperature of 55ºC. Twelve combinations were used in triplicate with 48 h, 96 h, and 288 h of HRT, including 3 blanks for each combination, considering a total of 144 reactors. The present work reports the optimal conditions that favour the highest production of volatile fatty acids (VFA) in the anaerobic digestion process from primary and digested sludge. The sludges used came from the Salitre WWTP in Bogotá under thermophilic conditions were obtained, where the best production and accumulated yield of VFA were 31224 mg COD/L, and 2,230 g COD/g VS respectively, produced with primary sludge at 14gSV/L; ph10± 0.5.
Keywords: VFAs, Acidogenic Fermentation, Polyhydroxyalkanoates, digested sludge, primary sludge.

1. Introduction

Sludge from wastewater treatment plants contains large amounts of organic matter; however, converting it into value-added products such as methane or hydrogen is very complex. Recently, methane production studies have explored the potential of volatile fatty acid VFA. There is currently an issue regarding what to do with the quantities of sludge generated in municipal wastewater treatment plants, as it has low efficiencies in the systems incorporated and is expected to increase significantly in the coming decades (Duan et al., 2012). It is also causing very high by-product costs in WWTPs; as pointed out by Wu et al., 2020, China alone produces about 60 million tonnes of sewage sludge annually. In Bogotá-Colombia, the Salitre wastewater treatment plant (WWTP) produces 148,455.58 m^3/month of primary sludge and 1051.73 m^3/month of digested sludge, so these volumes require the incorporation of efficient and sustainable technologies that can add value to them.
The production and type of VFAs depend on the fermentation operating conditions and the physicochemical characterization of the substrates. (Rizzioli et al., 2022). The optimal conditions that play an essential role in the anaerobic digestion process for obtaining VFA are temperature, pH, substrate-to-inoculum (S/I) ratio, and an organic load of sewage sludge (Parra Orobio et al., 2015). According to Parra (2015), low pH values cause the inhibition of VFA production, which is confirmed in the research of Pérez-Zabaleta et al., (2021), working with food waste at an alkaline pH close to 9 results in higher acetic acid content. In addition, the substrate conditions the production of VFA due to the content of proteins, carbohydrates, and lipids (Rodríguez de la Garza et al.,

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2012; Jankowska, et al., 2017; Yin et al., 2016). Due to the richness of nutrients, organic matter, and microorganisms contained in sewage sludge, it can be reused in bio fermentations, being eco-efficient technologies for the generation of value-added products such as volatile fatty acids (VFA) (Liang et al., 2021).
On the other hand, VFAs are renewable carbon sources for different processes, for example, the production of polyhydroxyalkanoates PHA bioplastics (Frison et al., 2015), which is considered an alternative to reduce the consumption of petroleum-based plastics (Xiong et al., 2012). In addition, it can have multiple uses thanks to its thermoplastic properties and is a promising option for using mixed microbial cultures (Rizzioli et al., 2022). However, previously the enrichment phase of the microbial culture that accumulates the biopolymer is necessary for an acidogenic fermentation for the VFAs bioproduction (Mendoza, 2017).
Therefore, the objective of this project was to evaluate the anaerobic fermentation conditions that maximize the yield in the production of VFAs under thermophilic temperature conditions at a laboratory scale. The VFAs obtained will be subsequently used as substrate in producing PHAs.

2. Material and methods

For the bioproduction of VFA, the present study used digested and primary sludge from the treatment plant of the Salitre WWTP in Bogotá. An experimental design with 12 treatment combinations was used, and every treatment combination was done in triplicate. In addition, to the combinations, blanks were used to establish the potential for VFA production. The anaerobic fermentation was carried out with organic loads of 4 g VS/L, 6 g VS/L, 10 g VS/L and 14 g VS/L, based on the concentration of volatile solids (VS) being adequate for the acceleration of the process as presented (Pittmann & Steinmetz, 2013). The pH values were 9 ± 0.5, 10 ± 0.5, 11 ± 0.5, and TRH hydraulic retention times of 48 h, 96 h, and 144 h. The granular inoculum used was obtained from the anaerobic digester of the Alpina dairy industry and treated with a heat shock following the methodology of Hernández et al., 2018. The temperature used in the study was thermophilic conditions at 55°C. Table 1 details the experimental design developed.

Table 1: Table of treatment combinations.

Combination	Organic Load (gVS/ L)	Substrate	pH
C1			9,5
C2	6		10,5
C3		Digested Sludge	11,5
C4	4		9,5
C5			10,5
C6			11,5
C7	14		9,5
C8			10,5
C9			11,5
C10	10	Primary Sludge	9,5
C11			10,5
C12			11,5

The basic physicochemical parameters analyzed were Total Solids (Method 2540B APHA SM), Volatile Solids dry and wet basis (Method ASTM D3174), Total Nitrogen Kjeldhal (Method ASTM D1426), Chemical Oxygen Demand (Method ASTM D1252), Volatile Fatty Acids (Method 5560D APHA SM), (Baird, 2017). The biogas production was quantified by volume displacement in an aqueous pH 9 solution. Additionally, the biogas quality was monitored by spot measurements using a Landtec Biogas 5000 infrared detector.
The VFA production yield was calculated with the ratio between the total VFA concentration in the effluent expressed in COD grams and VS fed grams, according to Liu et al. 2009 as shown in Equation 1.

$$VFA_{yield} = \frac{VFA_{out}}{VS_{in}} \quad (1)$$

3. Results and discussions

The experimental design was made based on the physicochemical characterization of primary and digested sludge and the inoculum. Table 2 shows the main characteristics. The sludges used in the study were kept in a freezer at -4°C to avoid microbiological degradation before testing.

Table 2: Physico-chemical characteristics of inoculum and primary and digested domestic wastewater sludge

Substrate	% Total Solids (TS)	% Organic Matter	% Volatile solids (SV)	% Total Nitrogen Kjeldahl	Chemical Oxygen Demand COD mg/L
Primary Sludge	4.88+0,01	69,21 +0,01	3.67 + 0,41	0,16 + 0,01	31600
Digested Sludge	2.98+0,01	74,24 +0,01	1.71 + 0,41	0,19 + 0,01	11600
Inoculum	5,38+0,49		4.39+ 0,41		

This study's highest concentration of VFA achieved was 4704 mg COD/L at a pH of 10.5 and an organic load of 14 g SV/L, with a hydraulic retention time of 96 hours. This result agrees with the ones reported by Pittmann & Steinmetz, 2013, where the highest VFA production was obtained on day 4. Additionally, alkaline pH provides a buffer effect, helping the bacterial community to produce more VFAs. Furthermore, studies report that alkaline pH enhances complex substrates' hydrolysis and increases organic matter degradation with primary sewage sludge substrates (Jankowska et al., 2017).

Figure 1a shows the temporal profile of the acidogenic batch fermentation for digested sludge, with the highest concentration of VFA obtained with organic loading of 4 g VS/L occurring on day 6, with a pH of 9.5 (1168 mg COD/L), corresponding to the C4 combination. Conversely, the best resulting production with organic loading of 6 g VS/L occurred on day 2, with a pH of 10.5 (1780 mg COD/L) corresponding to the C2 combination.

The bioproduction of VFAs using the primary sludge as substrate can be seen in Figure b. The results show that the highest concentration of VFA at an organic load of 14 g VS/L was on day 4 (4320 mg COD/L), at pH 11.5, in combination C9. Finally, using an organic loading of 10 g VS/L, the best concentration of VFA occurred on day 4, at pH 10.5 (4450 mg COD/L), in combination C11.

(a)

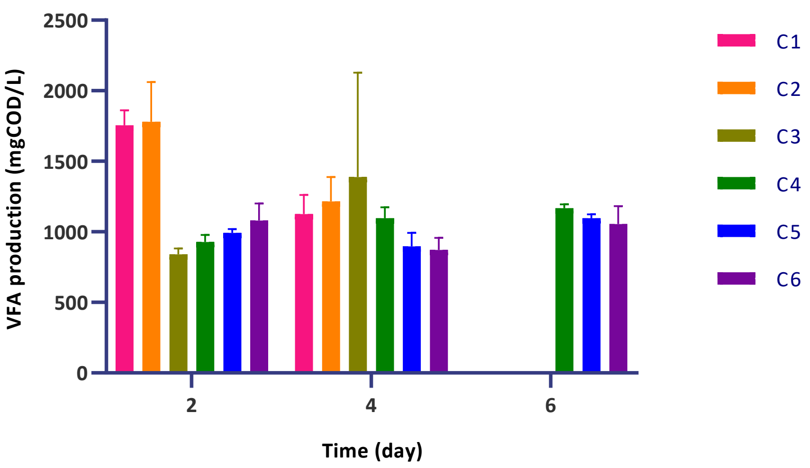

Figure 1: Acidogenic fermentation of (a) digested sludge and (b) primary sludge in terms of VFA production over 6 days (continue)

(b)

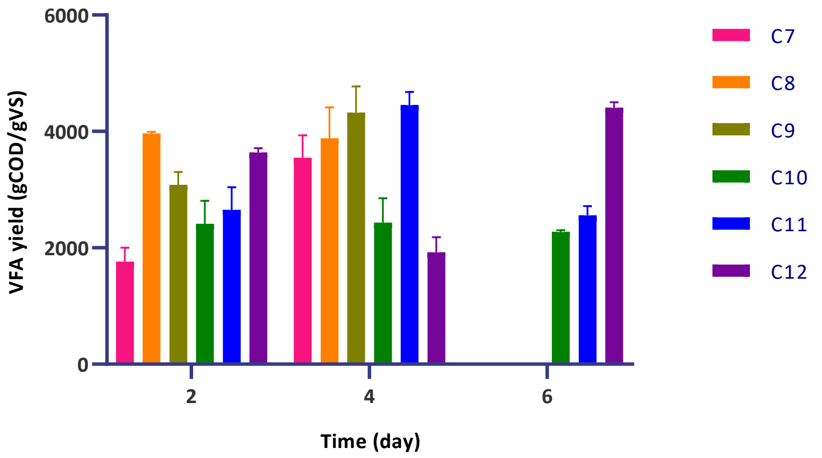

Figure 1: Acidogenic fermentation of (a) digested sludge and (b) primary sludge in terms of VFA production over 6 days.

According to the results of this experiment, the best VFA productions are obtained using the highest organic loads and alkaline pH, as well as hydraulic retention times greater than 4 days, which coincides with the research reported by Wu et al 2010; since when using substrates with high organic loads there is a greater amount of microorganisms, which favours fermentation.

Additionally, alkaline pH and thermophilic temperature facilitate the hydrolysis process, as these factors directly affect the microbial growth rate, modifying their metabolism by changes in their primary energy sources, carbon and protein synthesis, which facilitate VFA production (Xiong et al., 2012; Yu & Fang, 2003). On the other hand, the results with low VFA concentration are attributed to insufficient hydraulic retention time to achieve the hydrolysis phase ultimately. The reactors have a higher soluble protein and carbohydrate content to enhance VFA production in the acidogenic-acetogenic phase (Jankowska et al., 2015). A relevant operational parameter affecting the fermentation process in the hydrolytic-acetogenic phase for VFA production is the inoculum content responsible for the production efficiency (Atasoy et al., 2019).

On the other hand, according to Song et al., 2004, it was found that VFA yields performed better in digesters at thermophilic conditions than in mesophilic digesters, as well as the COD mg/L removal rate was more efficient, furthermore, it was reported that 55°C VFA degraded in a shorter period.

(a)

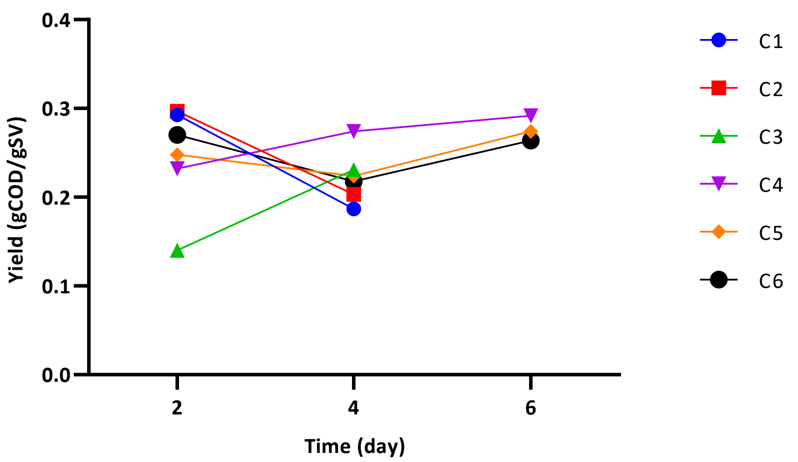

Figure 2: (a) Yield gCOD/g SV Digested Sludge; (b) Yield gCOD/g SV Primary Sludge (continue)

(b)

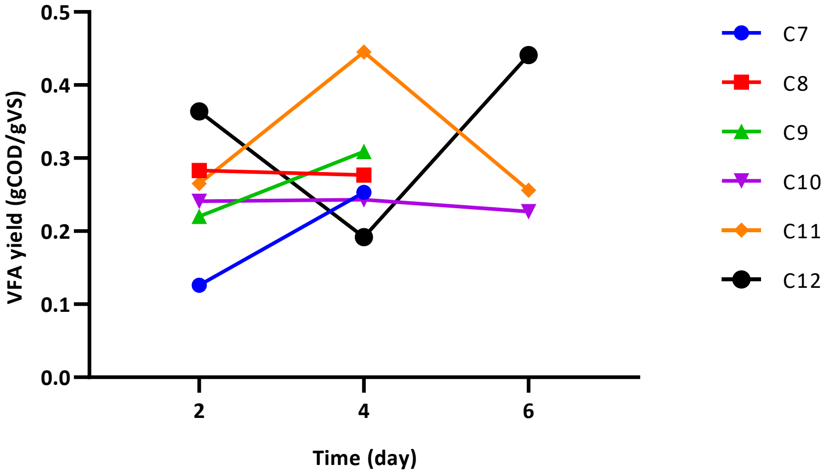

Figure 2: (a) Yield gCOD/g SV Digested Sludge; (b) Yield gCOD/g SV Primary Sludge

Figure 2 a and b shows the yields for digested sludge and primary sludge. The results it was reflected that an optimum hydraulic retention time was 96 hours, with 0.445 gCOD/gVS, using pH 10.5 and organic loading of 10gSV, which is in agreement with the results reported by Bahreini et al, 2020, where primary sludge fermentation generated an increase in VFA production yield from day 1 to 4. Furthermore, according to Feng et al., 2009 alkaline pH and thermophilic temperature are also key conditions for enhancing maximum VFA yield.

4. Conclusions

The results obtained regarding the production of VFAs contribute to current studies, using primary and digested sludge from a wastewater treatment plant as substrate and anaerobic sludge from a dairy industry as inoculum. The bioproduction of the best-performing VFAs can be used as a carbon source in producing PHAs. The highest VFA concentration and yield were 4450 mg COD/L and 0.445 g COD/g VS, respectively, corresponding to combination 11, which worked under an organic load of 14 g VS/L, pH 10.5 at a constant temperature of 55°C and with an HRT of 96 hours. These operating conditions prioritize the hydrolytic, acetogenic-acidogenic phase of the anaerobic fermentation process, maintaining a high production by the microorganisms, thanks to the adequate amounts of carbon sources for their retention.

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