https://doi.org/10.1590/S1413-415220230064

Scientific Paper

Two-phase anaerobic digestion of cassava wastewater with addition of residual glycerol for hydrogen and methane production

Digestão anaeróbica em duas fases de águas residuais de mandioca com adição de glicerol residual para produção de hidrogênio e metano

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ABSTRACT

Biogas production through co-digestion of two or more waste products has garnered increasing attention from researchers seeking to optimize this process. Biogas and methane production increase with the addition of glycerol to agro-industrial wastes during anaerobic biodigestion. However, the utilization of a two-phase process focused on hydrogen production has not been widely explored. This work aims to evaluate two-phase anaerobic biodigestion of cassava wastewater by adding residual glycerol and swine wastewater to enhance hydrogen and methane production. A pilot-scale biodigester was used during the acidogenic phase at 38.5°C, containing 4% glycerol. The effluent was submitted to methanogenic treatment, and the influence of temperature (36.0 to 39.0°C) and sodium bicarbonate concentration (2.0 to 6.0 g L-1) were evaluated. The results indicated that the optimum conditions during the methanogenic phase were 39.0°C with a sodium bicarbonate concentration of 5.0 g L^{-1}. The two-phase biodigestion produced 30.8 mL of (H_2) $RCOD^{-1}$ and 104.5 mL of (CH4) $RCOD^{-1}$. Thus, the substrates and inoculum used were adequate for the anaerobic biodigestion process, increasing the energetic efficiency of the process due to hydrogen production.

Keywords: anaerobic biodigestion; codigestion; biogas; biomethane; green hydrogen.

RESUMO

A produção de biogás por meio da codigestão de dois ou mais resíduos tem atraído atenção crescente de pesquisadores que buscam otimizar esse processo. A adição de glicerol aos resíduos agroindustriais durante a biodigestão anaeróbica tem demonstrado aumentar a produção de biogás e metano. No entanto, a utilização de um processo em duas fases focado na produção de hidrogênio ainda não foi amplamente explorada. Este trabalho visa avaliar a biodigestão anaeróbica em duas fases de águas residuais de mandioca, adicionando glicerol residual e águas residuais suínas para aumentar a produção de hidrogênio e metano. Um biodigestor em escala piloto foi utilizado durante a fase acidogênica a 38,5°C, contendo 4% de glicerol. O efluente foi submetido a tratamento metanogênico, e a influência da temperatura (36,0 a 39,0°C) e da concentração de bicarbonato de sódio (2,0 a 6,0 g L^{-1}) foi avaliada. Os resultados indicaram que as condições ótimas durante a fase metanogênica foram 39,0°C com uma concentração de bicarbonato de sódio de 5,0 g L^{-1}. A biodigestão em duas fases produziu 30,8 mL de H_2 por $RCOD^{-1}$ e 104,5 mL de CH_4 por $RCOD^{-1}$. Assim, os substratos e o inóculo utilizados foram adequados para o processo de biodigestão anaeróbica, aumentando a eficiência energética do processo devido à produção de hidrogênio.

Palavras-chave: biodigestão anaeróbica; codigestão; biogás; biometano; hidrogênio verde.

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Conflicts of interest: the authors declare no conflicts of interest.
Funding: none.
Received on: 05/18/2023 – **Accepted on:** 05/10/2024

INTRODUCTION

Due to world population growth, the demand for food and fuel for subsistence is on the rise. The agricultural and agro-industrial sectors have experienced rapid development, which has resulted in an exponential increase in waste generated from these activities. Conversely, on a global scale, there is an urgent need for energy transition, where sustainable energy sources, such as residual biomass from agroindustrial activities, stand out as an alternative to the energy scenario. This eco-alternative approach reduces environmental liabilities and adds value to the existing production chains (CREMONEZ *et al*., 2021).

Hydrogen is widely regarded as a fuel for the future due to its carbon-free energy carrier potential and ability to mitigate greenhouse gas emissions. It is considered a viable and carbon-neutral fuel, distinguished among other alternatives for its cleanliness, as its combustion results only in the release of water as a final product (SUDALAIMUTHU; SATHYAMURTHY, 2024). In this context, hydrogen production emerges as a promising alternative to renewable fuels, as it can be produced from various chemical and biological pathways and raw materials.

Currently, anaerobic fermentation for biological hydrogen production stands out as one of the most researched methods due to its straightforward operation mode and low energy consumption. It is conducted under low temperatures and pressures and has the advantage of effectively treating organic waste. However, waste treatment during hydrogen production is inefficient, as the anaerobic organic matter degradation produces large amounts of organic acids that remain in the liquid medium (LIU *et al.*, 2013).

Bonassa *et al.* (2021) reported that post-anaerobic digestion, the liquid effluent (referred to as digestate) predominantly contains ammoniacal nitrogen and organic carbon. Consequently, further treatment of the liquid phase is required to meet nitrogen discharge standards.

The waste produced during methane production can be effectively repurposed in the fermentation process to generate hydrogen, thereby optimizing the utilization of residual organic matter (SILVA *et al*., 2018). Moreover, the gases derived from this dual-phase approach can be utilized individually or combined to generate biomethane (CREMONEZ *et al*., 2021). This biofuel typically contains approximately 60% CH_4, 30% CO_2, and 10% H_2 (MEENA *et al.*, 2020), and is a subject of extensive interest by numerous automotive companies in the USA (RENA *et al*., 2020). Because of this, numerous studies aim to explore new routes to enhance the efficacy of organic waste remediation.

One of the ways to improve the efficiency in methane and hydrogen synthesis is adding biodegradable organic compounds into the process. These compounds, such as carbohydrates, proteins, aldehydes, fulvic acids, phenols, organic peroxides, and glycerol, play a pivotal role. Their high concentrations of nitrogen and carbon provide essential substrates that facilitate successful biodigestion in treatment systems utilizing microorganisms. Those microorganisms utilize available carbon as an electron acceptor for the decomposition of organic matter (MEIER *et al*., 2020). Meier *et al.* (2020) evaluated the biodigestion of cassava wastewater with the addition of residual glycerol, using swine wastewater as inoculum, for hydrogen production, and determined the ideal temperature and glycerol concentration for it; however, the effluent obtained still contained high organic matter contents.

The western region of the State of Paraná (Brazil) produces large amounts of waste, which justifies using cassava wastewater with the addition of residual glycerol using swine wastewater as an inoculum, in a two-phase anaerobic digestion process to produce hydrogen and methane. It is also important to note the significant challenge in conducting anaerobic digestion of residual glycerol without dilution, as its solids and organic load content exceed the ideal amounts for this process. In this regard, cassava wastewater has emerged as a promising diluent due to its low solids content and the presence of ammonia nitrogen, which facilitates the action of microorganisms in anaerobic digestion.

In this sense, this work assesses two-phase anaerobic digestion by utilizing the same substrates and parameters of the acidogenic phase defined by Meier *et al.* (2020), and to optimize the methanogenic phase focused on methane production, considering different temperatures and sodium bicarbonate concentrations (buffer) as variables.

MATERIAL AND METHODS

Substrate and inoculum

Residual glycerol and cassava wastewater were used as substrates for anaerobic digestion. The glycerol was sourced from the Federal University of Paraná (Palotina Sector). It was obtained as a by-product of biodiesel production from used cooking oil produced through homogeneous alkaline catalysis (transesterification), resulting in the presence of sodium hydroxide and methanol in its chemical composition. These compounds render glycerol reuse unfeasible and classify it as an environmental hazard if improperly discarded. Furthermore, methanol is toxic to most microorganisms. Hence, to remove the methanol present in the glycerol, it was heated to 85°C before being used in the process.

The cassava wastewater was obtained from a cassava processing industry in Terra Roxa, Paraná (PR), Brazil. It was obtained from a Canadian model biodigester operating with swine wastewater, installed in a farm with a capacity for 900 animals, located in Palotina (PR), Brazil.

Before utilization, the inoculum applied in the acidogenic-phase reactors underwent heat treatment at 100°C for 30 minutes, following the methodology adopted by Reyna-Gómez *et al.* (2021). According to Jung *et al.* (2021), the inoculum is often subjected to pre-treatment to restrict the development of unwanted microbial populations.

Biodigesters

The biodigester and gasometer system (Figure 1) were constructed following the specifications presented by Meier *et al.* (2020). The acidogenic-phase biodigester was built using terephthalate polyethylene, with a total volume of 65.0 L and a usable volume of 50.0 L. A 20% dead volume was maintained to prevent foam formation or backflow at the gas collection outlet due to pressure. Similarly, the gasometers were built from terephthalate polyethylene with a useful volume of approximately 5.0 L. In contrast, the methanogenic phase biodigester had a total volume of 2.5 L, with a usable volume of 2.0 L, while the gasometers featured a usable volume of approximately 1.5 L. The gasometers were immersed in a sealing acid-saline solution consisting of sodium chloride and sulfuric acid to prevent gas leakage and carbon dioxide dissolution in the biogas. Both acidogenic phase biodigester and gasometers were connected via silicone hose.

Biodigesters for destructive samples

Replicas of the biodigesters used for hydrogen production were built to investigate the initial degradation profile of the substrates. However, these replicas had

a volume of 0.30 L. They contained inoculum and substrate samples in the same proportions as the original reactor, with 20% dead volume and a useful volume of 0.24 L. Additionally, each replica included a gas outlet hose immersed in an aqueous solution to prevent oxygen from entering the system.

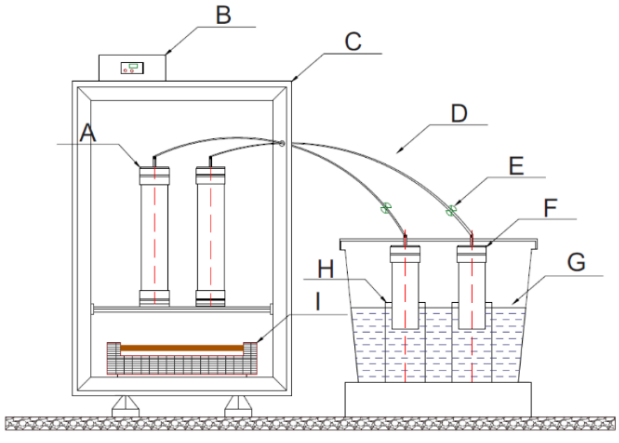
The biodigesters used for hydrogen production and the replicas used to obtain destructive samples were placed in a controlled-temperature oven (Figure 2) with a variation of 1.0°C. The hydraulic retention time was 90 hours for the acidogenic and 26 days for the methanogenic biodigester, based on the period when biogas production ceased (MEIER *et al.*, 2020).

Biodigester operation and experimental design

All biodigesters were operated in batch regimes. The acidogenic-phase biodigester was loaded with 10.0 L of inoculum subjected to thermal treatment and 40.0 L of substrate consisting of 4% glycerol and 96% cassava wastewater (v v^{-1}). The biodigester was placed in an oven at 38.5°C. The operating conditions to maximize the hydrogen production in the reactor for this phase were the same as the ones reported by Meier *et al.* (2020). Different concentrations of sodium bicarbonate (2.0, 4.0 and, 6.0 gL^{-1}) were added to the effluent in the acidogenic-phase biodigester and used as a substrate in the methanogenic-phase biodigester; the inoculum was also added, but with no thermal treatment. These biodigesters were placed in an oven under controlled temperatures (36.0, 37.5, and 39.0°C). A 3^2 factorial planning was used in the Statistica 7.0 software, focused on determining the ideal temperature for this phase, and the ideal concentration of sodium bicarbonate, a buffer that maximizes methane production. The central point experiments were performed in triplicate. Table 1 presents the experimental conditions used in each treatment.

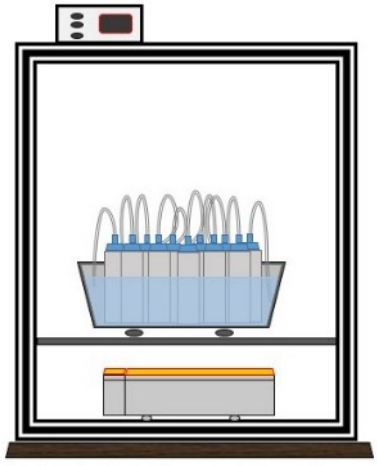
The volume of produced methane, methane concentration, biodigestion kinetic parameters (Gompertz model: total hydrogen volume, lag phase time, and maximum specific velocity), initial content removal percentage, total solids (TS), volatile solids (VS), and chemical oxygen demand (COD) were evaluated through the experimental planning.

After analyzing the experimental data, the desirability function (Statistica 7.0 software) was utilized to optimize the temperatures and concentrations of sodium bicarbonate required for maximizing methane production. Once the optimized conditions were determined, triplicate tests were conducted to compare the experimental results with the model predictions generated by Statistica 7.0 software.



Source: adapted from Cremonez *et al.* (2016).

Figure 1 - Scheme of the experimental system: (A) reactors; (B) thermostatic controller; (C) oven; (D) hoses for channeling the generated gas; (E) inlet gas connection; (F) gasometers; (G) acid-saline solution.



Source: the authors (2023).

Figure 2 - Scheme of the system for monitoring destructive samples.

Physicochemical analysis of substrates and effluents from the biodigesters

The physico-chemical characteristics of the biodigester substrates were analyzed according to the Standard Methods for Examination of Water and Wastewater (APHA, 1995). The initial contents of TS, VS, and COD, as well as their removal, were performed in triplicate.

The gas volume produced by the biodigesters was measured by the vertical displacement of the gasometers, with a subsequent correction for Normal Temperature and Pressure Conditions (NTP). The hydrogen content and volume were determined by collections, and the weighted average was calculated.

Gas chromatography was employed to determine the gas composition and volume of hydrogen and methane produced. A Shimadzu® 2010 system was used with a Carboxen™-1010 Capillary portion GC column, with argon as the gas carrier. The injector temperature was set to 200°C, and detection was conducted using a thermal conductivity detector (TCD) at 230°C. The methodology was adapted from Ekwenna *et al.* (2023).

Table 1 - Experimental conditions of treatments for methane production.

Treatment	Coded variables		Numeric variables	
	B ($g.L^{-1}$)	T (°C)	B ($g.L^{-1}$)	T (°C)
1	-1	-1	2	36.0
2	-1	0	2	37.5
3	-1	1	2	39.0
4	0	-1	4	36.0
5	0	0	4	37.5
6	0	1	4	39.0
7	1	-1	6	36.0
8	1	0	6	37.5
9	1	1	6	39.0

B: sodium bicarbonate; T: temperature.

The organic acids in the liquid phase (acetic, butyric, propionic, formic, and lactic) were quantified by varying the glycerol concentration at 40°C, a condition known to enhance hydrogen production. Analyses were carried out using high-performance liquid chromatography (HPLC) in a Shimadzu® system equipped with Aminex® HP-87H column and UV detector with SPD-20A diode arrangement set at a wavelength of 208 nm. The mobile phase consisted of acidified ultrapure water with 0.005 M sulphuric acid, following a methodology adapted from Park *et al.* (2020).

Kinetics of gas production

The kinetics data of hydrogen and methane productions were fitted to the non-linear Gompertz model (Equation 1). This model has been used to describe the kinetics of biogas production in anaerobic biodigestion processes and to characterize the kinetics of hydrogen production (SILVA *et al.*, 2018).

$$M(t) = Aexp\left[-exp\left(\frac{\mu_{max}}{A}e(\lambda - t) + 1\right)\right] \quad (1)$$

where: (M(t)) is the volume of biogas produced over time t (hours for the acidogenic phase; days for the methanogenic phase); (A) is the maximum gas volume (mL); (μmax) is the maximum specific velocity (mL h^{-1}); (λ) is the lag phase time (hours for the acidogenic phase; days for the methanogenic phase).

After determining the optimal conditions for hydrogen and methane production, the bi-sigmoidal kinetics of biogas generation was studied. This analysis employed the Gompertz equation (Equation 2), which comprises two components: 1. biogas production during the acidogenic phase and 2. biogas production during the methanogenic phase.

$$M(t) = AHexp\left[-exp\left(\frac{\mu_{maxH}}{AH}e(\lambda H - t) + 1\right)\right] + AMexp\left[-exp\left(\frac{\mu_{maxM}}{AM}e(\lambda M - t)1\right)\right] \quad (2)$$

where: (M) is the ratio between the volume of produced biogas and the volume of biodigester (mL L^{-1}) as a function of time (t); (AH) represents the maximum volume of biogas produced in the acidogenic biodigester (mL); ($\mu maxH$) is the biogas specific maximum velocity in the acidogenic biodigester (mL day^{-1}) L^{-1}; (λH) is the lag phase time of the acidogenic biodigester (days); (AM) is the maximum biogas volume produced in the methanogenic biodigester (mL L^{-1}); ($\mu maxM$) is the biogas specific maximum velocity in the methanogenic biodigester (mL day^{-1}) L^{-1}; (λM) represents the lag phase time of the methanogenic biodigester (days).

RESULTS AND DISCUSSION

Substrate and inoculum characterizations

Table 2 shows the results of the initial characterization of substrates and inoculum. The pH of the cassava wastewater (the main component of the tested mixtures) indicated the acidity of the reaction medium and was considered appropriate for digestion during hydrogen production. Since the optimal pH for biohydrogen production is determined to be 6.5, production ceases below an initial pH of 4.5 (RASHIDI *et al.*, 2024).

However, in the study by Silva *et al.* (2018), the pH was adjusted for the performance of the methanogenic phase of two-phase biodigestion. In this way, adding sodium bicarbonate is a viable alternative to adjust pH and buffer the liquid medium to a pH range that favors the action of methane-producing microorganisms.

The inoculum employed in this study underwent microbiological assessment as conducted by Meier *et al.* (2020). Sequencing analysis revealed that, of the four bacteria isolated, three were identified as belonging to the genus *Bacillus*, while one was categorized under the genus *Brevundimonas*. These microorganisms are known for their predominant trait of hydrogen production.

Anaerobic digestion (acidogenic phase)

The substrate mixture used resulted in a COD of 101.6 g/L^{-1}. Of these, 98.1 g/L^{-1} were derived from glycerol, and 3.5 g/L^{-1} from cassava wastewater. Hydrogen production is an attractive pathway from the energy point of view because it presents low energy consumption and enables the joint or sequential development of the methanogenic phase (CREMONEZ *et al.*, 2019). Despite the lower proportions compared to methanogenic digestion, acidic digestion contributes to wastewater treatment because it favors the reduction of organic loads and solid contents (ARANTES *et al.*, 2017).

The experimental hydrogen production data were fitted to the Gompertz model (Figure 3) and presented a correlation coefficient (R^2) of 0.998. The model obtained kinetic parameters of 17.9 h for the lag phase time, 2,327 mL.h^{-1} for maximum specific velocity and 433.74 mL $V_{Mixture}$ for the volume of produced hydrogen per liter of the inoculum/substrate mixture added to the biodigester. A high accumulated hydrogen production was found in a short period (approximately 10 hours), soon after the lag phase time estimated by the Gompertz model, ceasing the production after 35 hours. The period preceding the onset of gas production can be considered the time of the adaptation phase of the

Table 2 – Characteristics of the effluents studied.

Parameter	Cassava wastewater	Glycerol	Inoculum
pH	5.4	8.1	7.2
COD (g.L^{-1})	5.2 ± 0.4	876.0 ± 1.2	0.3 ± 0.1
TS (%)	6.3 ± 0.2	68.3 ± 0.4	2.1 ± 0.2
VS (%)	5.9 ± 0.3	61.7 ± 0.6	0.3 ± 0.1
TRS (%)	6.1 ± 0.2	-	-

COD: chemical oxygen demand; TS: total solids; VS: volatile solids; TRS: total reducing sugar.

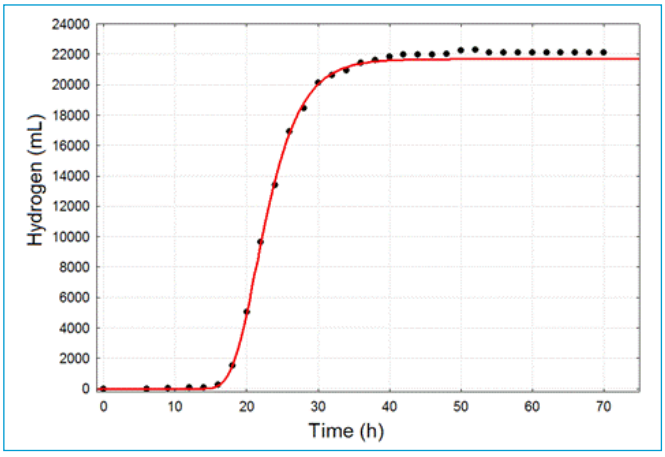


Figure 3 – Hydrogen production in the acidogenic biodigester.

microorganisms. This period is related to the acclimatization of microorganisms to the medium for the beginning of the digestion process.

Table 3 shows the effluent profile of the outlet of the acidogenic reactor. The results showed that the removal of solids and COD removal (CODR) was higher than 40%, which aligns with what is expected for a two-phase treatment system. Consequently, the volatile acids generated and remaining from the acidic digestion can be consumed in the methanogenic phase of the process. The high production of acids found is consistent with the pH (5.1) of the effluent. Nevertheless, the pH remained within the ideal range for the development of the acidogenic bacteria.

Figure 4 illustrates the organic acid production profile during acidogenic digestion using destructive samples. Regarding organic acid production, acids were detected within approximately 10 hours following the initiation of the digestion process. Butyric acid was found to be the predominant acid throughout the sampling period, followed by acetic acid. The molecular hydrogen in the acidic digestion process commonly originates from the production pathways of these acids by the conversion of glucose (WEI *et al.*, 2018).

The acidic digestion time was very shorter than that found for the methanogenic-phase reactors. This is due to the fast stability of acidogenic bacteria and the high activity of the cultures. Thus, shorter times for hydraulic retention than the time for the methanogenic lag phase were defined to prevent the proliferation of methanogenic microorganisms (ALGAPANI *et al.*, 2018; KOROGLU; OZDEMIR; OZKAYA, 2019).

It was observed that the total organic acid concentration began to decrease after 30 hours of digestion, indicating the completion of the conversion of sugars and the beginning of the consumption of the acids produced in the reactor. One hypothesis for this result is that the acids consisting of chains with more than three carbons were reduced to acetic acid. Subsequently, the acetic acid was metabolized and converted into H_2 and CO_2, which explains the decrease in total acid concentration while acetic acid concentration was stable.

Table 3 - Treatment of substrates in the acidogenic biodigester.

Biodigester effluent	
pH	5.1 ± 0.1
CODR (%)	43.9 ± 1.4
TSR (%)	47.3 ± 0.4
VSR (%)	48.6 ± 0.3

Anaerobic digestion – methanogenic phase

The experimental design used in this study utilized a significance level of 5%, with a F_{tabled} value of 4.95 based on the Snedecor F-distribution. Table 4

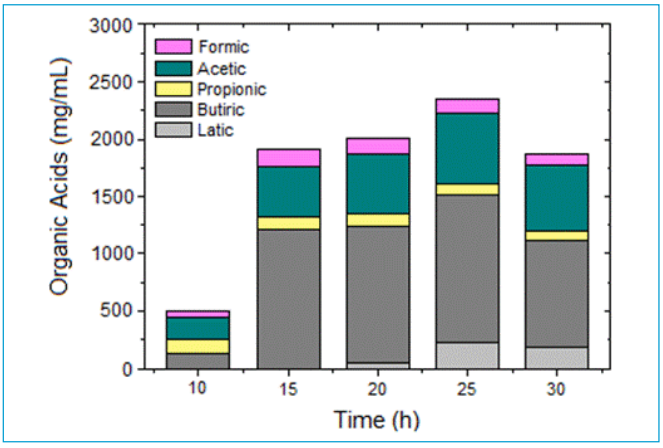


Figure 4 - Profile of organic acids for the acidogenic digestion.

Table 4 - Gas production and kinetic parameters obtained in the different treatments studied.

Treatment	Conditions		Methane production		Kinetic parameters		
	B ($g.L^{-1}$)	T (°C)	V (mL) $V_{Substrate}$ ($mL.L^{-1}$) $V_{CH4.}$	C ($g.L^{-1}$)	A (mL) ($mL.L_{Substrate}^{-1}$)	μ_{max} (h^{-1})	λ (d)
1	2	36.0	1,985.6 992.8	52.0	1,902.6 951.3	346.9	5.4
2	2	37.5	2,113.3 1,056.6	54.0	2,046.8 1,023.4	312.4	5.0
3	2	39.0	2,118.7 1,059.3	59.0	2,059.3 1,029.6	319.9	5.1
4	4	36.0	2,678.1 1,339.0	63.0	2,655.6 1,327.8	512.7	5.4
5	4	37.5	2,766.0 ± 17.0* 1,383.0 ± 8.5*	66.0 ± 1.0*	2,773.7 ± 19.2* 1,386.8 ± 9.6*	546.2 ± 4.5*	5.4 ± 0.3*
6	4	39.0	2,698.8 1,349.4	64.0	2,682.3 1,341.1	524.1	5.4
7	6	36.0	2,587.4 1,293.7	62.0	2,567.2 1,283.6	623.3	5.5
8	6	37.5	2,630.5 1,315.2	58.0	2,607.6 1,303.8	423.6	3.8
9	6	39.0	2,654.3 1,327.1	61.0	2,640.2 1,320.1	403.4	3.9

B: sodium bicarbonate; T: temperature; V: volume; C: methane concentration; A: maximum volume of methane; μ_{max}: specific maximum velocity; λ: lag phase duration time. Values with (*) are means followed by their respective standard deviation.

presents the experimental conditions for each treatment, along with the corresponding responses, including methane volume and concentration, and the kinetic parameters assessed. Methane contents ranged from 52 to 64%, with the highest content observed in the treatment that combined 4.0 g L^{-1} of sodium bicarbonate and a temperature of 39.0°C. Additionally, the central point yielded the most favorable results regarding methane production volume.

Regarding the parameters obtained from the Gompertz model (Table 4 and Figure 5), the R^2 of all treatments was higher than 0.99.

The time obtained for the lag phase was similar for most treatments. However, the treatment with 6 g L^{-1} of sodium bicarbonate had a more significant variation and lower results. Similarly, the treatment combining the addition of 6.0 g L^{-1} of sodium bicarbonate with a temperature of 36°C presented a higher maximum specific velocity.

Figure 5 indicates that, for all treatments, methane production ceased between the 12th and 18th day of digestion, as indicated by the stabilization of accumulated production points. Treatments with lower sodium bicarbonate content required longer digestion times to complete biogas production. This is because carbonates and bicarbonates act as buffers for the organic acids produced, which controls and prevents pH fluctuations, thus maintaining the system's stability (AKBAS; BILGEN; TURHAN, 2015). The growth rate of anaerobic microorganisms and the subsequent production of biogas are significantly influenced by the composition of the organic matter in the feedstock. Different microbial consortia selectively consume feedstock components, thereby neutralizing the formation of ammonia, which is essential for maintaining a neutral pH for cell growth during the digestion process. However, an excess of nitrogen can be toxic to bacteria due to ammonia overproduction. Therefore, it's essential to maintain a balanced nitrogen level to provide sufficient nutrients without causing ammonia toxicity and acidification during digestion (RABII *et al.*, 2019).

It is well-established that methanogenic microorganisms are most active when pH is close to neutral. Therefore, lowering the initial pH of the inoculum below 6 helps to decrease methanogen activity. Conversely, the use of an alkaline bicarbonate buffer effectively inhibits methanogen growth and boosts hydrogen production (IVANENKO *et al.*, 2024).

Several factors can decrease the performance of anaerobic digesters, including pH range, accumulation of ammonia, and volatile fatty acids, which inhibit the activity of methanogenic microorganisms. Besides ammonia, other factors such as sulfide, sodium, potassium, heavy metals, volatile fatty acids, long-chain fatty acids, and hydrogen can also affect methanogen activity. This inhibition

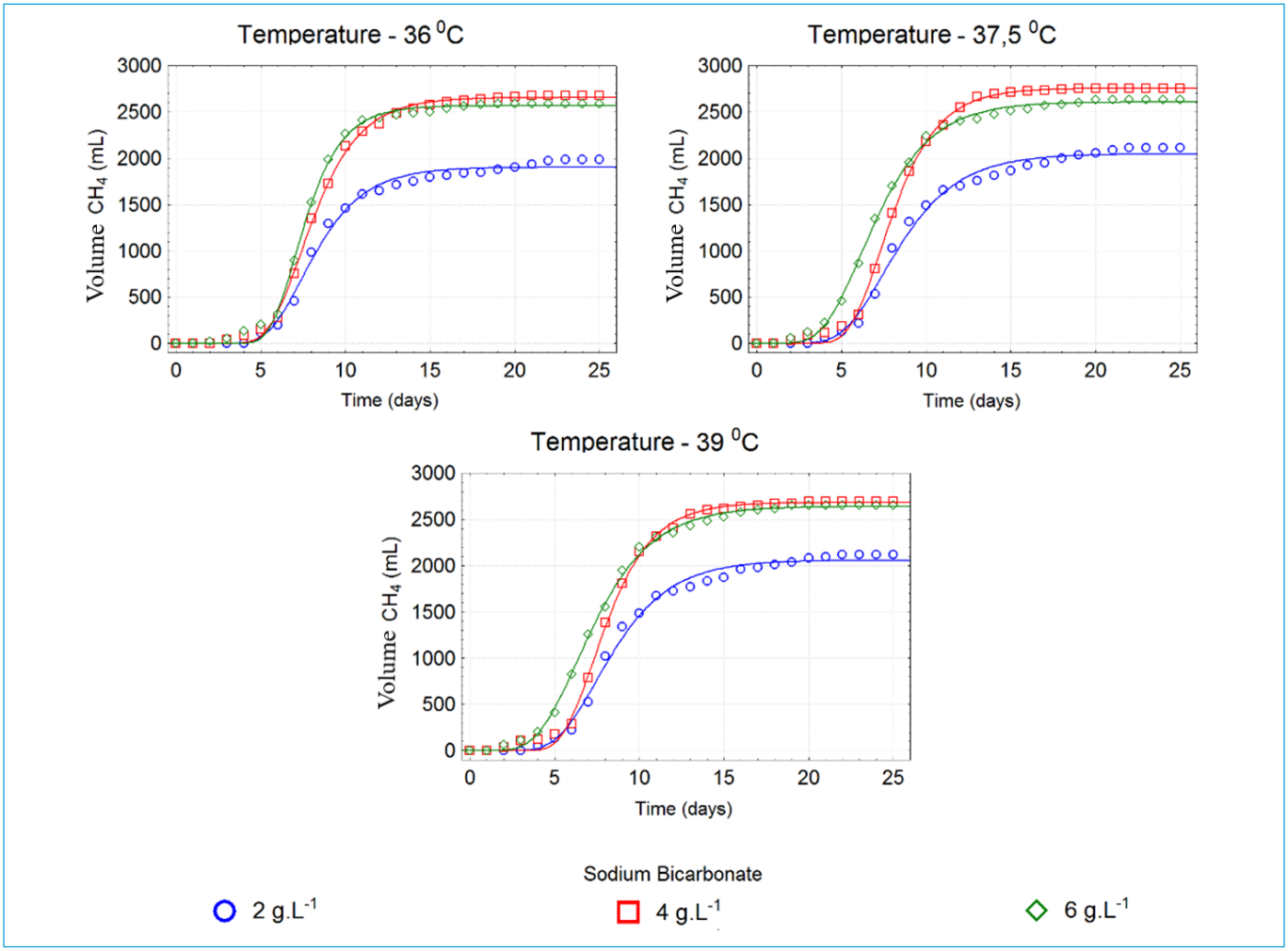


Figure 5 – Kinetics of methane production.

occurs due to an imbalance between hydrolysis and methanogenesis rates. Thus, a proper balance between these rates is essential for increasing methane production, since achieving rapid methanogenesis is necessary to prevent the accumulation of organic acid, which reduces the pH to a point that inhibits methanogenesis (RABII *et al*., 2019).

The process's stabilization time was approximately 12 days. According to Srisowmeya, Chakravarthy, and Nandhini (2020), this is one of the advantages of using two-phase biodigestion. One-phase biodigestion has stabilization times between 20 and 30 days, whereas two-phase biodigestion has a shorter stabilization time: approximately two to four days for the first phase and eight to ten days for the second.

Figure 6 shows the responses obtained for temperature and sodium bicarbonate interaction. The independent variable sodium bicarbonate had a significant effect on the responses of methane production and concentration. In contrast, the temperature variation did not significantly affect methane production and contents. Considering the surface response, the sodium bicarbonate percentages between 4.0 and 5.0 g L^{-1} were responsible for the best results.

It is worth noting that all temperatures studied were in the mesophilic range (between 20 and 40°C). Nevertheless, evaluations of the effect of temperature on psychrophilic and thermophilic ranges require experiments with larger temperature ranges so that other cultures of microorganisms can develop (CREMONEZ *et al*., 2019).

Regarding the effluents of the biodigesters, Figure 7 shows the surface response for the variables referring to the removal of organic loads. Results showed removals of TS, VS, and COD higher than 80% for all variables. Similar results were obtained for methane production and contents, where the best results for removing TS and VS contents and COD were found with the addition of close to 4.0 g/L^{-1} of sodium bicarbonate. Temperature variation presented no significant effect on the variables tested.

Ideal conditions for the methanogenic phase

The results of the statistical analysis and mathematical model for the volume of methane produced through the desirability function of the Statistica 7.0 software were used to determine the ideal temperature and sodium bicarbonate concentration to reach the maximum methane volume, which were 39.0°C and 5.0 g L, respectively. The response variables total solids removal (TSR) and removal chemical oxygen demand (RCOD) were disregarded since these variables presented calculated values lower than the F_{tabled} value.

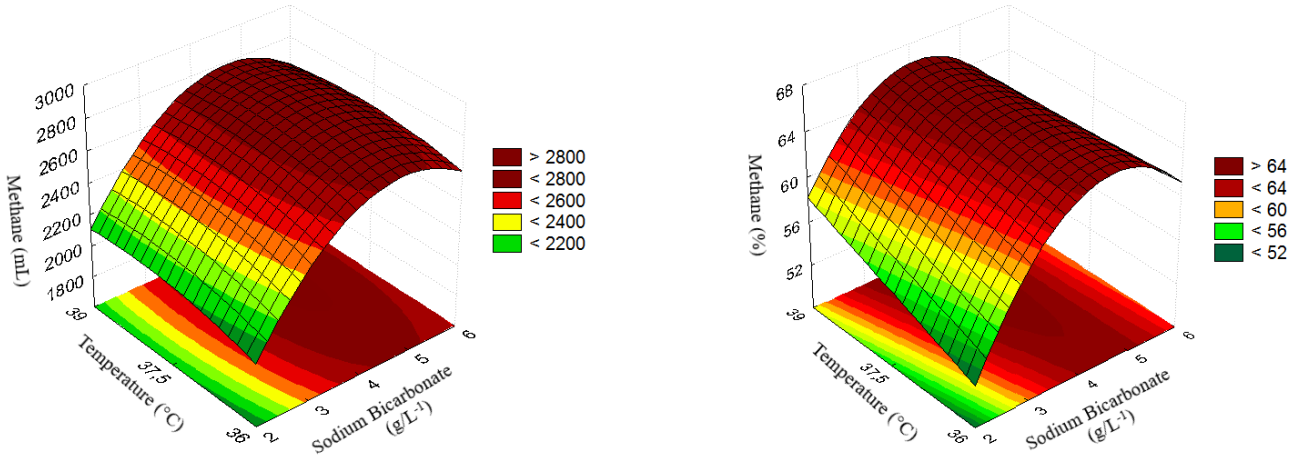
The results of the statistical analysis and mathematical model, conducted using the desirability function of software Statistica 7.0, were utilized to determine the optimal temperature and sodium bicarbonate concentration for maximizing methane production volume. These optimal conditions were determined to be 39.0°C and 5.0 g/L, respectively. The response variables, TSR and RCOD, were disregarded since these variables presented calculated values lower than the F_{tabled} value.

Table 5 shows the expected responses of the model when using these optimized conditions in the biodigestion process, and the results obtained in the tests (performed in triplicates) carried out under these conditions.

Considering the standard deviation of the experimental results, all values predicted by the mathematical model were within the range of the experimental values. However, the lag phase time, although similar, presented lower values than those predicted. This can be considered a positive result.

In optimized conditions, during the methanogenic digestion, the organic acids profile showed the presence of acids since the first verification of destructive samples, two days after the beginning of the digestion process (Figure 8).

The concentrations of total organic acids were favorable even at the beginning of the process, when it already presented the highest values, approximately 1,800 mg/L^{-1}. According to Qu *et al.* (2024), the maximum concentration of volatile acids that can inhibit the anaerobic digestion process is above 6,000 mg/L^{-1}.



Methane (mL)= 2758,99* + 275,76 B - 373,04 B^{2*} + 36,78 T* - 56,48 T^{2*} - 16,55 BT

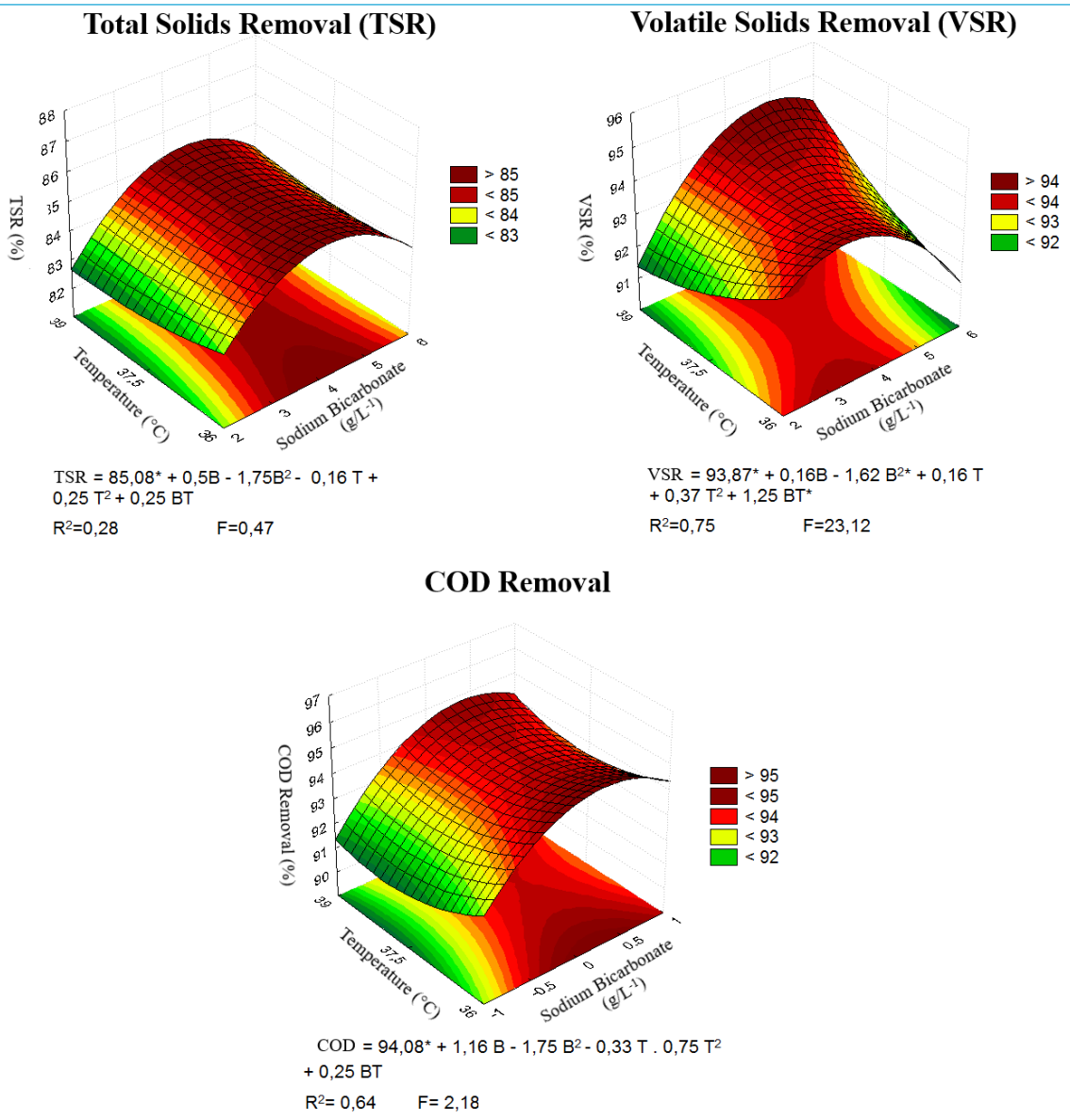
R^2= 0,994 F = 222,65

Methane (%) = 65,16* + 2,66 B* - 7,5 B^{2*} + 1,16 T - 2,00 BT

R^2=0,90 F = 60,62

T: temperature (°C); B: sodium bicarbonate (g/L^{-1}). Terms with superscript (*) asterisks are significant at 5% level.

Figure 6 – Surface response for methane production and concentration in the treatments studied.



Source: the authors (2023).
T: temperature (°C); B: sodium bicarbonate (g/L^{-1}). Terms with superscript asterisks are significant at 5% level.

Figure 7 – Surface response referring to the removal of organic loads in the dependent variables: removal of total solids, volatile solids, chemical oxygen demand.

Table 5 – Comparison between results from experimental tests and results predicted by the model for methane production.

Parameter	Model prevision	Experimental result*
Methane (mL)	2,775.6	2,819.4 ± 46.7
Methane (%)	64.8	64.5 ± 0.4
Lag phase duration time (d)	4.0	3.8 ± 0.1
Maximum specific velocity (d^{-1})	487.7	493 ± 12.1
TSR (%)	85.0	87.6 ± 3.8
VSR (%)	94.6	95.3 ± 2.6
CODR (%)	94.7	93.4 ± 3.7
Production of CH_4 L^{-1}_{waste}	1,732.5	1,758.1 ± 29.2

TSR: removal of total solids; VSR: removal of volatile solids; CODR: chemical oxygen demand removal. *Values are means followed by their respective standard deviation.

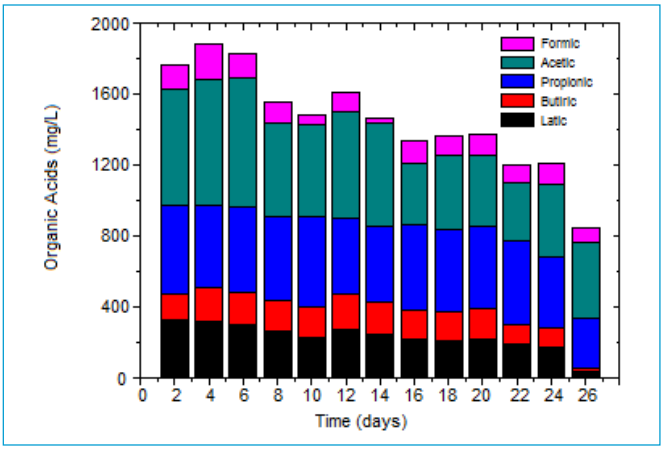


Figure 8 – Organic acids present in the effluent under the optimized conditions.

The presence of acetic acid predominated throughout the sampling period. According to Equation 3, when consumed, this acid can be converted directly into CH_4 and CO_2 (YE *et al.*, 2013), contributing to a high specific maximum velocity. The proportional consumption of organic acids occurred throughout the biodigestion process, which resulted in methane production with no major changes in the ratios between these acids in the liquid phase, indicating the stability of the process (WEI *et al.*, 2018).

Two-phase biodigestion evaluation

Figure 9 shows the kinetics of hydrogen production in the pilot-scale acidogenic biodigester, methane production in the methanogenic biodigester, and biogas production in the two-phase biodigestion (sum of the biogas produced in the two biodigesters under the optimized conditions).

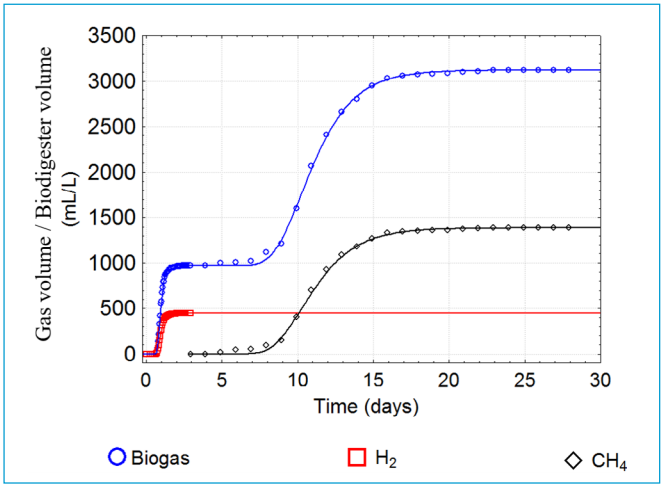


Figure 9 - Kinetics of gas production by two-phase biodigestion.

Table 6 shows the experimental data of the three cases fitted to the Gompertz model and the kinetic parameters obtained.The lag phase time of the acidogenic phase, as well as that of the biodigestion process, were shorter than that of the methanogenic phase. It was also observed that the acidogenic reactor produced hydrogen for approximately 35 hours and methane for approximately 12 days.

The specific productions of hydrogen and methane were obtained through the volumes of these gases produced in the pilot-scale acidogenic reactor and in the methanogenic reactor under the optimized conditions obtained by the desirability function of the Statistica 7.0 program (Table 7). Table 7 compares the results found in the present work with those of previous studies that also evaluated two-phase biodigestion for the treatment of cassava wastewater present in the literature. The hydrogen yield obtained was twice that reported by Chavadej *et al.* (2019). Although it was slightly lower than some yields reported in other studies, it exhibited a higher concentration of hydrogen in the produced gas. The methane yield obtained in this study was comparable to that found by Intanoo, Chaimongkol, and Chavadej (2016), while the yield reported by Chavadej *et al.* (2019) was approximately 2.5 times higher.

However, Chavadej *et al.* (2019) and Intanoo, Chaimongkol, and Chavadej (2016) used a semi-continuous flow reactor, which may have contributed to the increase in the production of hydrogen and methane since the results were obtained with a stabilized gas production.

These comparisons confirm the hypothesis that the addition of glycerol to cassava wastewater (bioavailable carbon sources) and the use of swine wastewater as an inoculum is a viable alternative for treating these effluents, since it presents the advantage of obtaining higher concentrations of hydrogen, thereby enabling its use in more efficient energy production processes than direct burning in boilers.

Table 6 - Kinetic parameters of the two-phase biodigestion.

Parameter	Hydrogen	Methane	Biogas
A – maximum volume of gas (mL.L^{-1})	433.8	1409.7	AH = 943.8 AM = 2,196.3
μmax – specific maximum velocity (L.mL^{-1})	46.5	246.5	$\mu maxH$ = 102.2 $\mu maxM$ = 393.4
λ – lag phase duration time (day)	0.75	6.8	(λH) = 0.73 λM = 6.8

(AH) maximum volume of biogas produced in acidogenic biodigester; $(\mu maxH)$ specific maximum velocity of biogas produced in acidogenic biodigester; (λH) lag phase duration time of acidogenic biodigester; (AM) maximum volume of biogas produced in methanogenic biodigester; $(\mu maxM)$ specific maximum velocity of biogas produced in methanogenic biodigester; (λM) lag phase duration time of methanogenic biodigester.

Table 7 - Yield evaluation of the two-phase biodigestion using cassava wastewater as substrate.

Substrate	Inoculum	H_2/RCOD (mL/g)	CH_4/RCOD (mL/g)	Reference
Cassava + residual glycerol	Swine wastewater	30.8 (46.5%)*	104.5 (64.5%)*	This study
Cassava + residual glycerol	Semi-continuous flow reactor sludge for cassava treatment	15.0 (43.0%)*	259.0 (70.5%)*	Chavadej *et al.* (2019)
Cassava	Anaerobic lagoon sludge for the treatment of cassava starch effluent	39.8 (36.4%)*	100.0 (63.6%)*	Intanoo, Chaimongkol, and Chavadej (2016)

*The values correspond to the concentration of H_2 or CH_4 in the gas produced.

CONCLUSIONS

The mixture of cassava wastewater and residual glycerol (bioavailable carbon sources) demonstrated significant degradation potential through anaerobic two-phase biodigestion, employing swine wastewater as inoculum. In addition to hydrogen production, using a methanogenic reactor in two-phase biodigestion facilitated the reduction of organic substrate loads while yielding methane gas, thereby enhancing the overall energy output of the process. Furthermore, it was observed that temperature variations had a relatively minor impact compared to the addition of sodium bicarbonate. Therefore, the buffering of the biodigester is essential to the process.

AUTHORS' CONTRIBUTIONS

Weiser Meier, T.R.: Conceptualization; Data Curation; Formal Analysis; Investigation; Methodology; Visualization; Writing – Original Draft; Writing – Review & Editing. Cremonez, P.A.: Methodology; Visualization; Writing – Original Draft; Writing – Review & Editing. Oliveira, C.J.: Methodology; Writing – Original Draft; Writing – Review & Editing. Teleken, J.G.: Funding Acquisition; Methodology; Project Administration; Supervision; Validation; Writing – Original Draft; Writing – Review & Editing. Palú, F.: Data Curation; Writing – Review & Editing. Campos, P.R.F: Data Curation; Writing – Original Draft; Writing – Review & Editing. Silva, E.A.: Writing – Original Draft; Writing – Review & Editing.

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