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Study of Anaerobic Co-digestion of Crude Glycerol and Swine Manure for the Production of Biogas

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Estudo da Codigestão Anaeróbia de Glicerol Bruto e Dejeto Suíno para Produção de Biogás

Resumo: Glicerol bruto e dejetos suínos são efluentes produzidos em larga escala pela indústria do biodiesel e pela suinocultura, respectivamente. O aproveitamento de tais efluentes para a produção de biogás contribuiria para o aumento da oferta de combustíveis renováveis e para a mitigação das emissões de gases de efeito estufa. Diante de tal perspectiva, o presente estudo avaliou a produção de biogás a partir da codigestão anaeróbia do glicerol bruto com o dejeto de suínos através do uso de metodologia de superfície de resposta (MSR). Foi empregado um planejamento composto central com dois fatores, quatro pontos axiais e quatro centrais. Os dois fatores avaliados foram a concentração do glicerol bruto e a concentração dos dejetos suínos, que foram examinadas na faixa de 4 a 10 g L^{-1} e 5 a 15 g L^{-1}, respectivamente. Lodo ativado obtido de estação de tratamento de esgoto urbano foi usado como inóculo. A razão carbono:nitrogênio (C/N) nos reatores variou de 17,9:1 a 63,6:1. Foram observados efeitos lineares positivos e significativos (p<0,05) para os fatores avaliados sobre a produção de biogás. A máxima produção de biogás observada foi de 521,5 mL por grama de COD inicial quando a codigestão foi conduzida com 4 g L^{-1} de glicerol bruto e 5 g L^{-1} de dejeto suíno após 35dias de processo. Os dados experimentais mostraram que o maior rendimento de biogás foi obtido com razão C/N de 29,4 e pH próximo a 6,5.

Keywords: Biodigestão; tratamento de efluentes; glicerina bruta; biometano.

Abstract

Crude glycerol and swine manure are large-scale effluents produced by the biodiesel industry and swine breeding, respectively. The use of such effluents for the production of biogas would contribute to increase the supply of renewable fuels and to decrease emissions of greenhouse gases. The present study evaluated the production of biogas from the anaerobic co-digestion of crude glycerol with swine manure through the use of response surface methodology (RSM). A central composite design was employed with two factors, four axial points and four central points. The two factors evaluated were crude glycerol and swine manure concentrations, which were examined in the range of 4 to 10 g L^{-1} and 5 to 15 g L^{-1}, respectively. Activated sludge obtained from an urban sewage treatment plant was used as inoculum. The carbon:nitrogen ratio (C/N) in the reactors ranged from 17.9:1 to 63.6:1. Positive, significant linear effects (p<0.05) on biogas production were observed for the two factors evaluated. The maximum biogas production observed was 521.5 mL per gram of initial COD when the co-digestion was conducted with 4 g L^{-1} of crude glycerol and 5 g L^{-1} of swine manure after 35 days. The highest biogas yield was obtained with a C/N ratio of 29.4 and pH close to 6.5.

Palavras-chave: Biodigestion; wastewater treatment; crude glycerin; biomethane.

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Study of Anaerobic Co-digestion of Crude Glycerol and Swine Manure for the Production of Biogas

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1. Introduction

Anaerobic digestion is a biological process that produces biogas from biodegradable waste through the action of bacteria in the absence or restriction of oxygen. This biological process is generally used for the treatment of organic waste because of its good performance in volume reduction, residue stabilization and the generation of biogas and biofertilizer.[1] The biogas generated by the anaerobic digestion of organic matter can contain between 45 and 70% of methane (CH_4). From the energy point of view, one kg of methane contains 55.5 MJ of energy and is equivalent to 1.2 kg of LPG, 1.2 kg of diesel or 3.7 kg of wood.[2,3] The anaerobic digestion of organic waste involves a series of metabolic routes (hydrolysis, acidogenesis, acetogenesis and methanogenesis) in which different types of microorganisms participate. In an environment with a pH between 6.5 and 7.5 and a C/N (carbon:nitrogen) ratio between 15 and 35, these microorganisms find the optimal conditions for the degradation of organic compounds and the production of methane.[1,4]

Two large niches in the world market are responsible for the generation of effluents with a large organic load, and they deserve attention from the sanitary, environmental and economic point of view. They are the swine breending and the biodiesel industry. The annual global consumption of pork is about 118 million tons.[5] Brazil is the fourth largest producer of pork in the world, with a total annual production of 3.47 million tons.[6] This situation results in the generation of huge quantities of swine manure (SM), which can cause environmental imbalance and damage to human health if it does not have an adequate destination for disposal.[7] With regard to the biodiesel market, around 30 billion liters of this fuel are produced worldwide.[8] Brazil is the second largest biodiesel producer in the world, reaching 3.8 billion liters in 2016.[9] It is estimated that approximately 10 kg of crude glycerol (CG) is generated for every 100 kg of biodiesel produced.[3,10] However, as a consequence of the high cost of CG purification, its allocation to markets such as the pharmaceutical, fine chemical, cosmetics and food markets is unprofitable.[11] A destination that is common and consonant with the legislation applied for the mentioned effluents would be anaerobic digestion with consequent production of biogas and biofertilizer.

Anaerobic co-digestion is a type of organic waste treatment where different substrates or effluents are mixed and processed together.[12] This method is used to improve the performance in anaerobic digestion,[13-16] provide a buffering effect that avoids possible inhibition of the process by alteration of pH, furnish nutrients absent in certain effluents, or adjust the C/N ratio to stimulate methanogenic activity and to enable a possible increase in the kinetics of biogas production.[13,17-19]

Some studies on biogas production have shown that the ideal C/N ratio for this purpose is between 20:1 and 30:1.[20] The exclusive use of SM for biogas production results in a low conversion efficiency of the organic compounds into methane because of the low C/N ratio, which also favors the production of ammonia.[21] The use of CG, which has a high relative carbon content, mixed with animal waste would adjust the C/N ratio to more desirable values. However, co-digestion experiments with SM and CG already reported in the scientific literature did not address the C/N parameter or explore C/N values that were limited and below the ideal values.[22,23]

The evaluation of the substrates or effluents destined for biogas production should seek to clarify the synergistic effects and avoid possible antagonistic effects during anaerobic codigestion. In this sense, the response surface methodology (RSM), which uses a set of mathematical and statistical methods to evaluate the relationships between multiple independent variables and one or more responses, is a very useful tool.[24-27] This study proposed to determine the influence of concentrations of crude glycerol and swine manure on the process of anaerobic co-digestion through the use of response surface methodology for the production of biogas and treatment of the effluents involved.

2. Material and Methods

2.1. Reagents, substrates and inoculum (activated sludge)

All the reagents and solvents used in this work were analytical grade, P.A.-A.C.S. The analytical standards used for liquid chromatography had a minimum purity of 99%. The crude glycerol used in this study was recovered after the transesterification of residual frying oil with sodium methoxide, and it was donated by the Laboratório de Produção de Biodiesel, Biolubrificantes e Biograxas of the Universidade Federal dos Vales do Jequitinhonha e Mucuri - UFVJM. Swine manure was obtained fresh from the swine facilities of the Animal Science Course of the same university. All effluents were maintained at -4 °C until use. The biogas production was accomplished using the mixed anaerobic microflora present in the activated sludge from the effluent treatment plant in operation at the JK Campus of the UFVJM in Diamantina, MG, Brazil, as inoculum.

2.2. Characterization of crude glycerol, swine manure and activated sludge

Crude glycerol, swine manure and activated sludge were characterized in terms of chemical oxygen demand (COD), total solids content (TS), volatile solids (VS) and pH using the analytical techniques proposed by the American Public Health Association.[28] All the analyses were performed in triplicate. The percentage of carbon, nitrogen and hydrogen in crude glycerol and swine manure was determined by elemental analysis (CHNS/O TruSpec® Micro Analyzer, LECO, USA).

2.3. Experimental planning for anaerobic co-digestion

The assays for the anaerobic co-digestion study were delimited by an experimental design using a central composite design, as presented in Table 1, containing two factors, four axial points and four central points. The factors evaluated were the concentration of crude glycerol (CG) and the concentration of swine manure (SM).

Table 1. Matrix of the central composite design 2^2 containing four axial points and four central points, used to study the anaerobic co-digestion of crude glycerol with swine manure

Variable	Factors	Axial (-α)	Minimum (-1)	Central point (0)	Maximum (+1)	Axial (+α)
x_1	Crude Glycerol (g L^{-1})	2.75	4.00	7.00	10.00	11.24
x_2	Swine Manure (g L^{-1})	2.93	5.00	10.00	15.00	17.07

All the assays were performed in batch mode using 50-mL glass cylinders and a silicone septum cap. The reactors were inoculated with 3 mL of activated sludge, which was equivalent to 120 mg of volatile solids. After addition of the activated sludge, the reactors received different amounts of the CG and SM substrates to reach the concentrations determined by the experimental design detailed in Table 2.

Table 2. Concentrations of crude glycerol (CG) and swine manure (SM) assigned by the central composite design used as planning to the anaerobic co-digestion assays

Assay	Factors	
	CG (g L^{-1})	SM (g L^{-1})
1	4.00	5.00
2	4.00	15.00
3	10.00	5.00
4	10.00	15.00
5	2.75	10.00
6	11.24	10.00
7	7.00	2.93
8	7.00	17.07
9	7.00	10.00
10	7.00	10.00
11	7.00	10.00
12	7.00	10.00

After adding the substrates, 2.0 mL of nutrient solution modified from information contained in the work of Aquino *et al*. (2007)[29] was added (Table 3). The final volume in the reactor was adjusted to 25 mL with distilled water, and the pH was determined. The flasks were closed to maintain an anaerobic environment and incubated in a thermostated bath at 30 ± 1.0 °C until the co-digestion terminated, as indicated by the end of the production of gas. The volume of biogas produced during the fermentation process was monitored every 24 hours for a 40 day period.

Table 3. Nutrient solution used as a supplement in biogas production trials

Macronutrient	Concentration (mg L^{-1})	Micronutrient	Concentration (mg L^{-1})
NH_4Cl	1112.0	$FeCl_3.6H_2O$	5.00
$(NH_4)H_2PO_4$	132.5	$ZnCl_2$	0.13
$(NH_4)_2HPO_4$	44.50	$MnCl_2.4H_2O$	1.25
$MgCl_2.6H_20$	250.00	$(NH_4)_6MO_7O_{24}.4H_2O$	1.60
$CaCl_2.2H_2O$	189.00	$AlCl_3.6H_2O$	0.13
$NaHCO_3$	2500.00	$CoCl_2.6H_2O$	5.00
-	-	$NiCl_2.6H_2O$	13.00
-	-	H_3BO_3	3.00
-	-	$CuCl_2.2H_2O$	8.00
-	-	HCl	1,00

The dependent variables or response factors of choice for the experimental analysis were the normalized volume of biogas produced (NmL) and the percentage reduction of COD. Statistica 7.0 software (StatSoft) was used for the analysis of the responses to allow the description of the results by means of the adjustment to a linear mathematical model (Equation 1) or a quadratic model (Equation 2) used for the generation of response surface curves and the determination of possible interactions.[25,30] Data were submitted to analysis of variance considering a level of significance (α) of 0.05. The coefficient of determination (R^2adj) was used as a parameter of adequacy of the mathematical models generated by a least squares regression to the phenomena evaluated.

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{1\,2} x_1 x_2 + \varepsilon \qquad \textbf{Eq. 1}$$

Where

Y= value of the dependent variable;

x_1 *e* x_2 = independente variables

β_0, β_1, β_2, $\beta_{1\,2}$ = parameters of the regression model for each variable;

ε= error term (effects not explained by the model).

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum \sum_{i<j}^{k} \beta_{ij} x_i x_i + \varepsilon \qquad \textbf{Eq. 2}$$

Where
Y= value of the dependent variable;
x_1 *e* x_2 = independent variables;
β_0,β_i,β_{ii}, β_{ij}= parameters of the regression model for each variable;
ε= error term (effects not explained by the model).

For the description of the mathematical, quadratic or linear models, the parsimony principle was adopted, excluding the statistically non-significant variables from the model. In addition to the response surface methodology, the Pearson correlation coefficient (ρ) was calculated crossing the C/N (carbon mass:nitrogen mass) and I/S (mass of VS from inoculums / mass of VS from substrates) parameters with the cumulative biogas response (NmL), biogas by mass of the total oxygen demand (NmL $gCOD_{total}^{-1}$), biogas by mass of total volatile solids (NmL gVS_{total}^{-1}), average biogas production rate (NmL $gCOD^{-1}$ d^{-1}) and percentage reduction of COD variables.

2.4. Quantification of the volume of biogas

The gas volume gauging system was adapted from the work of Aquino *et al.* (2007)[29] and was composed of a 500-mL inverted glass vial containing 3 mol L^{-1} solution of NaOH, whose function was to capture CO_2. The vial was adapted with a cover with an opening for the entrance of the gas produced in the anaerobic reactor and another opening for the exit of the liquid displaced by the gas (Figure 1). Measurements were performed every 24 hours after the first day of incubation by the liquid displacement method, in which the liquid displaced by the gas (Figure 1) was collected in a graduated cylinder, and the volume was then converted to the standard biogas volume (NmL) under normal conditions of pressure and temperature (1 atm, 0 °C), according to the Ideal Gas Law.

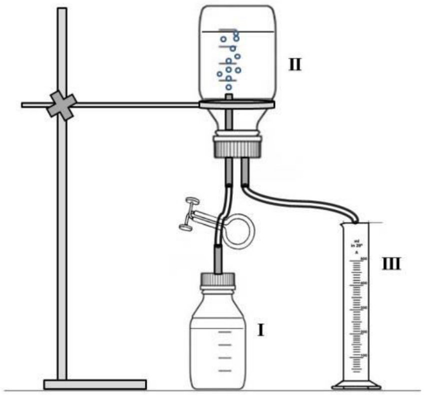


Figure 1. Apparatus for determining the volume of biogas produced: (I) reactor with exit for the biogas, (II) flask with 3 mol L^{-1} NaOH to remove CO_2 and (III) graduated cylinder for measuring the volume of liquid displaced by the biogas

2.5. Quantification of glycerol and organic acids

Glycerol and organic acids were quantified by high performance liquid chromatography (HPLC) using the Shimadzu Prominence UFLC 20A system, equipped with a Rezex ROA-Shodex™ column (300 x 7.5 mm), maintained at 60 °C, and coupled to a UV/Vis detector for organic acid analysis at 220 nm and a refractive index detector in series for measuring the glycerol. The automatic injector was programmed for 5-μL injections. A 0.0025 mol L^{-1} H_2SO_4 solution was used as the eluent at a flow rate of 0.6 mL min^{-1}. The identification of glycerol and organic acids, as well as their quantification, was performed with the use of external standards.

3. Results and Discussion

3.1. Characterization of crude glycerol (CB), swine manure (SM) and activated sludge (inoculum)

The results of the physical-chemical and elemental characterization of CG, SM and activated sludge are presented in Table 4. The alkaline pH of the CG results from the fact that it is a byproduct of transesterification of vegetable oil by homogeneous catalysis employing sodium methoxide, which results in an alkaline pH. The pH value is an important indicator for evaluating the stability of anaerobic digestion systems because it affects acidogenic and methanogenic microorganisms.[31,32] The concentration of volatile solids in CG was about 80% higher than that contained in SM. The percentage of carbon in CG was about 40% higher than that determined for SM. On the other hand, no significant amount of nitrogen was found in CG (%N <0.05). The percentage of carbon found in CG (88%) is not characteristic of this effluent, which, according to Thompson and He (2006)[33], can vary between 24 and 38%. This excess carbon may indicate the presence of unconverted free and saponified fatty acids from the transesterification process and the presence of unrecovered methyl esters.[34] SM contained 25% of proteins that would contribute as a source of nitrogen. According to Marone *et al*. (2015)[25] and Dennehy *et al*. (2011),[31] the addition of complementary nutritional components to waste co-digestion is a widely applied procedure to increase biogas production because the combination of different substrates can provide balanced nutrients, reduce costs related to pH control and the adjust the C/N ratio required to optimize the biogas production process.

Table 4. Physical-chemical and elemental characterization of crude glycerol (CG), swine manure (SM) and activated sludge (inoculum) used in the anaerobic co-digestion process

Parameters	CG	SM	Inoculum
	Mean ± Standard Deviation		
pH	10.30 ± 0.10	6.51 ± 0.54	6.73 ± 0.15
TS (g L^{-1})	870.34 ± 0.10	199.86 ± 0.10	80.95 ± 0.11
VS (g L^{-1})	870.10 ± 0.10	167.55 ± 0.10	40.72 ± 0.92
VS/TS (%)	99.97 ± 0.10	83.90 ± 0.10	50.30 ± 0.56
COD (g L^{-1})	1974.02 ± 3.10	137.83 ± 1.34	11.92 ± 0.59
Carbon (% m m^{-1}))	88.04 ± 0.05	49.62 ± 0.05	ND
Nitrogen (% m m^{-1})	<0.05	4.08 ± 0.05	ND
Hidrogen (% m m^{-1})	11.08 ± 0.05	6.50 ± 0.05	ND
Protein (% m m^{-1})	<0.05	25.55 ± 0.05	ND

TS: Total Solids; VS: Volatile Solids; COD: Chemical Oxygen Demand; ND: Not Determined.

3.2. Characterization of the initial conditions of the anaerobic co-digestion assays.

Although the pH values of the CG and SM effluents were individually significantly different (Table 4), pH values for the combination of the effluents under the different assay conditions were in the range of 7.1 to 7.6 (Table 5). No post-mixing pH adjustment was required because some authors report that this range is recommended for an efficient performance of methanogenic bacteria.[35,36]

The mixture of the CG and SM substrates used in this study contained 4.39 to 11.78 g L^{-1} of TS; 4.07 to 11.45 g L^{-1} of VS; and 6.12 to 19.41 g L^{-1} of COD (Table 5). The I/S ratio (mass of VS from inoculums / mass of VS from substrates) (Table 5) varied from 0.44 to 1.16 and is in line with the minimum value recommended by Labatut et al. (2011)[37] to ensure the onset of the anaerobic digestion, which, according to the authors, would be 0.5. The C/N ratio in the present study ranged from 17.88 to 63.63 (Table 5). The C/N ratio reflects the relationship between carbon sources and nitrogen sources that make up the fermentation medium. An elevated C/N ratio leads to low concentrations of free ammonia in the system. According to Zhang et al. (2013)[38] and Elsayed et al. (2015)[39], the most widely used C/N ratios in anaerobic digestion have been in the range of 10 to 35. There is no nitrogen (% N <0.05) in crude glycerol, and the combination with the swine manure was determinant in yielding C/N ratios higher than 12:1.

Table 5. Initial parameters of biogas production assays carried out with different concentrations of crude glycerol and swine manure

Assay	C/N Ratio ($g\ g^{-1}$)	I/S Ratio ($gVS\ gVS^{-1}$)	COD_{total} ($g\ L^{-1}$)	TS_{Total} ($g\ L^{-1}$)	VS_{Total} ($g\ L^{-1}$)	$pH_{initial}$
			Mean ± Standard Deviation			
1	29.37	1.16	7.17 ± 0.35	4.48 ± 0.13	4.32 ± 0.08	7.20
2	17.88	0.83	8.98 ± 0.79	6.48 ± 0.29	5.99 ± 0.13	7.15
3	55.22	0.52	16.56 ± 0.35	9.70 ± 0.39	9.54 ± 0.37	7.27
4	26.50	0.45	18.37 ± 0.26	11.70 ± 0.80	11.21 ± 0.67	7.51
5	18.06	1.23	6.12 ± 0.18	4.39 ± 0.37	4.07 ± 0.36	7.40
6	36.35	0.44	19.41 ± 0.35	11.78 ± 1.15	11.45 ± 1.07	7.00
7	63.63	0.76	11.49 ± 0.78	6.68 ± 0.70	6.58 ± 0.74	7.57
8	20.97	0.56	14.05 ± 0.10	9.50 ± 1.07	8.95 ± 0.97	7.19
9	27.22	0.64	12.77 ± 0.47	8.09 ± 0.27	7.77 ± 0.27	7.48
10	27.22	0.64	12.77 ± 0.47	8.09 ± 0.27	7.77 ± 0.27	7.36
11	27.22	0.64	12.77 ± 0.47	8.09 ± 0.27	7.77 ± 0.27	7.50
12	27.22	0.64	12.77 ± 0.47	8.09 ± 0.27	7.77 ± 0.27	7.60

C/N: mass of carbon / mass of nitrogen; I/S: mass of VS from inoculums / mass of VS from substrates; TS: Total Solids; VS: Volatile Solids COD: Chemical Oxygen Demand.

3.3. Evaluation of biogas production and reduction of COD

The progress curves for the production of biogas are presented in Figure 2. No lag phases were observed under any condition tested that indicated any restriction for the immediate onset of gas production. This observation led to the belief that there was a rapid adaptation of the microorganisms to the fermentation medium. The highest gas production rates were observed from the beginning of the fermentation until the sixth day, (Figure 2). This period was followed by a period in which the gas production occurred at a slower but steady rate. After the 35th day of fermentation, all the systems entered a steady state. According to Kafle and Kim (2013),[40] microbial degradation begins with substrates with a high soluble carbohydrate content, followed by proteins and subsequent degradation of lignocellulose, which is more recalcitrant.

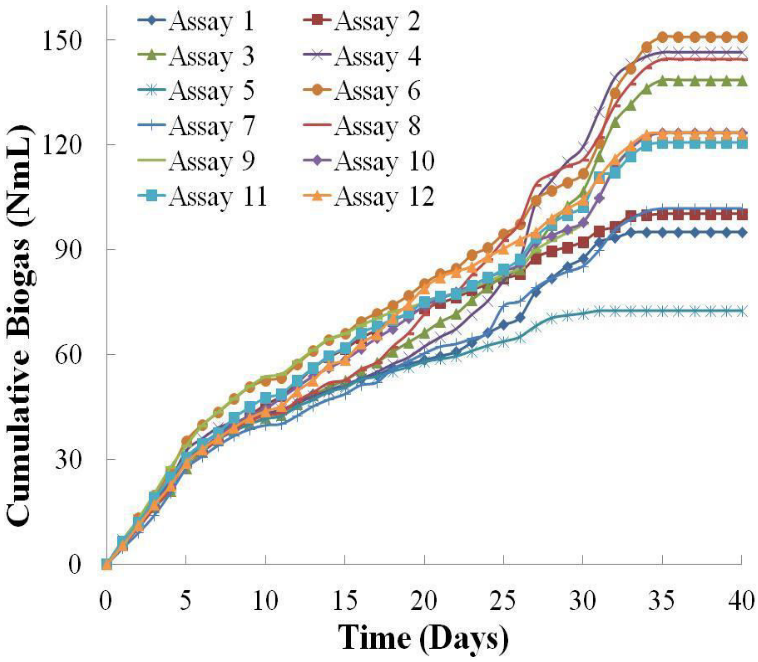


Figure 2. Profile of the biogas production in the anaerobic co-digestion assays

The biogas production was in the range 278.19 to 521.46 NmL per gram of COD_{total} or 458.38 to 834.57 NmL per gram of VS_{total} (Table 6). The average daily rates of biogas production ranged from 8.64 to 14.75 NmL $gCOD^{-1}$ d^{-1} (Table 6). The best biogas yields were observed in assays 1, 2 and 5 with the production of 521.46 NmL $gCOD^{-1}$, 430.02 NmL $gCOD^{-1}$ and 473.70 NmL $gCOD^{-1}$, respectively. Mean production rates were 14.75 NmL $gCOD^{-1}$ d^{-1}, 12.40 NmL $gCOD^{-1}$ d^{-1} and 13.16 NmL $gCOD^{-1}$ d^{-1} (Table 6). In assay 1, the ratio of SM to CG was 5:4. Astals *et al.* (2011)[14] obtained 215 NmL $gCOD^{-1}$ in an anaerobic codigestion process with SM and CG substrates mixed in a ratio of 4:1. Kafle and Kim (2013)[40] produced biogas from the combination of SM (77%) and apple residues (33%) with a yield of 398 NmL $gCOD^{-1}$ and 505.46 NmL $gCOD^{-1}$, under mesophilic and thermophilic conditions, respectively. The results obtained in the present study under mesophilic conditions were close to those obtained by Kalfe and Kim (2013) using thermophilic conditions.

The highest yields of biogas per unit mass of COD_{total} were observed in trials 1, 2 and 5 (Table 6), in which the C/N ratios were 29.4, 17.9 and 18.1, respectively (Table 5). Assays 3, 6 and 7, which had C/N ratios ≥ 36 (Table 5), were among those with the lowest biogas yields per gram of total COD (Table 6). Several authors have reported that the optimum C/N ratio for anaerobic digestion is in the range of 20 to 35.[36,41,42] The nitrogen limitation may be a hindrance to the efficient biodigestion of crude glycerol, whereas a carbon limitation could restrict the efficiency of the conversion of swine manure to biogas. Therefore, the combined use of SM, which has a high nitrogen content (4 % m m^{-1}), with CG may have contributed to the observed values for biogas production. However, the C/N ratio alone did not show a significant linear effect on the biogas production because the Pearson correlation coefficients (ρ) with the evaluated responses were negligible or weak ($\rho < 0.5$) (Table 7). On the other hand, the I/S ratio had a significant linear effect on biogas production (Table 7). The value of ρ for the correlation of the I/S ratio with biogas production per gram of COD_{total} was 0.925. Therefore, the I/S ratio exerted an effective and positive contribution to the yield of biogás per gram of COD. The same effect was observed on the biogas yield per gram of VS_{total}. The effect exerted by the I/S ratio on the accumulated biogas volume was negative (ρ = -0.926). A possible explanation for this observation is that microorganisms may have been suppressed by a restriction in nutrients, which may have occurred in trials 1, 2 and 5 because these trials involved the lowest CG intake.

Table 6. Final parameters from anaerobic co-digestion assays carried out with different concentrations of crude glycerol and swine manure

Assays	Final parameters					
	Accumulated biogas (NmL)	Biogas yield (NmL $gCOD_{Total}^{-1}$)	Biogas production rate (NmL $gCOD^{-1}$ d^{-1})	Biogas yield (NmL gSV_{Total}^{-1})	Final pH	COD Reduction (% m m^{-1})
1	93.45	521.46	14.75	834.57	6.80	32.74
2	96.55	430.02	12.40	595.92	7.00	53.27
3	126.53	305.62	9.29	521.76	6.48	77.38
4	139.24	303.14	8.87	475.96	6.65	70.49
5	72.45	473.70	13.16	660.37	6.94	30.18
6	134.98	278.19	8.64	458.38	6.54	79.92
7	95.62	332.91	9.85	572.63	6.16	71.31
8	131.18	373.41	11.41	552.25	6.46	60.53
9	113.90	356.76	10.75	563.14	6.37	64.01
10	113.36	355.06	10.74	560.46	6.36	66.16
11	112.04	350.94	10.49	553.96	6.34	68.32
12	116.00	363.31	10.73	573.48	6.48	67.14

VS: Volatile Solids COD: Chemical Oxygen Demand. The standard deviation on the main response (accumulated biogas) was 1.4% based on the analysis of the center point tests (n = 4)

Because the pH values measured at the end of the 40 days of fermentation varied from 6.16 to 7.00 (Table 6), it can be concluded that the mixture of the effluents contributed to the stabilization of the pH, which suggests that no inhibition of methanogenesis resulted from the acidification of the medium.

Regarding the reduction in the organic load (COD) after the anaerobic co-digestion, the decrease in COD (Table 6) was greater in the assays where there was a higher volume of biogas, and a strong, positive Pearson correlation was observed (Table 7). Interestingly, the highest biogas yields per unit mass of COD_{total} (Table 6) were observed in the tests in which the decrease in organic load was the lowest, and a very strong, negative Pearson correlation was observed (Table 7). The Pearson correlation between the reduction of COD and the average rate of biogas production was also very strong and negative (Table 7). It is likely that the high rates of initial conversion of organic matter into biogas might have depleted some nutrients and affected the reduction in COD. It is also true that the assays with the lowest decrease in COD were those with a lower CG load. This fact might have amplified the toxicity observed in anaerobic digestion systems of swine manure with low C/N ratios.[43]

Table 7. Pearson correlation coefficients for the correlation between the C/N and I/S parameters and the response variables evaluated.

Variables	Accumulated biogas (NmL)	Biogas yield (NmL $gCOD_{total}^{-1}$)	Biogas yield (NmL gVS_{total}^{-1})	Biogas production rate (NmL $gCOD^{-1}$ d^{-1})	COD Reduction (% m m^{-1})
C/N Ratio	0.111	-0.464	-0.196	-0.476	0.511
I/S Ratio	-0.926	0.925	0.882	-0.462	-0.935
COD Reduction (% m m^{-1})	0.773	-0.966	-0.860	-0.943	1.000

C/N: mass of carbon / mass of nitrogen; I/S: mass of VS from inoculums / mass of VS from substrates; TS: Total Solids; VS: Volatile Solids COD: Chemical Oxygen Demand.

3.4. Response surface curve analysis for the production of biogas

The adjustment of the polynomial model generated for the production of biogas as a function of the combination of different concentrations of CG and SM yielded a determination coefficient (R^2adj) of 0.90. The ANOVA test showed that the quadratic effects and interaction of CG and SM concentrations were not significant at p = 0.05 (Table 8). However, the linear effects of CG and SM concentrations were significant and positive (Figure 3). For these reasons, the linear regression for model adjustment that explained the behavior of gas production was used in the second moment. The adjustment of the linear model generated for the experimental data (Equation 3) resulted in a determination coefficient (R^2adj) of 0.89, which indicated that the mathematical model was adequate for the biological phenomenon studied and that it was capable of predicting the answers within the limits evaluated for each independent variable. The correlation coefficient (R^2) for the values observed experimentally and those predicted by the adjusted linear model was 0.91 (Figure 4).

Table 8. Analysis of variance (ANOVA) of the polynomial model obtained from biogas production data after the end of the anaerobic co-digestion process

Factors	SS	df	MS	F	p-value
CG (L)	3369.16	1.00	3369.16	86.37	0.00
CG (Q)	87.72	1.00	87.72	2.25	0.18
SM (L)	546.27	1.00	546.27	14.00	0.01
SM(Q)	8.12	1.00	8.12	0.21	0.66
CG & SM	23.08	1.00	23.08	0.59	0.47
Error	234.06	6.00	39.01		
Total SS	4286.06	11.00			

SS: Sum of Squares; df: degree of freedom; MS: Mean Squares; CG: Crude Glycerol; SM: Swine Manure.

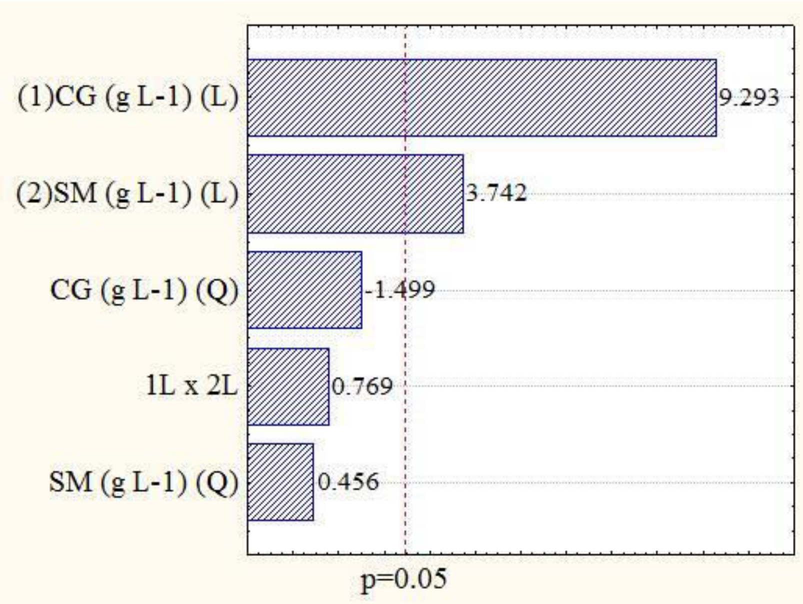


Figure 3. Pareto chart with the linear, quadratic and interaction effects of the concentration of CG and SM on the biogas production

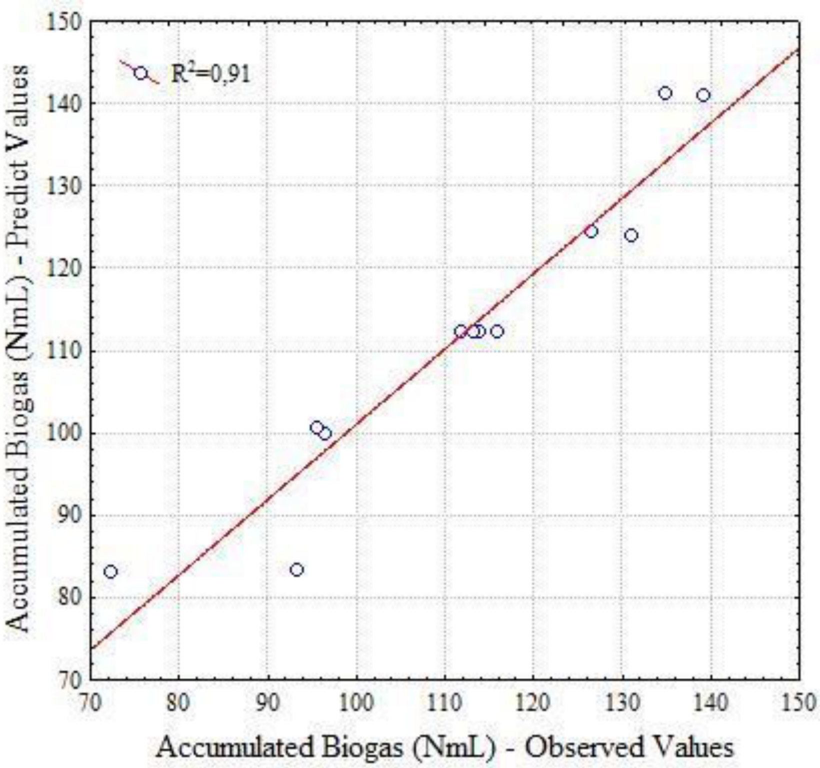


Figure 4. Chart of observed *vs*. predict values from accumulated biogas production at the end of anaerobic co-digestion process

$$Y = 47.70 + 6.84x_1 + 1.65x_2 \qquad \textbf{Eq. 3}$$

Where

Y is the biogás value (NmL)

x_1 is the CG concentration (g L^{-1})

x_2 is the SM concentration (g L^{-1})

In the analysis of the response surface graph generated from Equation 3 (Figure 5), was possible to observe the positive effect of DS and GB concentrations on biogas production. The highest values for biogas production were observed in the region of the graph that combines concentrations of CG and SM greater than 10 g L^{-1} and 15 g L^{-1}, respectively. Production values greater than 139 NmL of biogas were observed in this region of the graph. Because there was no maximum inflection in the surface curve, it is believed that a biogas production gain is possible with an increase in the concentration of CG and SM.

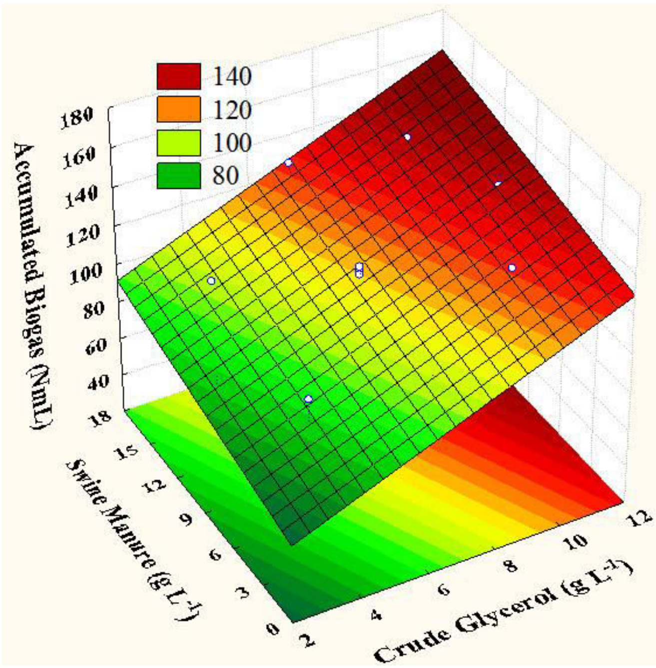


Figure 5. Surface curve adjusted to accumulated production of biogas in response to different mixtures of CG and SM after 35 days of anaerobic co-digestion

3.5. Evaluation of anaerobic codigestion for the treatment of crude glycerol

The treatment of the data obtained for the COD-dependent variable resulted in an adjusted model with a determination coefficient (R^2adj) equal to 0.91 when the linear, quadratic and interaction effects were considered. According to the Pareto graph (Figure 6), only the linear and quadratic effects of SM concentration were not significant (p> 0.05). The effect of interaction between CG and SM was negative and significant (p <0.05) (Figure 6). New data modeling was performed by removing the non-significant componentes, which resulted in Equation 4, with R^2_{adj} = 0.93. The ANOVA evaluation (Table 9) confirmed that the linear and quadratic effects of CG concentration and the interaction between CG and SM concentrations were significant.

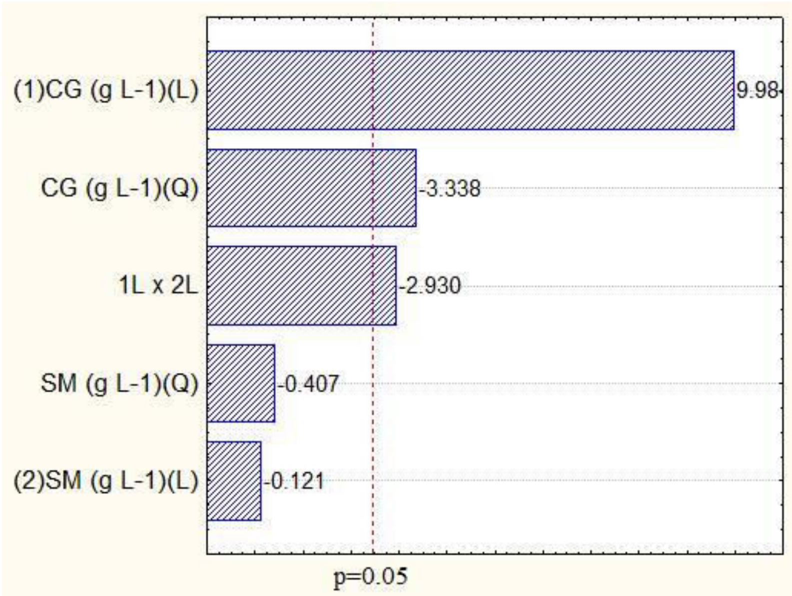


Figure 6. Pareto chart with the linear, quadratic and interaction effects of the concentration of CG and SM on the COD reduction

Table 9. Analysis of variance (ANOVA) of the polynomial model (Equation 4) obtained from COD reduction data after the end of the anaerobic co-digestion process

Fatores	SS	df	MS	F	p-value
CG (L)	2181.97	1.00	2181.97	129.03	0.00
CG (Q)	241.86	1.00	241.86	14.30	0.00
CG & SM	187.96	1.00	187.96	11.11	0.01
Erro	135.28	8.00	16.91		
Total SS	2750.63	11.00			

SS: Sum of Squares; df: degree of freedom; MS: Mean Squares; CG: Crude Glycerol; SM: Swine Manure.

$$Y = -5.48 + 15.3x_1 - 0.67x_1^2 - 0.04x_1x_2 \qquad \textbf{Eq. 4}$$

Where

Y = Decrease in %COD;

x_1 e x_2= concentration of CG and SM, respectively.

The effect of the SM concentration on the decrease in the COD of the effluent mixture after anaerobic co-digestion (Figure 7, obtained using Equation 4) was negligible within the assessed limits. In addition, the decrease in COD was proportional to the load of CG up to the limit of 10 g L^{-1}. Considering that the concentration of CG had a significant negative ($p < 0.05$) quadratic effect, it is probable that concentrations of CG greater than 10 g L^{-1} could inhibit codigestion.

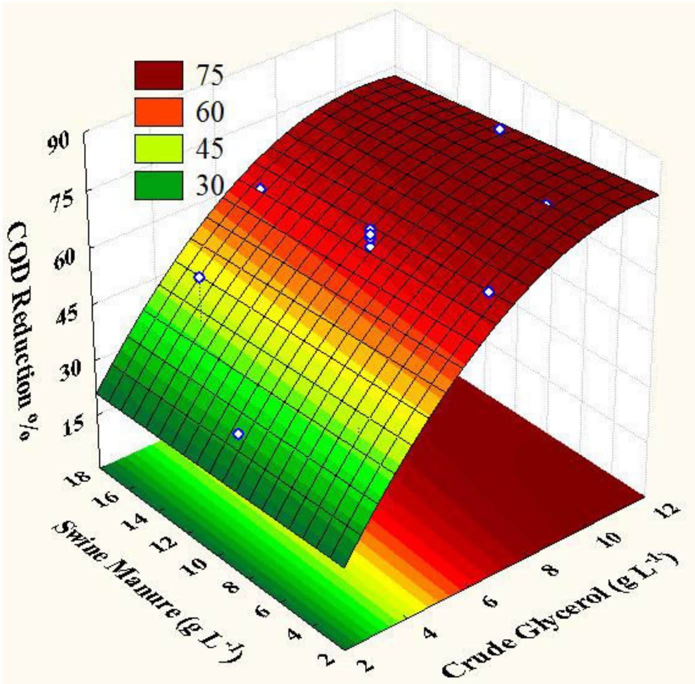
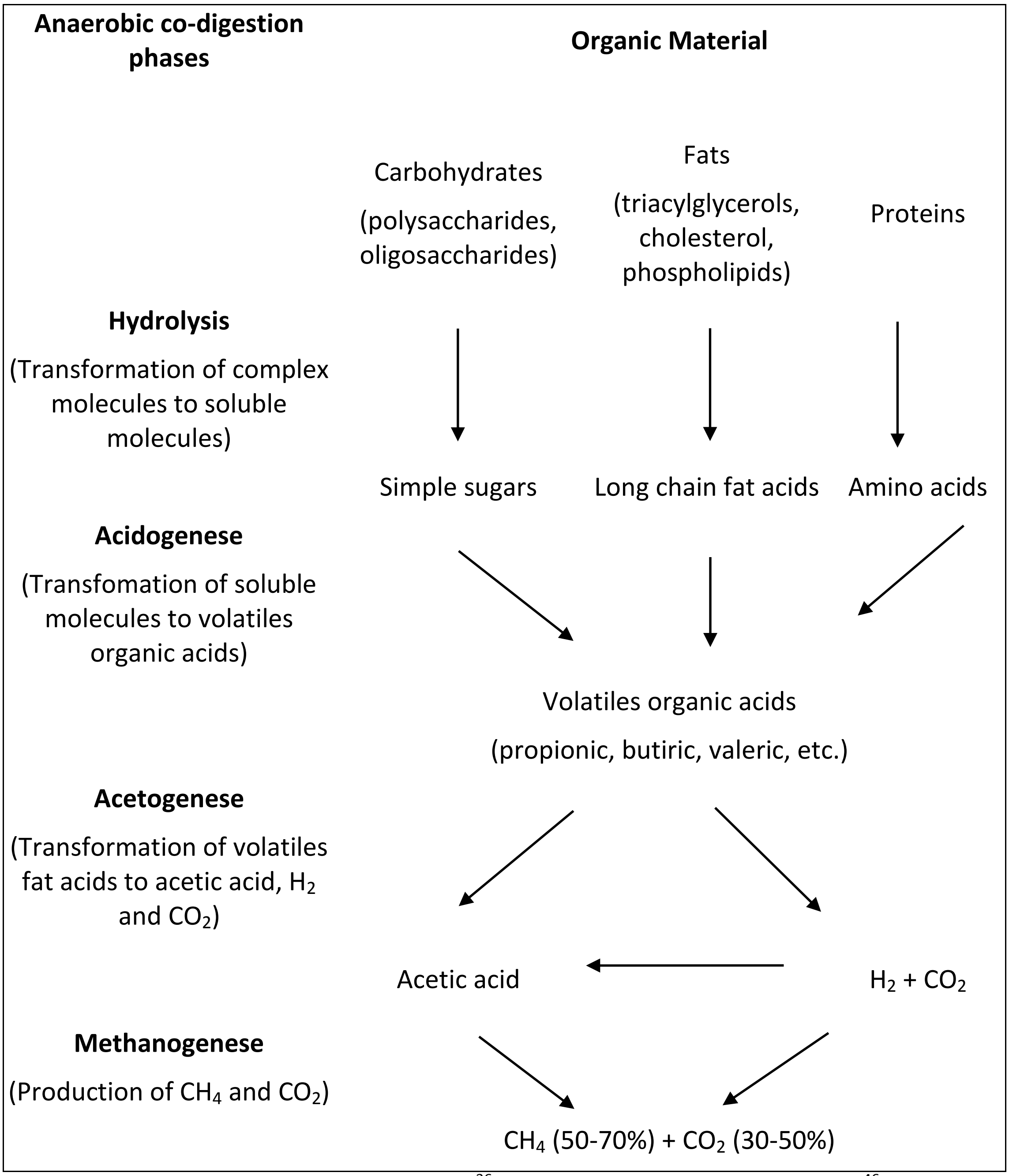


Figure 7. Surface curve adjusted to COD reduction in response to different mixtures of CG and SM after 35 days of anaerobic co-digestion

The COD decreased from 1941 mg L^{-1} to 390 mg L^{-1} under the condition of assay 6. The greatest reduction in organic load occurred with the CG concentrations of 10 to 11.2 g L^{-1}, independently of the SM values evaluated. In this region of the response surface curve, there was a 70.5 to 79.9% decrease in the COD values (Figure 7).

3.6. Production of organic acids

Volatile organic acids are key intermediate metabolites that are produced during anaerobic digestion. Acetic, propionic, butyric, isobutyric, valeric and isovaleric acids are commonly produced during the hydrolysis and acidogenesis steps. Low volatile organic acids concentrations indicate a balance between hydrolysis/acidogenesis and methanogenesis that favors methane production (Figure 8). The condition of assay 6 was the only condition in which the accumulation of acetic acid (48 g L^{-1}) was observed after 35 days of the process. This assay stood out from the others by the greater amount of GB used (11.2 g L^{-1}) (Table 2) and by the smaller amount of biogas produced (278.2 NmL $gDQO_{Total}^{-1}$) (Table 6). According to TIAN *et al*. (2015)[44] and Marchaim and Krause (1993)[45], a high concentration of free fat acids can inhibit methanogenic bacteria.



Modified from Mao *et al.*, (2015)[36] e Kondusamy e Kalamdhad, (2014)[46]

Figure 8. Biogas production stages during conventional anaerobic biodigestion

According to some authors,[15,17,18,23] propionic acid might be the strongest inhibitor of biogas production among the several free fat acids. During the digestion process, the degradation of propionic acid might be much slower than that of acetic acid, which is converted to methane and carbon dioxide (Equation 5). The propionic acid can be further degraded to acetic acid before the methane gas is generated (Equation 6, Figure 8).

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad \Delta G^0 = -31.0 \text{ kJ mol}^{-1} \qquad \textbf{Eq. 5}$$

$$CH_3CH_2COOH + 4H_2O \rightarrow 2CH_3COOH + CH_4 + CO_2 + 3H_2 \quad \Delta G^0 = +76.1 \text{ kJ mol}^{-1}$$ **Eq. 6**

The concentration of propionic acid tolerated by methanogenic bacteria is below 1 g L^{-1}.[15,17,23] The results obtained from the analysis of volatile fatty acids showed that no propionic acid existed at the end of the process. This observation suggests that, if produced at some time during the anaerobic digestion, it was produced at a concentration below that tolerated by the methanogenic bacterias or it was consumed during the generation of acetic acid and, as a final product, methane gas.

4. Conclusion

The results obtained in this study showed that the codigestion of the crude glycerol with the swine waste favored the production of biogas, probably because it corrected the nitrogen restriction peculiar to crude glycerol. The combination of the two effluents that yielded the best result was 4 g L^{-1} of crude glycerol and 5 g L^{-1} of swine manure, with the production of 521.5 mL of biogas per g of COD_{total} or 834.6 mL of biogas per g of VS_{total}. The mixing of the two effluents ensured adequate buffering of the medium without further intervention. The relation between the inoculum size and the total quantity of volatile solids appeared to be a significant factor for the efficiency of biodigestion, and this fact is noteworthy.

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