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Co-digestion of crude glycerin associated with cattle manure in biogas production in the State of Paraná, Brazil

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ABSTRACT. The addition of different concentrations of crude glycerin, associated with cattle manure, on the volumetric production of biogas is analyzed. Different concentrations of crude glycerin (2, 4 and 6% $m\ m^{-1}$) were added as supplement in anaerobic co-digestion of dairy cattle waste, in laboratory batch biodigesters (3.5 L usable volume). The biodigesters were operated under mesophilic conditions (30ºC), with 30-day hydraulic retention time (HRT). Total solids (TS), volatile solids (VS) and chemical oxygen demand (COD) were analyzed to determine the efficiency of the process in the removal of organic matter and its effect on biogas production. The addition of 4% glycerin provided a larger production of biogas, approximately 9.307 mL. The efficiency in COD removal decreased in treatments with glycerin, with highest reduction (68%) in the control treatment. There was a 90 and 118% increase respectively for Gli4 and Gli6% treatments. VS reductions Gly 0, Gly 2, Gly 4 and Gly 6% treatments were 18.17, 61.60, 24.36 and 44.83%, for the respective treatments.

Keywords: bio-digestion, cattle manure, biogas, crude glycerin, biodiesel.

Avaliação da codigestão de glicerina associada a esterco bovino para produção de biogás no Estado do Paraná, Brasil

RESUMO. O objetivo do trabalho foi avaliar o efeito da adição de diferentes concentrações de glicerina bruta associada com esterco bovino, sobre a produção volumétrica de biogás. A adição de diferentes concentrações de glicerina bruta (2, 4 e 6% $m\ m^{-1}$) foi utilizada como suplemento na codigestão anaeróbia de dejetos bovinos de leite, em biodigestores laboratoriais de batelada (3,5 L de volume útil). Os biodigestores foram operados em condições mesofílicas (30ºC), com tempo de retenção hidráulica (TRH) de 30 dias. Foram analisados os sólidos totais (ST), sólidos voláteis (SV) e demanda química de oxigênio (DQO), a fim de determinar a eficiência do processo na remoção da matéria orgânica e seu efeito na produção de biogás. A adição de 4% de glicerina resultou na maior produção de biogás, cerca de 9,307 mL. A eficiência na remoção de DQO diminuiu nos tratamentos com adição de glicerina, sendo a maior redução no tratamento controle, de 68%. Nos demais tratamentos houve um aumento de 90 e 118% para os tratamentos Gli 4 e Gli 6% respectivamente. As reduções de SV para os tratamentos Gli 0, Gli 2, Gli 4 e Gli 6%, foram de 18,17, 61,60, 24,36 e de 44,83%, para os respectivos tratamentos.

Palavras-chave: biodigestão, dejetos bovinos, biogás, glicerina bruta, biodiesel.

Introduction

Increasing global demand for energy, the scarcity of renewable natural resources and its environmental implications have stimulated the search for more sustainable alternatives to collaborate with the energy matrix and reduce the environmental impacts caused by the combustion of fossil fuels.

The burning of fossil fuels and biomass derivatives increases carbon dioxide emissions into atmosphere and, together with the growing threat of global warming and environmental degradation, stimulates policies for the study of renewable energy (Pereira et al., 2011; Salim, & Shafiei, 2014).

Several researchers and governments claim that the current energy matrix of the planet must change to reduce the dependency on fossil fuels, mainly coal, petroleum and natural gas which in 2013 accounted for approximately 81.6% of global energy consumption (International Energy Agency [IEA], 2014).

Renewable fuel, such as biofuels, is one of the strategies for changing the global energy matrix. The use of biodiesel and ethanol in internal combustion engines and of biogas in the generation of electricity should be highlighted in Brazil.

When compared to petroleum diesel, biodiesel has significant environmental advantages too. The

2015 studies by the National Biodiesel Board (2015), which represents the biodiesel industry in the United States, and by Environmental Protection Agency [EPA] (2015), demonstrated that the burning of biodiesel emits an average of 48% less carbon monoxide (CO), 47% less particle matter (which penetrates into the lungs) and 67% less hydrocarbons.

According to the Brazilian National Agency of Petroleum (Agência Nacional do Petróleo, Gás Natural e Biocombustível [ANP], 2015), about 45% of the energy and 18% of the fuels consumed are renewable in Brazil. Brazil is the global pioneer in the use of biofuels, with a position emulated by many countries which search for renewable energy sources as strategic alternatives to petroleum.

Biodiesel production is predominantly processed by catalyzed transesterification, a reaction which results in other products or co-products, such as crude glycerin. Crude glycerin has proved to be a co-substrate in the anaerobic treatment of different types of organic residues. However, a few considerations and inhibiting factors must be considered (Kolesárová, Hutňan, Bodík, & Špalková, 2011).

Although refined glycerin has several industrial applications, it is mainly used in the production of cosmetics and drugs. However, glycerin obtained from the process of production and the obtainment of esters (approximately 10 kg of glycerin are generated for every 100 L of biodiesel produced) has several impurities such as water, fatty waste, catalyst residues and a high chemical oxygen demand (Santibañez, Varnero, & Bustamante, 2011).

Several research works report the use of glycerin, derived from the production processes of biodiesel and aimed at increasing the biogas prod in anaerobic bio-digestion of agro-industry waste (Serrano., Siles, Chica, & Martin, 2014; Athanasoulia, Melidis, & Aivasidis, 2014; Martín, Fernández, Serrano, & Siles, 2013; Astals, Nolla-Ardèvolb, & Mata-Alvareza, 2013; Rivero, Solera, & Perez, 2014).

The advantages of glycerin, a high-energy molecule with great carbon and hydrogen availability, are its high digestibility and storage for long periods. Glycerin also presents adequate pH rates to anaerobic processes, coupled to high carbon content which ensures a better C/N (Carbon/Nitrogen) relationship in the mixture, avoiding digestion inhibition due to N excess and biogas production increase from 50 to 200% (Serrano et al., 2014; Rivero et al., 2014).

Astals, Ariso, Galí, and Mata-Alvarez (2011), using refined glycerin, and Astals, Nolla-Ardèvol, and Mata-Alvarez (2012), using crude glycerin, evaluated, among other characteristics, the production of biogas and the corresponding methane concentration associating glycerin manure in anaerobic co-digestion. The two studies showed positive rates when compared to biogas production volume with samples using swine manure only. In fact, their production was 380% higher than that of control (Astals et al., 2012). The concentration of CH_4 in the biogas generated in each sample reached 100% more when compared to control (Astals et al., 2011).

Panpong, Srisuwan, O-Thong, and Kongjan (2014) evaluated biogas production, including glycerin anaerobic co-digestion associated with wastewater from canned sea fruit industry and also obtained increased production of biogas and methane concentration. CH_4 concentration in the sample with the best performance was 48.18% higher than that of control inoculated only with waste water.

Robra, Serpada, Oliveira, Neto and Santos (2010) also evaluated glycerin in the anaerobic co-digestion of cattle manure. Congruent to the study by Astals et al. (2011), one of the characteristics evaluated was the volume of biogas produced when compared to samples containing only cattle manure. The above indicated that in samples with glycerin to produce the two types of biogas when methane concentrations were higher than control, Robra et al. (2010) observed an increase in the concentration of methane in the biogas generated. The higher the proportion of glycerin in the samples, the greater was the concentration of CH_4 in the generated biogas, reaching 14.6% more than that of control.

Biogas is a gaseous mixture (fuel) produced from the anaerobic fermentation of organic matter. The ratio of each gas in the mixture depends on several parameters, such as the type of digester and the substrate to be digested. Moreover, the mixture is essentially formed by methane and carbon dioxide, and its calorific power is directly related to the amount of methane in the gaseous mixture (Deublein & Steinhauser, 2008). Biogas derived from the anaerobic digestions of solid or liquid residues is a source of alternative energy. It also contributes largely to the solution of environmental issues since it potentially reduces the impacts of the polluting source (Neupane et al., 2015).

Increase in global demand for animal protein modernized the Brazilian agricultural sector since it increased the volume of waste generated with serious health and environmental risks. An efficient alternative would be the use of this waste as substrate in anaerobic bio-digestion.

Brazil is the second largest producer and consumer of biodiesel in the world, after the United States. In 2014, Brazil produced and consumed 3.42 billion liters, while USA produced and consumed 4.8 billion liters (Agência Nacional do Petróleo, Gás Natural e Biocombustível [ANP], 2015). Law 13.033 (Brasil, 2014) published in 2014 authorized a 7% increase in the biodiesel mixture in conventional diesel. Consequently, ANP predicted a production of 4.3 billion liters of biodiesel for 2015, and large amounts of glycerin in the market, completing 0.43 billion kg.

Due to the increase in glycerin amount and to the correct disposal of this potentially polluting co-product (while generating energy in the form of biogas), current assay evaluates an increase in the volumetric production of biogas by the addition of crude glycerin as co-substrate in bio-digestion of cattle manure, operated in small scale batch biodigesters.

Material and methods

The experiment was conducted in Guarapuava, Paraná State, Brazil, 25°23'S and 51°28' W; altitude 1098 m. Cattle manure was collected from the dairy cattle sector of the Agricultural School Arlindo Ribeiro by scraping the yard and the product transported for immediate use. The animals were fed on corn silage twice a day, and spent the rest of the time on pastureland. Inoculum consisted of iodine, derived from the municipal sewage treatment system, with approximately 4% total solids. The use of 40% (m m^{-1}) inoculum of the total sample volume was based on studies by Xavier and Lucas Jr. (2010).

Crude glycerin was collected at the micro biodiesel plant on the outbuildings of CEDETEG (Center of Technological Development of Guarapuava) in Guarapuava, Paraná State, Brazil. Crude glycerin was derived from methyl transesterification of soy oil, stored in plastic barrels at room temperature from where samples were collected and stored in PET bottles.

Laboratory-scale biodigesters were built from 100 mm PVC tubes, sealed at both ends with PVC-cap connections, totalizing a volume of 5 liters for each biodigester. They are immersed in a 1.5 m^3 liter tank, with water heated to 30ºC, maintained by electrical resistances controlled by a thermostat. An agitation system ensured homogeneity of the water temperature, and the tank was surrounded by 15 mm styrofoam plates to reduce heat exchange with the environment.

Transparent polyurethane tubes connected the reactors to the gasometers, which were also constructed from 75 mm PVC tubes turned into 100 mm tubes which were sealed by water seal. Sample taking occurred on top of each gasometer. Tables 1, 2 and 3 show the materials employed for the construction of reactors, gasometers, heating system, agitators and boxes where the reactors and the gasometers were allocated. Equipments, such as agitators, thermostat and resistors, characterized as costly material, were provided by the University laboratory.

Table 1. Materials used for the construction of reactors.

Materials	Dimensions	Amount
PVC pipe	100 mm Ø x 30 cm	16 units
PVC pipe	50 mm Ø x 10 cm	16 units
Connection Cap	100 mm Ø	16 units
Connection Cap	50 mm Ø	16 units
Simple glove	100 mm Ø	16 units
Simple reduction	100 mm Ø x 50 mm Ø	16 units

Table 2.- Materials used for the construction of gasometers.

Materials	Dimensions	Amount
PVC pipe	100 mm Ø x 60 cm	16 units
PVC pipe	75 mm Ø x 60 cm	16 units
PVC pipe	25 mm Ø 60 cm	16 units
Connection Cap	75 mm Ø	16 units
Transparent polyurethane tube	1,8” x 1.0 mm	85m
Water box	1.5 m^3	1unit

Table3. Materials for the construction of the heating system.

Materials	Dimensions / power	Amount
Electrical resistance of copper for immersion	1500 W	2 units
Digital thermostat	Rectangular or circular	1 unit
Agitators	0.5 CV / by agitation	2 units
Thermal insulation plates	1250 x 625 x 15 mm	10 units
Water tank	0.1 m^3	1 unit

Biogas volume was measured by vertical displacement of the gasometers, posteriorly corrected for Standard Conditions for Temperature and Pressure (SCTP). Operated at a batch system, the biodigesters were supplied with cattle manure diluted into water up to the usable volume of 3.5 L, without nutrient or pH corrections. The initial supply was calculated by expressions employed by Steil, Lucas Jr., & Oliveira (2002), resulting in a substrate with approximately 2% TS. Figure 1 shows the construction scheme of the digester, whereas the reactor model and gasometers are demonstrated in Figure 2.

Four different substrate proportions were used (manure + glycerin), totalizing four samples: 0, 2, 4 and 6% m m^{-1} of crude glycerin concentration. The biodigesters with their respective samples were randomly distributed and connected to their respective gasometers.

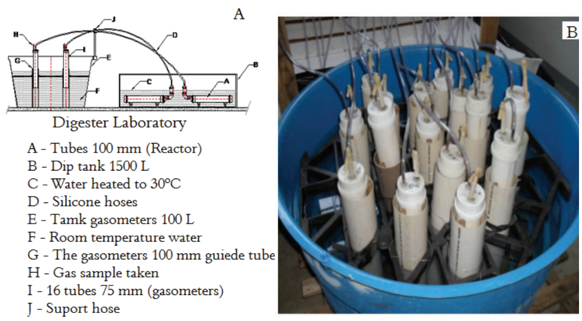

Figure 1. Reactor Picture: Biodigester Scheme (A), Biodigester (B).

Figure 2. Reactor model (A); gasometer model (B).

The experimental design was completely randomized, with the four treatments featuring three replications each. Tukey's test was applied at a 5% level of probability with ASSISTAT 7.6 beta.

The substrate was properly diluted and collected in samples to determine Chemical Oxygen Demand (COD), Total Solids (TS) and Volatile Solids (VS) prior to the loading of the biodigesters, following APHA methodology (American Public Health Association [APHA], 1999). The analysis was performed by the Central of Analysis of UNICENTRO. These parameters were again analyzed by the end of the 30-day TRH to evaluate the efficiency of the biodigesters in the removal of the organic fraction and in the biogas generation.

After inoculation, the biodigesters remained at rest for 24 hours until temperature reached 30ºC. The biogas generated during this period was discarded and the generated volume was monitored as from this point. The vertical displacement of the gasometers was measured daily, whereas the generated volume of the biogas was later calculated. Methane concentration was measured twice a week by a biogas alkaline washing solution, adding up to six samples. The great advantage of this method was its simplicity and its dismissal of chromatograph. In fact, it may be applied in any laboratory without a costly infrastructure.

Results and discussion

In current experiment, cattle manure and the inoculum, the total volatile solids, varied between 55.8 and 74.3%.

Rates for total solids, total volatile solids, pH and COD are given in Table 4.

Table 4. Analysis of substrates prior to the digestion process.

Affluent medium	Gly 0%	Gly 2%	Gly 4%	Gly 6%
Total Solids (mg L^{-1})	16,240	16,780	20,624	28,064
Fixed Total Solids (mg L^{-1})	7,176.66	4,312	5,812	9,784
Total Volatile Solids (mg L^{-1})	9,063.33	12,468	14,812	18,280
pH	6,43	6,56	6,68	6,5
COD	6,869.33	3,323	2,936,6	3,967
SV (%)	55.8	74.3	71.8	65.1

There were 32.24, 59.13, 22.43 and 34.91% reductions of total solids after the bio-digestion process respectively for 0, 2, 4 and 6% glycerin treatments.

Astals et al. (2011) evaluated five glycerin treatments associated with manure 0 DS, 80 DS, 60 DS, 40 DS and 20% DS, using as an inoculum the anaerobic manure after digestive action and respectively obtained 21.1, 78.1, 91.1, 81.1 and 51.6% reductions of total solids.

The sample with the highest ST reduction rate was 60% DS (40% Gly). Even with smaller reduction rates, other treatments with glycerin obtained a reduction greater than control, perhaps due to the fact that the manure had limitation, or rather, the imbalance of its nutrient content. The low C/N ratio decreased the activity of microorganisms (Astals et al., 2011).

Nuchdang and Phalakornkule (2012) compared the anaerobic breakdown of glucose into glycerin from biodiesel produced from cooking oil, together with manure, at the proportion of 80% crude glycerin: 20% waste, and obtained a total reduced solid for the sample with glycerin plus manure between 88 and 98%.

In their study, Nuchdang and Phalakornkule (2012) used as inoculum the granules from sieved waste water of a preserve fruit industry. Due to monosaccharides and disaccharides in its sugar composition, it may have been one of the causes of the great reduction of total solids since glycolysis is almost a central metabolic pathway of many organisms entirely dependent on glucose (Lehninger, Nelson, & Cox 2008).

Larsen, Gomes, Gomes, Zenatti and Torres (2013) conducted a study on anaerobic digestion of wastewater from the industrialization of cassava associated with crude glycerin. Samples were inoculated with sludge from the anaerobic lake treatment system in cassava industrialization. Each of the four evaluated samples was fed on different cassava batches: 3.05 (0% Gly), 9.32 (2% Gly), 14.83 (3% Gly) and 13.59 (2% Gly) g COD L^{-1} d^{-1}. The reduction of ST for each treatment was 81.19 ± 2.96, 75.47 ± 6.43, 68.79 ± 9.79 and 55.58 ± 12.96 (%), respectively. Treatments with glycerin had lower ST reduction when compared to control (0% Gly) perhaps due to the viscous characteristic of the effluent from the cassava industry. In fact, the mere addition of 3% glycerin (m m^{-1}) caused the clogging of the laboratory scale reactor feed system (Larsen et al. 2013).

Regarding to the Chemical Oxygen Demand (COD), there was a reduction of 68% in the control treatment and 38% in the treatment with glycerin 2%.

Nuchdang and Phalakornkule (2012) reported that COD reduction of glucose is higher when compared to glycerin and that HRT glycerin must be increased for a more complete digestion. A possible reason for this fact is that glucose metabolism builds towards glycolysis, while glycerol metabolism requires three additional enzyme activities before entering the glycolysis via. The sample analyzed with glycerin associated with manure (80% glycerin and 20% of pig manure) for the efficiency of COD removal was fairly stable while remaining well above 80%.

COD increase after the bio-digestion process in the reactors with glycerin was probably due to the increase in the organic load caused by glycerin.

Astals et al. (2011) reported a 2% decrease in COD reduction between the control sample and the 20% glycerin sample. On the other hand, the other samples (40, 60 and 80%) presented a higher reduction with regard to control sample. Further, there was a reduction of 61.4% ± 2 for the 80% glycerin sample. Astals et al (2012) obtained a COD reduction of 55.7% ± 3.2 for the sample without glycerin and 84.9% ± 2 for the sample associated with glycerin.

Nuchdang and Phalakornkule (2012) registered that the efficiency of COD removal was rather stable, remaining above 80%, in the sample analyzed with glycerin associated with pig manure (80% glycerin and 20% of pig manure).

Larsen et al. (2013) studied the behavior of anaerobic digestion of sewage from cassava industrialization, associated to glycerin, 3:05 (0% Gly), 9:32 (2% Gly), 14.83 (3% Gly) and 13:59 (2% Gly) g COD L^{-1} d^{-1}, and obtained COD reduction 0:42 ± 96.41, 98.69 ± 0.69, 98.59 ± 91.54 ± 23.88 and 00:12 (%), respectively. All samples revealed a better performance than control (0% Gly). Further, sample with 13:59 (2% Gly) g COD L^{-1} d^{-1} obtained the lowest removal rate due to increase in Volatile Acids / Total Alkalinity (VA TA^{-1}) proportion in the reactor (Larsen et al., 2013).

According to the literature, when in batch reactors there is an increase in the concentration of glycerin in the substrate, there is a significant decrease in the COD removal efficiency.

Tukey's test was applied when statistical differences between treatments were verified, as Table 5 shows. Although Gly and Gly 2% treatments were not different from each other, Gly 4 and Gly 6% treatments showed difference since Gly 4% treatments had the highest biogas production.

Table 5. Total quantity of biogas production from cattle manure and crude glycerin.

Treatment	Total amount (mL)
Treatment Gly 0%	7063.871 ab
Treatment Gly 2%	6827.790 ab
Treatment Gly 4%	9307.838 a
Treatment Gly 6%	4123.253 b

Averages followed by the same letter were not statistically different. Tukey's test was applied at 5% level of probability.

For the different treatments with glycerin, the VS concentration before and after bio-digestion were analyzed: there was a reduction of VS and a certain production of biogas, shown in Table 6.

Table 6. Reduction of volatile solids (VS) and biogas production.

Treatment	Affluent SV (mg L^{-1})	Effluent SV (mg L^{-1})	Substrate volume (L)	VS Reduction (kg)	Biogas quantity (m^3)	Biogas production on the basis of SV (m^3 kg^{-1})
Gly 0%	9,063.33	7,416.33	3.5	0.00576	0.00706	1.225
Gly 2%	1,246.8	4,786.66	3.5	0.02688	0.00683	0.254
Gly 4%	1,481.2	11,203.33	3.5	0.01263	0.00931	0.737
Gly 6%	1,828.0	10,083.33	3.5	0.02869	0.00412	0.144

As Table 6 demonstrates, Gly 0% treatment (control treatment) and Gly 4% treatment produced a higher amount of biogas per kg of removed volatile solids when compared with the others.

Weber, Zenatti, Feiden and Monique (2014) used a vertical continuous flow digester to generate biogas from cattle manure without the addition of inoculum, and obtained 0.627 m^3 kg^{-1} biogas per removed VS.

Nartker, Ammerman, Aurandt, Stogsdil, Hayden, and Antle (2014) analyzed glycerin samples associated to sewage sludge, varying the percentage of glycerin from 0 to 70%, but maintaining the same amount of sewage sludge, obtained a larger biogas production per removed VS in the 69% sample, with a production of 0.92 m^3 kg^{-1}, when compared to control sample (0.35 m^3 kg^{-1}).

VS removal rates (%), the production of biogas according to different treatments in co-digestion with glycerin, and the relation of biogas formation by VS reduction are highlighted in Figure 3.

Figure 3 reveals that the highest VS reduction occurred in the Gly 2% treatment, whilst the lowest reduction occurred in Gly 0% (control treatment).

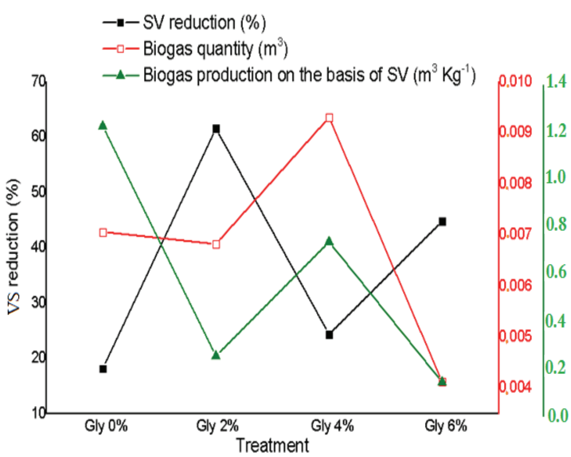

Figure 3. Biogas production, removal of VS and their relationship.

Astals et al. (2011) used pig waste plus synthetic glycerin and obtained a higher reduction in the 60 and 80% ratio of glycerin among the evaluated samples (0, 20, 40, 60 and 80%), 93.0 and 90.9 % respectively. Astals et al. (2012) evaluated samples with only pig manure and other samples associated with crude glycerin, all under mesophilic conditions, and had a higher reduction of volatile solids of 4.1% ± 1.7 for samples with only pig manure and 77.7% ± 1.5 for samples with pig manure associated with crude glycerin.

Nuchdang and Phalakornkule (2012) reported that among the analyzed samples (100% glucose, 100% glycerin and 80% glycerin and 20% pig manure), the reduction of volatile solids for the sample of glycerin + pig manure during the assay period varied between 10 and 82%.

The highest biogas production occurred in the treatment with Gly 4%, and the lowest production occurred with Gly 6%. The relationship of biogas production according to VS removal showed that there was a good relationship in Gly 0%, and a weak one in the Gly 6% treatment.

The experiment´s retention time was fixed at 30 days, but Vedrenne, Béline, Dabert, and Bernet (2008) in their experiment with different types of animal waste (pigs, cows, calves and ducks) recommended bio-digestion for a much longer period (80 days). A longer hydraulic retention time could imply a higher rate of reduction of the organic matter.

Figure 4 shows that biogas production started immediately and the availability of easily degradable carbon, derived from the addition of crude glycerin at the beginning of the process, caused peaks of biogas production during the first days.

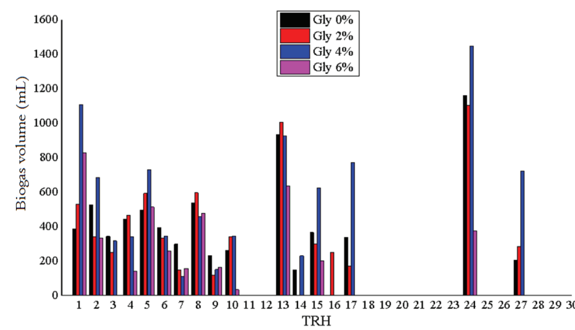

Figure 4. Biogas production of treatments with glycerin.

Once the carbon was consumed, the biogas production began to decline in the reactors with glycerin. A 2% addition already presented signs of instability in the anaerobic bio-digestion process, in reactors with 4% glycerin, or rather, a high biogas production, when compared to control, in the three days after the addition of glycerin. However, the system collapsed after this period.

It must be emphasized that it was not possible to monitor daily biogas production between the 18th and 23rd day of the experiment. Peak on the 24th day corresponded to the accumulated production during the period.

Figure 5 shows average daily production in the formation of biogas due to waste decomposition and glycerin.

In the control treatment (Gly 0%), biogas production had a stable behavior during the early days, with a production peak on the 24th day, and a declining tendency until the end of the experiment, after 30 days (Figure 4**)**.

The treatment with 2% glycerin had a similar behavior to that of control, with a slower biogas production on the first days, a production peak on the 13th day, and a decline until interruption on the 28th day.

Robra et al. (2010) used cattle manure associated with glycerin. Different from current study, the treatment was inoculated with effluent from a digester used for the production of biogas by cattle manure.

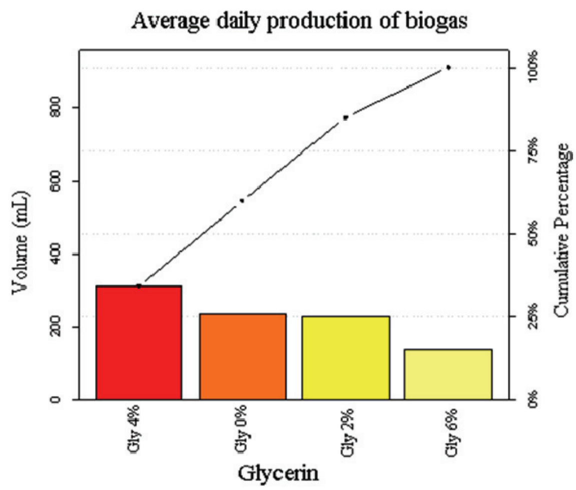

Figure 5. Average daily production of biogas for treatments Gli0 (control), Gli2, Gli4 and Gli6%.

The authors studied three glycerin concentrations associated with cattle manure 0 (control), 5, 10 and 15% (m m^{-1}), yielding 268.6, 825.3, 825.7, 387.9 mL g^{-1} biogas respectively. Samples with glycerin produced more biogas than control sample.

Panpong et al. (2014) used wastewater from the canned seafood industry, associated with crude glycerin with anaerobic digestion. Treatments were studied at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10% (m m^{-1}) ratio of glycerin, and a control treatment with only inoculated wastewater. The authors reported that the addition of 1% (m m^{-1}) glycerin increased biogas production but maintained stable biodegradation process.

The treatments 2 – 10% (m m^{-1}) increased C/N ratio although, at the same time, overloaded the system by inhibiting the process of anaerobic methanogenic co-digestion, thereby decreasing biogas production (Panpong et al., 2014).

Nghiem et al. (2014) used in their study crude and purified glycerin, associated with sludge from a sewage treatment plant, using as inoculum the same digested sludge. The authors studied treatments with 0.63 and 3% (m m^{-1}) glycerin associated with sewage sludge and reported that the treatment with the lowest proportion of glycerin had the highest biogas production volume when compared to treatment with 3% (m m^{-1}).

According to Nghiem et al. (2014), treatments with raw and purified glycerin (99%) showed no significant differences in the biogas production and other characteristics when compared to their respective treatments.

Fountoulakis, Petousi and Mañios (2010) reported behavior similar to that described in studies by Nghiem et al. (2014). They analyzed the addition of 1 and 3% (m m^{-1}) glycerin associated with sewage sludge from a treatment plant. The addition of 1% (m m^{-1}) had best results when the sample with 3% (m m^{-1}) glycerin was compared. Control sample produced biogas 751 ml d^{-1}, and the addition of 1% (m m^{-1}) production increased amount to 1253 ± 163 ml d^{-1}, while treatment 3% (m m^{-1}) inhibited the biogas before the 50th HRT day within a 60-day study period.

Konrad et al. (2010) added glycerin in spaced time periods according to decline in the biogas production and observed that there was a peak in biogas production posterior to the addition of glycerin. However, results linked to methane percentage were low immediately after the application of glycerin, with percentages between 50 and 70% only on the second day after the addition.

Treatment with 4% glycerin had the highest production among treatments; initially the produced biogas volume was higher, although there was a stabilization tendency on the following days.

Treatment with 6% crude glycerin presented clear signs of organic overload, with consequent buildup of volatile fatty acids (VFAs) and caused an inhibition in the activity of methanogenic bacteria. This fact may be confirmed by low biogas production and pH 5.02 at the end of the experiment.

Benabdallah, Astals, Galí, Macé, and Mata-Alvarez (2009) registered that behavior was due to the high level of volatile fatty acids produced by nitrogen limitation.

According to Nuchdang and Phalakornkule (2012), VFAs concentration is one of the most important in the control of anaerobic digestion parameters. The conversion rate from VFAs to acetic acid affects methanogenesis and bacteria amount. Afterwards, it affects the degradation rate of acetic acid and methane production (Yuanyuan, Yanlin, Jianbo, & Liang, 2009).

According to Aquino and Chernicharo (2005), the kinetic differences between acidogenic, methanogenic and ketogenic microorganisms cause an imbalance between production and consumption of intermediate products during organic load conditions. The buildup of carbon dioxide contributes towards the increase in the concentration of dissolved carbonic acid by increasing the production of ions (H^+) in the medium.

According to Kolesárová et al.(2011), the inhibitory effects from the composition of the substrate must be taken into consideration in the anaerobic treatment of biodiesel co-products. In the case of anaerobic digestion of crude glycerin, its high salinity may negatively affect methanogenic microorganisms, such as the presence of relatively high concentration of sodium and potassium salts, resulting from the catalysts often used in biodiesel production.

Kryvoruchko et al. (2006) analyzed the concentrations of volatile fatty acids (VFAs) at the beginning, middle and end of the experiment to identify the possible causes in the inhibition of the methane formation. The study was performed with samples containing 0, 3, 6, 8 and 15% crude glycerin. Kryvoruchko et al. (2006) reported that samples with 6 and 15% glycerin presented high concentration when compared to the sample with 0% glycerin, and the highest when compared to the other propionate and butyric acid samples, which probably caused the inhibition of methane production.

Astals et al. (2011) noted that among the studied samples (0, 20, 40, 60 and 80%), the samples with 80% glycerin and 20% pig manure had significant amounts of acetic, propionic and butyric acid, a phenomenon that illustrates poor digestion conditions. It was also the sample with the lowest methane production.

Dealing with the same amount per sample (80% glycerin and 20% pig manure), Nuchdang and Phalakornkule (2012) registered that the ratio of propionic and butyric acid in the sample did not indicate any imbalance in anaerobic decomposition.

Nartker et al. (2014) also observed that, when exceeding the 70% glycerin ratio in the sample associated with sludge, the acetic and propionic acids ratio inhibited anaerobic decomposition.

Another important consideration on the crude glycerin bio-digestion is the concentration of substances rich in nitrogen. Since there is low nitrogen concentration in crude glycerin, it might be necessary to supplement the reactor with nitrogen. Urea is the external source which is normally used. Figure 6 expresses the rates of accumulated biogas production during the experiment.

Gly 0% treatment obtained an increasing biogas production until the 9th day; followed by a small stagnation period and by a peak on the 13th day, again followed by long linear periods. This is probably due to the initial consumption of easily biodegradable compounds, making available to methanogenics only the most complex compounds, with slower degradation. The production totaled approximately 7,063 mL.

For the treatment of 2% glycerin, reaching an accumulated production with approximately 6,827 mL, the results were very close to the production of the control treatment.

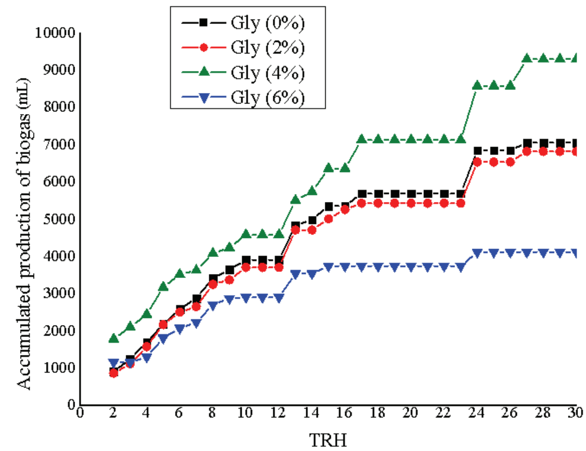

Figure 6. Accumulated production of biogas in 30-day TRH.

In fact, there was no statistical difference between treatments, according to Tukey's test at 5% probability.

Treatment with the addition of 4% crude glycerin provided the highest volumetric biogas production, totaling 9,307 mL. On the other hand, treatment with the addition of 6% glycerin had the worst performance, with a total production of 4,123 mL biogas. Low performance was probably due to inhibition in the activity of methanogenic microorganisms caused by pH decrease and by the buildup of intermediate compounds (VFAs).

According to Figure 7, the average readings of methane (CH_4) due to digestion and treatments are observed.

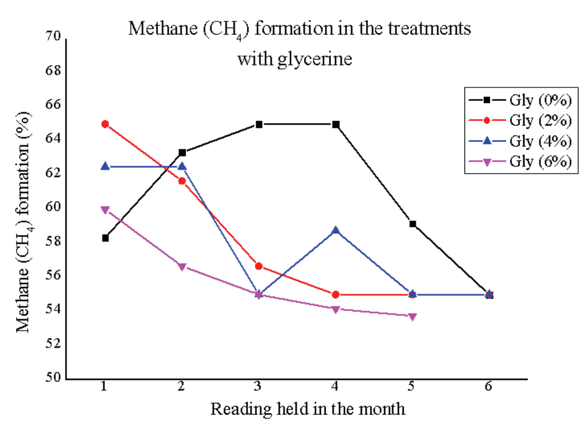

Figure 7. Methane concentrations for Gly 0% treatments, Gly 2, Gly 4 and Gly 6%.

Methane content in biogas for the control treatment (0%) was stable during the whole evaluated period, or rather, the average content of 65% methane. However, methane concentration in the biogas with addition of 2% crude glycerin was different, with an initial average of 65%. Over time, a significant decrease and a consequent increase in CO_2 concentration occurred.

In the case of Gly 4%, the methane content was stable during the whole period, averaging 62.5%, which was gradually reduced to 55%, till the end of the experiment.

Table 7 demonstrates statistical data referring to samples with methane (CH_4).

Table 7. Statistic data for methane rates

	Samples	Readings	Sum	Average	Standard Deviation
Statistic data	16	6	365.84	61.25	4.09

Figure 7 shows that, in the case of Gly 6% treatment, methane concentration was initially high, followed by an abrupt drop, till the end of the experiment. During the last days, this treatment did not present a significant biogas production. In fact, the collection of a sufficient sample to determine methane concentration was impossible.

Figure 8 reveals that it is possible to observe the monthly average of CH_4 formation for different glycerin concentrations.

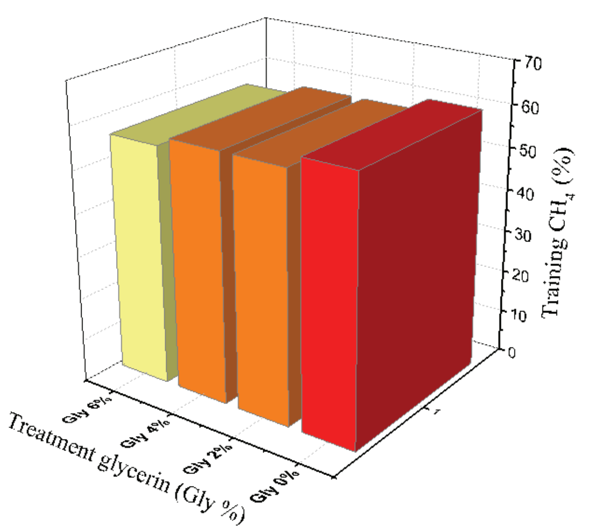

Figure 8. Monthly average of CH_4 production according to different treatments with glycerin.

As Figure 8 shows, monthly average production of methane decreased according to increase in glycerin concentration.

Treatments by Robra et al. (2010), namely, 5, 10 and 15% (m m^{-1}) glycerin associated with cattle manure, showed increased production of CH_4 by the control treatment. Further, treatment 15% (m m^{-1}) produced 14.6% more than control treatment, a distinct behavior from current study.

Astals et al. (2011) observed that until the 16^{th} day of treatment (0, 20, 40, 60 and 80% glycerin), the methane production remained similar in all samples. Samples with 20 and 40% glycerin reduced production after this period. According to Astals et al. (2012), the sample which most produced methane was treatment with 20% glycerin and 215 mL g^{-1} COD, about 125% more than the control treatment. The sample which produced less was treatment with 80% glycerin and 88 mL g^{-1} COD.

Conclusion

Under the experimental conditions used and based on the results, crude glycerin derived from biodiesel production, without any type of pre-treatment, is a viable substrate for the anaerobic co-digestion with cattle manure.

The addition of 4% (m m^{-1}) resulted in a significantly larger biogas production, although the efficiency in the removal of organic matter has been seriously compromised. If the goal is solely biogas production, without taking into consideration the quality of the generated effluent, the above concentration is the one that best meets this requirement.

However, it must be underscored that the addition of 6% of glycerin causes instability to the process due to the probable buildup of volatile fatty acids, creating a toxic environment for methanogenic microorganisms and causing an interruption in biogas production.

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