Crude glycerin in anaerobic co-digestion of dairy cattle manure increases methane production

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ABSTRACT: Anaerobic digestion of crude glycerin (CG) along with animal waste has been an excellent option for increasing the production of biogas and methane to achieve efficiency in the treatment of both residues. This study aimed to evaluate improvements in specific productions of biogas and methane, reductions in solid and fibrous components in substrates prepared with dairy cattle manure and CG (containing 14 % glycerol). With these residues, experimental substrates were prepared and placed in 25 batch digesters. Initial content of the TS in the influent was 4 % and CG was added in increasing doses (0, 5, 10, 15 and 20 % relative to total solids (TS) of the influent). Results were submitted to ANOVA and orthogonal contrasts to assess the effects of linear and quadratic order and thereby estimate the optimal CG doses through the adjusted models. The highest values for specific production of methane (0.19 and 0.26 L g^{-1} of TS and volatile solids (VS) added, respectively) were reached with the CG inclusions of 6 and 8 %, respectively. Total production of biogas with the inclusion of 6 % CG was 11 % higher when compared to the control treatment. The largest reduction in VS (48 %) was achieved with the addition of 4 % CG. Addition of CG at levels between 3 and 8 % improved the efficiency of the process of anaerobic digestion with dairy cattle manure.
Keywords: glycerol, biogas, anaerobic digestion, solids

Introduction

Dairy-cattle farming is one of the most traditional activities in rural areas in Brazil. In 2012, 23.2 million cows were milked for a total of 34.2 billion liters (IBGE, 2013). Waste produced by dairy cattle has high organic and pathogen loads, but it can be treated by anaerobic biodigestion. However, biogas and methane yields are below those of waste processing from other species due to fibrous constituents that are both hard to break down and limit degradation (Zhang et al., 2013). Joint digestion, or co-digestion, of such waste with high-energy residues has been successfully employed to break down fibrous constituents and increase biogas and methane production.

Crude glycerin is a by-product of biodiesel production and has high polluting potential. High amounts of the substance produced in Brazil – 290,000 tons in 2013 (ANP, 2014) – is, therefore, cause for concern. Use of this residue is compromised by its low quality, high moisture content, excessive lipids, and high concentrations of sodium and other elements. Nonetheless, in recycling processes, the glycerol provided to the digestive medium may contribute to improving the process as long as the concentrations of the other constituents, or of the glycerol itself, do not outnumber those microorganisms able to deal with them.

Benefits of the co-digestion of sheep waste with increasing doses of crude glycerin containing 39 % glycerol were verified by a study carried out by Orrico and Orrico (2015). Biogas yield increased with the inclusion of up to 15 % glycerin, which was the maximum level employed, reaching potentials of 421.05 L kg^{-1} volatile solids (VS) added and 83 % methane in batch biodigesters. Athanasoulia et al. (2014) carried out co-digestion of sewage sludge with increasing additions of glycerin and found increases in biogas production immediately after the inclusion. However, biodigester stabilization was not reached, i.e., pH and biogas production soon dropped. Initial increases in biogas production were credited to the better use of soluble organic material in the substrates when glycerol was added, which produced volatile fatty acids in high concentrations and compromised methanogenic activity.

Anaerobic co-digestion was used in this study with the aim of assessing the increase in specific productions of biogas and methane and enhanced reduction of solid and fibrous constituents by adding crude glycerin to substrates prepared with dairy cattle waste.

Materials and Methods

The study was conducted in Dourados, MS, Brazil (latitude 22°11′55″ S, longitude 54°56′7″ W and 452 m of altitude). According to the Köppen climate classification, the climate is humid mesothermal - Cwa, with average temperature and precipitation between 20 and 24 °C and 1250 and 1500 mm respectively.

A completely randomized design was adopted in five treatments with different levels of inclusion of crude glycerin: 0, 5, 10, 15 and 20 % (relative to total solids (TS) of the substrate) in association with dairy cattle manure and five replicates (digesters) per treatment.

The crude glycerin was donated by a biodiesel plant located in Dourados, which uses soybean oil as the main raw material. The glycerin consisted of 95 % TS, with a composition as follows: 14 % glycerol, 78 % lipids and 6 % methanol, and COD (chemical oxygen demand) equal to 1532 g of $O_2 L^{-1}$ glycerin.

Dairy cattle manure was collected by scraping the stalls' floors during the animals' confinement period for milking. The manure pile was homogenized after collection and presented 19 % of TS of which 83 % were volatile (VS), and 57 % neutral detergent fiber (NDF). COD was equal to 592 g of $O_2 kg^{-1}$ of manure and the most probable number of total and fecal coliforms was 3.48×10^8 per 100 g of manure.

Before the preparation of the input substrate, an inoculum was prepared and considered ready when reached and kept a constant maximum concentration of methane (83 %). In the influent of all treatments, the inoculum represented 13 % of TS. At the time of loading the digesters, the inoculum consisted of 1 % TS, with 67 % volatiles, and COD equal to 293 g of $O_2 L^{-1}$, but coliforms were not detected.

Substrates composed of dairy cattle manure, glycerin, inoculum and water for dilution were prepared so that all treatments had TS content of 4 %. The digesters remained 176 days at room temperature, protected from sunlight and rain throughout the experimental period.

Digesters were built basically with two straight PVC cylinders with 150- and 100-mm diameters and a container with 65-mm diameter for storage of the material to be fermented with an average 1.3-L capacity of substrate in fermentation in each one. Cylinders with 100- and 150-mm diameter were inserted into each other, so that the space between the outer wall of the inner cylinder and the inner wall of the outer cylinder could hold a volume of water ("water seal"). The cylinder with a 100-mm diameter had one end closed and one opening for the release of biogas and was kept submerged in the water seal to provide anaerobic conditions and store the gas produced (represented by Sunada et al., 2014).

The daily volume of biogas produced was determined by measuring the vertical displacement of gasometers according to their cross-sectional area. Gas volume was corrected according to standard conditions for temperature and pressure.

Biogas composition was evaluated weekly to determine the volume of methane produced. Analysis of the biogas composition was perfomed using a Finnigan GC-2001 gas chromatograph equipped with a Porapack Q and molecular sieve column, and thermal conductivity detector in GA-21Plus gas analyzer. Specific productions of biogas and methane were calculated to determine the volume (liters) and the amounts (kg) of TS and VS added in the digesters.

Analyses of TS, VS and hydrogenic potential (pH) were carried out at the beginning and the end of the process (influent and effluent) according to the methodology described by APHA (2012). The NDF and hemicellulose contents were measured in accordance with the methodology proposed by Mertens (2002).

Results generated in the test of anaerobic co-digestion were analyzed by ANOVA and orthogonal contrasts were used to evaluate the effects of linear, quadratic and cubic order. These analyses were performed using the R statistical computer package (version 3.1.0 for Windows).

Results and Discussion

The mean values used to estimate the behavior of the parameters assessed and to obtain predictive models are presented in Table 1. As shown in Figure 1, there was a quadratic effect ($p < 0.05$) according to the prediction models estimated for reductions of TS and VS during the co-digestion of substrates prepared with dairy cattle manure and increasing levels of crude glycerin.

The maximum reduction (37 %) of TS was reached at the level of 5 % glycerin, while the best reduction of VS (48 %) occurred at the optimum inclusion dose of 4 % glycerin in the TS of the influent. Although TS and VS reductions were improved by the inclusion of glycerin in the substrates, increases in these degradations were only 3 and 1 %, respectively, when compared to substrates without added glycerin. Reductions in organic material in digestion are essential to reducing the polluting capacity of the waste, and they also represent the potential for

Table 1 – Mean reduction of the solid and fibrous constituents, of specific biogas and methane productions, of the proportion of methane in biogas, and pH values during anaerobic co-digestion of crude glycerin with dairy cattle waste in batch biodigesters.

Doses	Reductions (%)				Productions (L g^{-1}) Biogas				Methane (%)	pH	
					Biogas		Methane				
	TS	VS	Hem	Cel	TS ad	VS ad	TS ad	VS ad		Ini	Fin
0	35.6	47.3	19.7	35.8	0.25	0.35	0.17	0.24	68.1	7.8	7.7
5	37.8	48.4	22.8	35.0	0.27	0.36	0.20	0.27	74.0	8.0	7.8
10	35.3	46.1	24.5	34.7	0.26	0.35	0.19	0.26	73.0	8.0	7.7
15	33.1	42.9	26.8	35.2	0.22	0.30	0.16	0.21	71.0	8.0	7.6
20	29.2	38.1	34.9	37.2	0.18	0.23	0.12	0.16	67.0	8.0	7.3
CV (%)	1.2	1.3	2.6	2.0	1.1	2.0	1.9	1.6	1.8	0.6	1.0

TS = total solids; VS = volatile solids; Hem = hemicelluloses; Cel = cellulose; Ini = initial; Fin = final; CV = coefficient of variation.

biogas and methane production. However, the amounts reduced during the process are dependent on the composition of the substrates.

Glycerin used in this study contained high levels of lipid (78 %) and the increase of organic load in digestion caused by the inclusion of this fraction in substrates may have compromised the solids reductions. Gigestion of lipids in an anaerobic medium improves the yields of methane and biogas production, in addition to providing greater degradability of the mixtures in digestion. But, concentrations higher than 670 g of COD per liter of influent may cause accumulation of long chain fatty acids and also volatile fatty acids, lowering the pH and directly impairing methanogenic activity (Cirne et al., 2007). According to the experimental conditions of this study, the substrates prepared with 0, 5, 10, 15 and 20 % glycerin resulted in COD of 453.0, 838.0, 1202.1, 1587.2 and 1856.9 g of O_2 per liter, which represent a range of values that may have limited the conditions of digestion.

Superior results of solids reduction were reported by Orrico Junior et al. (2010) when evaluating manure of cattle fed diets with different ratios of forage-concentrate in batch digesters with hydraulic retention time (HRT) of 120 days. Reductions of 46 % of TS and 62 % of VS were obtained from manure generated with 60 % forage, while with 40 % forage the reduction of TS was 61 % and VS 63 %. These high values reached by the authors can be attributed to the quality of diets, especially forage, since fibrous constituents are a limiting factor in the degradation of substrates and the presence of glycerin may have changed the conditions of the medium in digestion.

Based on biogas yield at 70 days of retention time during the experimental period, the treatment without added glycerin had already produced 95 % of the total volume of biogas and the substrates with inclusion of 5, 10, 15 and 20 % glycerin had produced 91, 82, 41 and 36 % of the total volume of biogas, respectively. Therefore, the organic matter degraded in less time and for this reason, the experiment could have been finished earlier for the treatments with 0, 5 and 10 % glycerin, since at 112 days the three treatments reached 100 % of biogas production. However, according to the concentrations of methane in the biogas (Figure 2), the dose of 6 % glycerin promoted the highest volumetric yield of methane (11 L), which was 11 % superior to the treatment without crude glycerin. The highest methane concentrations were achieved firstly in the treatments with glycerin, demonstrating that, despite the degradations of the solids in digestion having taken longer, substrates containing glycerin sped up the production of methane. One of the most important parameters in biogas production is the increase of methane concentration with the addition of glycerin. This is an expected and desirable outcome because the fuel gas is a major indicative of efficiency in the waste digestion (Orrico and Orrico, 2015).

Reductions in the fibrous constituents (cellulose and hemicellulose, Figure 3) indicated that the greatest degradations of cellulose occurred according to the addition of glycerin in the composition of the substrates in digestion, but they were lower than hemicellulose, prob-

Figure 2– Concentrations of methane in the biogas produced from anaerobic co-digestion of dairy cattle manure and crude glycerin in batch digesters. TS = total solids.
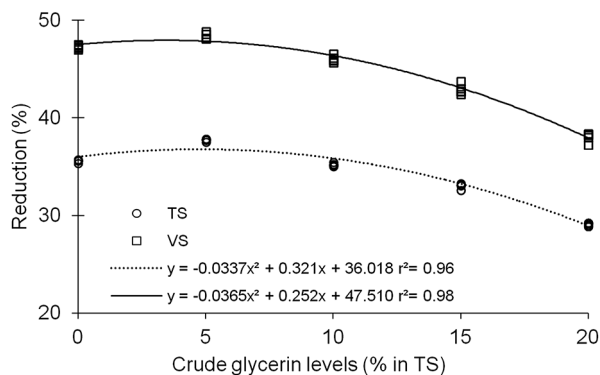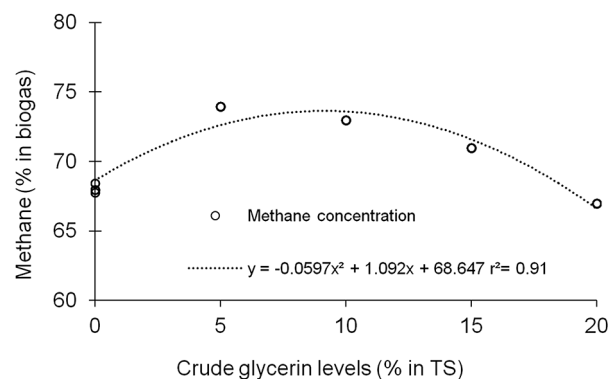

Figure 1 – Reductions of TS (total solids) and VS (volatile solids) in substrates prepared with dairy cattle manure and increasing levels of crude glycerin in batch digesters.
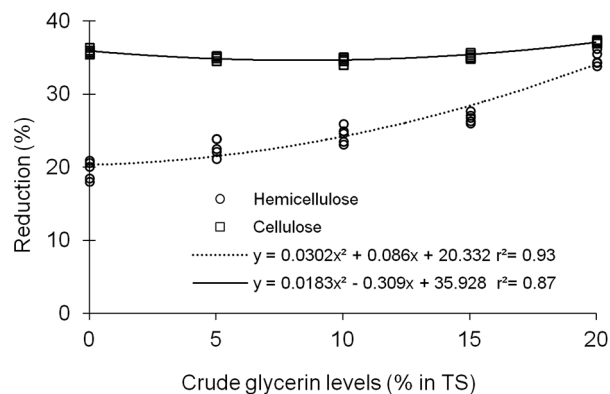

Figure 3 – Reductions of cellulose and hemicellulose in substrates prepared with dairy cattle manure and increasing levels of crude glycerin in batch digesters. TS = total solids.

ably because cellulose is associated with lignin, which makes it a constituent of hard degradation. There was a quadratic effect on hemicellulose reduction when the highest reduction (35 %) reached the maximum dose of glycerin (20 %). There was also high statistical correlation ($p < 0.01$) between the reductions of hemicellulose and final pH values (Figure 4), indicating that the acidification of the medium promoted by the addition of glycerin to the composition of the substrates affected the reductions due to the hemicellulose solubilization in an acid medium.

The acidification of the medium according to the increase in glycerin doses is due to microbial metabolism in the digesters. In substrates that contain carbohydrates of rapid degradation, a rapid increase in the population of bacteria also occurs. It is responsible for the processes of hydrolysis and acidogenesis and the doubling time of these microorganisms is on average 2-3 h (Shida et al., 2009). In addition to 14 % glycerol, glycerin had large amounts of lipids (78 %). Lipids also quickly degrade into organic acids (such as propionic and butyric) that are oxidized into acetate and subsequently generate methane. The drop in pH occurs because the archaea responsible for assimilation of the acids have a much slower doubling time of 1 to 9 days (Jetten et al., 1992), generating an acid accumulation in the medium.

According to the results of specific productions of biogas per added solids (Figure 5A), the dose of 6 % crude glycerin promoted the highest yield of biogas per TS (0.267 L g^{-1}), while the addition of 4 % glycerin generated the maximum yield of biogas per VS (0.364 g L^{-1}). For the specific production of methane (Figure 5B), the maximum yields of 0.196 and 0.264 L of methane per gram of TS and VS added were reached with the optimal doses of glycerin inclusion of 8 and 6 % (in relation to the substrate TS), respectively. These values represented increases of 13 and 10 % when compared with the results of the treatment without the addition of glycerin.

When using cattle manure fed only roughage, Alvarez et al. (2006) obtained inferior results of specific

production of methane of 0.131 L g^{-1} VS added, which is probably related to the shorter time that the material remained in digestion (HRT of 50 days) and the differences in animal diets, since without the inclusion of crude glycerin, the authors achieved a production of 0.239 L g^{-1} VS added. In contrast, Moller et al. (2004) using cattle manure fed 55 % roughage obtained better results (0.207 L g^{-1} VS) than Alvarez et al. (2006), but still lower than the current study, which may also be attributed to the composition of the residue and the beneficial effect of glycerin inclusion.

Crude glycerin inclusion greater than 6 % caused a decline in specific production of methane per VS added, which is probably indicative of overload of organic material in the medium in digestion. This outcome may be associated with the poor quality of the crude glycerin due to a high content of lipid. As stated by Long et al. (2012), lipids can have a negative effect on methanogenic archaea in high concentrations or loading rates, because they are composed of triglycerides and long chain fatty acids.

The rapid degradation of substrates in digesters results in a greater release of acids in the medium, which consequently inhibits methane production (Astals et al., 2011), possibly in virtue of the elevated sensitivity of methanogenic microorganisms to the changes in the medium and their slower growth rate (Jetten et al., 1992).
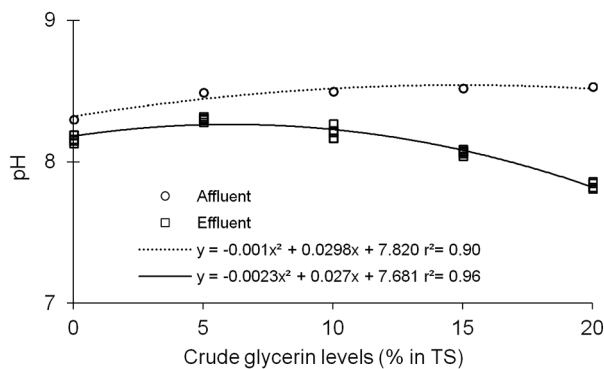
Figure 4 – pH behavior of the influent and effluent for evaluation of anaerobic co-digestion of crude glycerin with dairy cattle manure in batch digesters. TS = total solids.
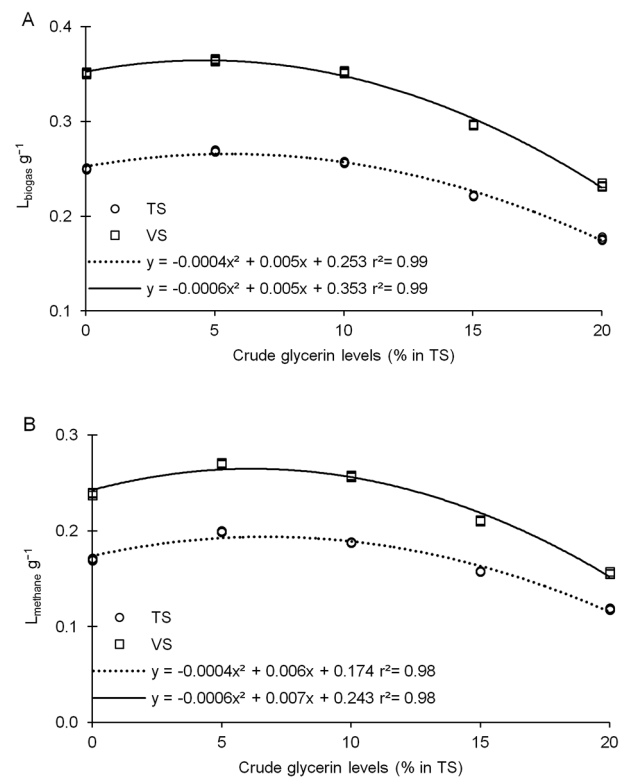
Figure 5 – Behaviors and predicting models of the effect of glycerin levels on the specific productions of biogas (A) and methane (B) per total solids (TS) and volatile solids (VS) added.

According to Jensen et al. (2014), the inclusion of glycerol in co-digestion has numerous advantages relating to its biodegradability, which improves the efficiency of this process. However, the amount added to the system must be taken into account, since it increases the organic load and may lead to overload and failure of the process. The aforementioned authors reported an optimal dose of 2 % glycerol in relation to the volume of digesters during co-digestion of sewage sludge, resulting in a 50 % increase in methane production. In addition, glycerol inclusions above this value became limiting even under ideal conditions of temperature and agitation. Thus, the recommendation made by these authors was based on the inclusion of high purity glycerin (98 % glycerol), which can be somewhat impractical for the preparation of substrates in Brazil, since the glycerin generated in the country may contain reduced glycerol content, as occurred in this study. Even when crude glycerin with low glycerol content was used, a positive effect was found for inclusions at 3 and 8 % in co-digestion with dairy cattle waste. At this inclusion level, the maximum reductions in TS and VS (37 and 48 %, respectively) and the highest specific productions of biogas and methane (reaching 0.364 and 0.294 L biogas and methane, respectively, per gram of VS added) were found. It is clear that these results are beneficial to the environment and can also improve the profitability of production units with power generation from renewable sources and production of organic fertilizers in terms of quality of pasture and forage.

Acknowledgments

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