Acta Scientiarum
http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v39i2.34007

Influence of manure concentration as inoculum in anaerobic digestion of vinasse

Carlos Eduardo de Farias Silva[1*] and Ana Karla de Souza Abud[2]

*[1]Centro de Tecnologia, Universidade Federal de Alagoas, Campus A. C. Simões, s/n., 57072-970, Maceió, Alagoas, Brazil. [2]Departamento de Tecnologia de Alimentos, Universidade Federal de Sergipe, São Cristóvão, Sergipe, Brazil. *Author for correspondence. E-mail: eduardo.farias.ufal@gmail.com*

ABSTRACT. Vinasse, main residue of the sugarcane industry, has high pollutant content, being subjected to the use in biogas production due to the high content of organic matter non-toxic to microbial action. For a consolidated process, it is necessary to study parameters that influence the process, in which the amount of inoculum is one of the major factors in the biological process of biogas production. This study investigated the influence of the amount of manure as inoculum (0.5 to 5.5%) during the biodigestion process, evaluating variables such as chemical oxygen demand (COD), pH, biogas production, methane concentration, total solids and total phosphorus and nitrogen contents, as well as microbiological analysis in the sludge remaining in the digester. Biodigestion occurred normally, with hydraulic retention time (HRT) of 20 days, with an acidogenic phase, subsequent stabilization of pH and biogas production. The vinasse had COD and total solids reduced during biodigestion by around 67 and 40%, respectively. Biogas production was increased after the fifth day. Among the three studied conditions, there was no significant increase in efficiency of inoculum use and it can be used the lowest amount, 0.5 % (m v^{-1}).

Keywords: biodigestion, manure, vinasse, biomethane, inoculum.

Influência da concentração de esterco como inóculo na digestão anaeróbia da vinhaça

RESUMO. A vinhaça, principal resíduo da indústria sucroalcooleira, possui alto teor poluente, sendo passível seu uso na produção de biogás devido ao grande teor de matéria orgânica não tóxica à ação microbiana. Para que haja um processo consolidado, é necessário estudar parâmetros que influem em seu processo, sendo a quantidade de inóculo um dos principais fatores no processo biológico de produção de biogás. Este estudo verificou a influência da quantidade de esterco bovino como inóculo (de 0,5 a 5,5%) durante o processo de biodigestão, avaliando variáveis como demanda química de oxigênio (DQO), pH, produção de biogás, concentração de metano, sólidos totais e teores de fósforo total e de nitrogênio total, além de análises microbiológicas no lodo restante no biodigestor. A biodigestão aconteceu normalmente, com um tempo de retenção hidráulica (TRH) de 20 dias, verificando-se a fase acidogênica, posterior estabilização do pH e produção de biogás. A vinhaça teve seus parâmetros de DQO e de sólidos totais diminuídos durante a biodigestão, em torno de 67 e 40%, respectivamente. Houve crescente produção de biogás após o quinto dia. Percebeu-se que, dentre as três condições estudadas, não houve significativa elevação da eficiência de uso de inóculo, podendo ser usada a menor quantidade, 0,5% (m v^{-1}).

Palavras-chave: biodigestão, esterco bovino, vinhaça, biometano, inóculo.

Introduction

Brazil is the largest producer of sugarcane (*Saccharum* spp.) in the world, followed by India and Australia. On average, 55% sugarcane produced in Brazil is transformed into ethanol and 45% into sugar (Bebé, Rolim, Pedrosa, Silva, & Oliveira, 2009). The expansion of this crop in the state of Alagoas occurred through an adaptation to the natural conditions, becoming the main agribusiness economic activity. In the national scenario, in the 2015/2016 growing season, the state of Alagoas ranked seven in bioethanol production (União dos Produtores de Bioenergia [UDOP], 2016).

In the last decade, there has been a significant increase in ethanol production, reflecting the growth in ethanol production in the United States from corn and the Brazilian supply, as well as the greater demand for renewable fuel (Brasil, 2004, Silva & Bertucco, 2016). Vinasse is the main residue of the sugar and alcohol industry, characterized as an effluent from the alcohol production after the must fermentation and wine distillation, being usual its application in the fertigation of sugarcane itself.

At the same time that it has a high fertilizing value, has a high polluting power, around one hundred times greater than domestic sewage, due to its organic matter content, low pH, elevated corrosivity and biochemical oxygen demand (BOD), ranging from 20 to 35 g L^{-1}. This polluting power makes it highly harmful to the fauna, flora, microfauna and microflora of freshwater, as well as frighten the marine fauna that comes to the Brazilian coast for breeding (Freire & Cortez, 2000, Moraes et al., 2014), while the high fertilizing value can be used in the cultivation of traditional crops. Due to this, the environmental perspective has been emphasized and, in some cases, the application of vinasse has been challenged by its effects on the soil in places close to groundwater, although several works show increased productivity due to its application as fertilizer, especially as a source of potassium (Paula, Holanda, Mesquita, & Carvalho, 1999, Brito, Rolim, & Pedrosa, 2005, Barbosa, Arruda, Pires, Silva, & Sakai, 2012, Silva & Abud, 2016).

The alternative forms of biomass conversion into secondary energy include the anaerobic biodigestion of waste, whose emergence occurred with the first oil crisis and with the pressure of sanitarians for cleaner production and more appropriate effluent treatment. The process leads to BOD reduction, biofertilizer production, small sludge or biomass production, consuming only 10% of the required by an aerobic process, low operating and investment costs, as well as the possibility of decentralized treatment systems (Chernicharo, 1997, Cortez, Silva, Lucas Junior, Jordan, & Castro, 2007). The study on biodegradable substrates is essential for a selection of suitable substrates, propitious to higher yield of the process, as well as the residue used (Yusuf, Debora, & Ogheneruona, 2011).

Anaerobic conversion takes place in a biodigestor, a closed chamber where biomass is placed, which can be any effluent rich in organic matter, and the inoculum, that contains methanogenic anaerobic bacteria, such as manure. Biomass provides the substrate and the biodigestor provides the anaerobic condition for bacterial proliferation, resulting in the metabolism of biogas and, what is left of the substrate, a biofertilizer, richer in NPK mineralized nutrients, essential to plants. According to Sganzerla (1983), the main reason for the great fertilizing capacity of the biofertilizer lies in the fact that the biomass digestion drastically reduces the carbon content present in the biomass, almost exclusively losing carbon in the methane form (CH_4). The concentration of inoculum is important for the process and varies according to the material used to achieve an active and adequate microbial population (Raposo, De La Rubia, Fernandez-Cegri, & Borja, 2012). According to De Vrieze et al. (2015), the type of inoculum affects the microbial population in the process and can influence the physicochemical biofertilizer composition and the capacity and quality of biogas production.

However, in several situations, it is not enough to have in hand data regarding the amount of biogas produced. It is also essential to know the quality of this biogas, referring to parameters that can be submitted to the process, that is, to have the exact notion of the components variation influencing the biogas production during the fermentation process, since, based on these data, it is possible to verify and characterize its variation in the fermentation and to suggest the quality control of the gas to be purified and stored (Galbiatti, Caramelo, Silva, Gerardi, & Chiconato, 2010, Moraes, Zaiat, & Bonomi, 2015).

The present study aimed to evaluate the use of bovine manure as inoculum in the vinasse biodigestion process, using 0.5, 3.0 and 5.5% of manure. The whole process was characterized, controlling the pH in the acidogenic phase and following the chemical oxygen demand, besides the formation and composition of the biogas formed. At the end of the process, the biofertilizer was characterized and the sludge formed was microbiologically investigated.

Material and methods

Obtaining and characterizing sugarcane vinasse

The vinasse was kindly provided by Usina Cachoeira and the trials were conducted at the Chemical Engineering Laboratory of the Center of Technology at the *Universidade Federal de Alagoas*. It was collected still warm, stored in an ice bath and taken to the laboratory, where it was identified, conditioned in plastic bottles and stored in freezer. For the physicochemical characterization, vinasse was analyzed for pH, total fixed and volatile solids, chemical oxygen demand (COD), total nitrogen, ammonia nitrogen, chloride, phosphorus, total acidity and density, according to the Standard Methods for the Examination of Water and Wastewater (American Public Health Association [APHA], 1995).

The pH was determined using potentiometer and electrode, calibrated in buffer solutions of pH 4.0 and 7.0. The analysis of total solids was performed by gravimetry, based on the results obtained by means of the difference in weight before and after the sample evaporation in an oven at 105°C. Fixed total solids were determined after calcination in a muffle at 550°C for one hour. The difference between total solids and total fixed solids resulted in total volatile solids. Chemical oxygen demand (COD), which corresponds to the chemical oxidation of organic matter, was obtained by means of a strong oxidant, potassium dichromate ($K_2Cr_2O_7$), in acidic medium, at high temperature. The standard COD curve for the spectrophotometric analysis was performed with solutions of potassium biphthalate ($C_8H_5O_4K$) of known concentration. The analysis of the phosphorus content was made by the ascorbic acid method, from spectrophotometric readings after digestion with concentrated acid, by constructing a standard curve with solutions of potassium phosphate (KH_2PO_4) of known concentration. The chloride content determination and the total acidity were determined by volumetry, titrating the samples with the standard solutions of 0.01 N $AgNO_3$, having a 5% potassium chromate solution by precipitation by the Mohr method and with 0.1 N NaOH, using 1% phenolphthalein as the alcoholic solution by neutralization, respectively. The density was calculated from the mass/volume ratio, obtained by weighing aliquots of 10 mL vinasse on an analytical balance of four decimal places, at 30°C.

Both ammonia nitrogen and total nitrogen (Kjeldahl) were made from the distillation of the sample in a nitrogen distiller, collected in 4% boric acid with mixed indicator, followed by titration with the standard solution of 0.1 N HCl. The difference between the two analyses is the sample digestion in concentrated sulfuric acid and catalytic mixture ($Se + CuSO_4 + NaSO_4$) at 350°C for 3 hours before the ammonium distillation to total nitrogen (TKN). Ammonia nitrogen measures only the nitrogen present as ammonia in the effluent, while the total also evaluates the nitrogen from the acid hydrolysis of other compounds, mostly organic, as proteins, promoted by digestion.

Biodigestion experiment

The trials were carried out in duplicate using bovine manure as inoculum and plastic biodigesters (0.6 L usable volume and 0.2 L headspace), according to Angelidaki et al. (2009), with two connections at the top of the reactor (one for the biogas collection syringe and one for pH adjustment and sample collection). The pH adjustment, when necessary to keep it neutral, was done using NaOH during the acidogenic phase. The reactor heating was provided by Nova Tecnica NT 235 bath. The reactors were homogenized once daily for sample collection.

The inoculum concentrations used were 0.5, 3.0 and 5.5% (m v^{-1}), since the bovine manure has about 80 g volatile solids (VS) kg of material^{-1} (Barros et al., 2009). In this study, the amount of manure used as inoculum ranged from 0.5 to 5.5%, that is, a load between 10 and 100 g of $COD_{vinasse}$ g $VS_{inoculum}^{-1}$ (50 as mean value), based on studies by De Vrieze et al. (2015) and Esposito et al. (2012), in order to evaluate the interactions of the different inoculum concentrations on the production of biogas and biofertilizer.

The hydraulic retention time (HRT), considered the time required for organic matter degradation (Diesel, Miranda, & Perdomo, 2002), was determined according to biogas production, finalizing the biodigestion when the biogas volume was constant.

Physicochemical and microbiological characterization of biofertilizer

Physicochemical analyses performed in the biofertilizers consisted of chemical oxygen demand (COD), total fixed and volatile solids, phosphorus and total nitrogen content, according to the aforementioned methodologies in the characterization of fresh vinasse.

Microbiological analyses for total and fecal coliforms were performed using the most probable number (MPN) technique in 3 tube series with the 2% brilliant green bile broth (VB) and *Escherichia coli* (EC) media, respectively, incubated in water bath for 37.5 and 45°C for up to 48 hours. For the sludge formed on the vinasse surface inside the biodigestor, standard plate count was performed with nutrient agar (NA), MacConkey and Sabouraud agar media to evaluate, respectively, heterotrophic mesophilic bacteria, Gram-negative bacteria and fungi (molds and yeasts), using serial dilutions of the order of 10^5 and 10^6 for better adjustment in the count, incubated at 30°C in a bacteriological incubator for 5 days.

Concentration of carbon dioxide and methane

The determination of carbon dioxide, by the Alfakit biogas kit, developed in partnership with *Embrapa Suínos e Aves*, was carried out by adapting the Orsat method, in which a basic solution reacts with carbon dioxide, causing it to precipitate to measure its concentration. The methane gas was obtained indirectly, estimated by the difference in CO_2 result. The concentrations obtained for CO_2 and CH_4 are presented in percentage (%), with an accuracy of ± 5%, and are relative, since it takes into account the existence of only these two compounds, that is, the concentration of methane is obtained by the difference between 100% and the percentage of CO_2.

Using the Origin 6.0, a non-linear regression was performed with the modified Gompertz equation based on studies in the literature (Yusuf et al., 2011, Patil et al., 2012). Equation 1 aims to model the biogas production and obtain constant parameters that are characteristic to the process in Equation 1.

$$B(t) = B.\exp\left(-\exp\left[\left(\frac{R_b}{B}\right)\left[(\lambda - t)\right.\right.\right. \quad (1)$$

where:
$B(t)$ (mL) is the amount of biogas produced at any given time t (day),
B is the potential of biogas production (mL),
Rb is the maximum production rate achieved during the biogas production (mL day^{-1}) and λ is the latency period of the production process (day).

Statistical analysis

Data were subjected to Analysis of Variance (ANOVA) and multiple mean comparison tests, using the Tukey's test (Assistat Software version 7.6) and the Statistica software version 8.0, with a 95% confidence level. It was also evaluated the coefficient of determination (r^2), which indicates the proportion (%) of variation among the variables, and the Chi-square test (Chi^2), one of the most popular parametric tests, used to analyze behavior/performance of two groups/populations.

Results and discussion

The hydraulic retention time (HRT), which may vary between 20 and 50 days, depending on the substrate and the manure used, was 20 days. For bovine manure, the range is usually between 30 and 45 days, with an average of 35 days (Diesel et al., 2002, Arruda, Amaral, Pires, & Barufi, 2002). In biogas production with swine manure, the HRT range is around 15 and 36 days, and it was observed by Orrico Junior, Amorim, and Lucas Junior (2009) that within 36 days, there is a greater removal of COD, about 60 to 80%. Amaral et al. (2004) examined the biogas production from dairy cattle waste, and found that between 20, 30 and 40 days of HRT the major total solids reduction was achieved in 40 days. Quadros et al. (2010), using goat and sheep manure, indicated 45 days as the ideal HRT. In all these studies, based on animal waste as inoculum, prevails the characteristics of the effluent, which is what will be decomposed and will result in biogas.

Results of physicochemical analyses are listed in Table 1 and show that vinasse has all of the harmful characteristics to the environment, as cited by Freire and Cortez (2000) and Lora and Andrade (2009), as high COD, low pH and high total solids content.

Table 1. Physicochemical characterization of sugarcane vinasse.

Determinations	Results
pH	4.47
Total solids (mg L^{-1})	55728 ± 3785
Total fixed solids (mg L^{-1})	16921 ± 128
Total volatile solids (mg L^{-1})	38807 ± 3657
COD (mg O_2 L^{-1})	38911.2
Chloride (mg Cl L^{-1})	378.7 ± 41
Phosphorus (mg P L^{-1} in PO_4^{3-})	17.56 ± 0.88
Ammonia nitrogen (mg N L^{-1} in NH_3)	15.9 ± 5.5
Total nitrogen (mg N L^{-1})	711 ± 153
Total acidity (%)	6.54 ± 0.33
Density (g cm^{-3})	1.015 ± 0.005

It was possible to collect gas and the biodigestion was interrupted only when gas formation was constant, at an HRT of 20 days. The results of COD monitoring over time are illustrated in Figure 1.

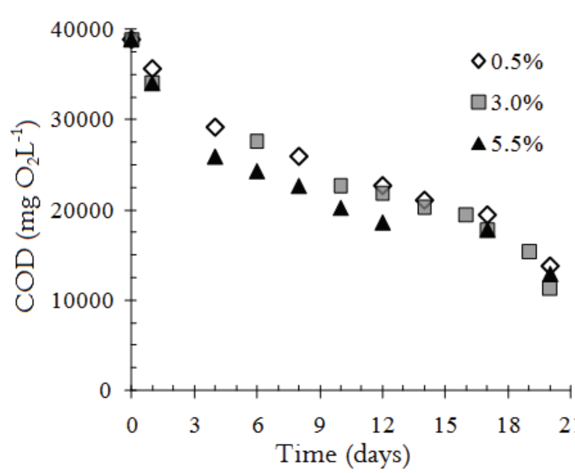

Figure 1. COD analytical monitoring during the biodigestion.

There was no significant difference in COD reduction between the three inoculum concentrations used, reaching a mean of 68 ± 3%, with COD reducing a little between the 5th and 12th day. According to Von Sperling (2007), the reductions in vinasse BOD must be around 70%, a value close to that found for COD reduction of the effluent that underwent microbial degradation, indicating reduction, in its majority, of the biodegradable part.

The control pH was 7.0 ± 0.5 and a negative variation (ΔpH < 0) indicated a drop, making the environment more acidic. Anaerobic digestion can be summarized in four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Patil et al., 2012, Costa, Barbosa Filho, & Giordano, 2014), where acidogenesis is the process in which there is the formation of organic acids that cause a sharp decline in the pH of the medium, helping to promote then the acetogenesis, which forms the main quantities of substrates necessary to the last step, the methanogenic, of methane production. Acidogenesis ceased after the 3rd day. Biogas production started from the 6th day, just after the end of the acidogenesis, being the production almost similar in the three concentrations studied, as shown in Figure 2.

Biogas production, around 175 mL of biogas $L^{-1}_{vinasse}$, was practically similar in all three trials. The theoretical values for methane production per g of COD depend on the substrate used. For bovine manure, the values turn around 0.025 to 0.1 L g^{-1} $COD_{removed}$ (Amaral et al., 2004, Oliver, Neto, Quadros, & Valladares, 2008); for pig farming, around 0.061 to 0.45 L g^{-1} $COD_{removed}$ (Lamo, 1991), and for vinasse from 0.3 to 0.365 L g^{-1} $COD_{removed}$ (Lamo, 1991, Salomon, & Lora, 2009).

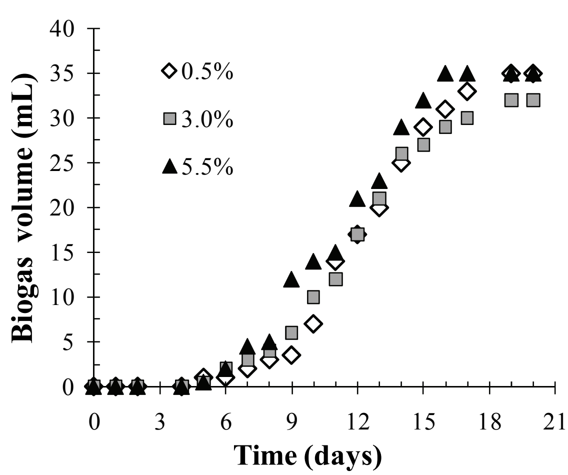

Figure 2. Biogas collection along the biodigestion process.

The characterization of the biogas formed can be visualized in Table 2. It should be noted that the Alfakit biogas kit performs the quantification of CO_2 and does a 100% subtraction to find the value of CH_4, disregarding the 5% relative to other components, such as ammonia, hydrogen sulfide, hydrogen, water, oxygen, among others (Oliver et al., 2008, Salomon, & Lora, 2009, Patil et al., 2012, Costa et al., 2014). The values found are not far from those mentioned in the literature, especially regarding the CO_2 content. There was a statistically significant difference between the trials with the lowest and the highest (5.5%) concentrations of manure. Differences associated with biogas production, as well as composition may occur when using a very low or very high substrate/inoculum ratio (De Vrieze et al., 2015).

Considering the COD removed (Table 3) and using for the vinasse employed the relation 0.365 L g^{-1} $COD_{removed}$, the biogas collected in the trials represented only about 10% of the theoretical value. The removal of COD/BOD is a combination of the demand for biogas formation with the required demand for cell proliferation and the retained demand in biofertilizer and final sludge. Therefore, given the batch production, low conversion efficiency was reached, indicating the need to use a greater part of the organic matter for cell proliferation. As it has been commonly used continuous or semi-continuous biodigesters, the cultures established by the proliferation of the first stage are more active in the next digestions, achieving greater efficiencies.

Table 2. Characterization of biogas collected during biodigestion.

Biogas characterization			
Trial	% CO_2	% CH_4	C_{H2S} (ppm V)
5.5%	37.5	62.5[a]	20
3.0%	22.5	77.5[b]	20
0.5%	27.5	72.5[b]	20
(Quadros et al., 2010)	34.4	57.8	-
(Salomon & Lora, 2009)	25 - 40	40 - 75	-
(Lora & Andrade, 2009)	30.5	66.5	-

Different letters between the tests indicate significant differences by Tukey's test at 5% level.

In the physicochemical characterization of fresh and biodigested vinasse (Table 3), similar values were detected for parameters analyzed between the three inoculum concentrations at the end of biodigestion, a fact also validated by the statistical analysis ofTukey test, where the inoculated trials did not differ statistically from each other at the 5% probability level, except for the phosphorus content.

The total solids concentration used was between 6 and 11%, suitable for the anaerobic digestion process. For Rajendran, Aslanzadeh, and Taherzadeh (2012), this value should be between 7 and 9%, while Amaral et al. (2004) cites 8% and Arruda et al. (2002), 10%.

During the anaerobic fermentation process, carbon of the organic matter is transformed into methane (CH_4), corroborating the results obtained. For nutrients such as nitrogen, phosphorus and potassium, however, there is greater preservation, with concentration due to the low or no de-assimilation of these nutrients in gaseous form. The total nitrogen present was concentrated around 80 ± 5% and, for total phosphorus, 16 ± 7% in the biofertilizer collected, in a similar way for the trials, except for the total phosphorus content in 5.5% of manure.

Table 3. Physicochemical characterization of biodigestion process.

Analysis	Fresh vinasse	Biodigested vinasse (Biofertilizer)		
		0.5%	3.0%	5.5%
COD (mg O_2 L^{-1})	38911.2	13781.1	11349.1	12970.4
Phosphorus (mg P L^{-1} in PO_4^{3-})	$17.56^a \pm 0.88$	$19.90^{ab} \pm 1.34$	$19.31^{bc} \pm 1.76$	$21.95^c \pm 1.34$ CV (%) = 11.81
Nitrogen (mg N L^{-1})	$711^a \pm 153$	$1253.9^b \pm 213$	$1359.1^b \pm 8.1$	$1278.0^b \pm 55.5$ CV(%) = 11.74
Total solids (mg L^{-1})	$55728^a \pm 3785$	$35660^b \pm 85$	$33155^b \pm 346$	$34880^b \pm 226$ CV (%) = 4,54
Total fixed solids (mg L^{-1})	$16921^a \pm 128$	$22630^b \pm 806$	$23045^b \pm 290$	$25048^b \pm 200$ CV (%) = 8.55
Total volatile solids (mg L^{-1})	$38807^a \pm 3657$	$13030^b \pm 721$	$10110^b \pm 636$	$9832^b \pm 26$ CV (%) = 3.83

CV (%) – coefficient of variation between the means of the studied variables. The letters a, b, and c indicate the statistical analysis performed by Tukey's test using Assistat Software version 7.6. Means followed by different letters are significantly different at the 5% probability level.

The levels of coliforms in the biodigested effluents (Table 4), compared to the literature data, show homogeneity, presenting a 90-95% reduction in the amount of total and fecal coliforms. The Environmental Protection Agency (Environmental Protection Agency [EPA], 1985) states that the survival time of pathogens, such as *Escherichia coli*, in fecal coliforms, is from 4 to 55 days, evidencing the concern with the resulting microbial load in the biofertilizer and its ability to contaminate soils and surface water.

Table 4. Microbiological analysis of total and fecal coliforms in biofertilizer.

Analysis of coliforms (MPN mL^{-1})	% Inoculum	Total	Fecal
HRT 20 days Bovine manure - Batch model	0.5	> $1.1.10^5$	$1.1.10^5$
	3.0	$2.1.10^4$	$6.4.10^3$
	5.5	$1.1.10^5$	$1.1.10^5$

Amaral et al. (2004) used bovine manure in a Chinese digester and observed in a 20 day-HRT, $2.4.10^4$ MPN mL^{-1} total coliforms and $4.7.10^3$ MPN mL^{-1} fecal coliforms in the biofertilizer. Orrico Junior et al. (2009), in a 22 day-HRT with swine manure in an Indian model, verified $4.6.10^4$ to $1.1.10^5$ MPN mL^{-1} total coliforms and $1.5.10^4$ to $1.1.10^5$ MPN mL^{-1} of fecal coliforms in the biofertilizer. Quadros et al. (2010), in an ongoing process using a Canadian biodigestor with sheep and goat manure, reached above 98% reduction in 20 day-HRT.

Table 5 lists the results obtained from the plate counts for the sludge sample on the surface of the biodigested effluent, indicating that the behavior of the three tests did not diverge. In the nutrient agar medium, the dilutions of the order of 10^{-6} were insufficient to make a plate count, and the maximum value was extrapolated. For both MacConkey and Sabourard agar, results were obtained at 10^{-6} dilutions. This information can be important, since an analysis of the population diversity was not possible, at least, the concentration of groups (total heterotrophic mesophilic, Gram-negative bacteria and yeast and molds) is presented.

Table 5. Plate count of microorganisms from the surface sludge of the biodigester.

Count (FCU.g^{-1})	Inoculum condition		
	0.5%	3.0%	5.5%
Nutrient agar(total heterotrophic mesophilic)	> $3.76.10^8$	> $3.81.10^8$	> $3.79.10^8$
MacConkey agar(Gram-negative bacteria)	$3.30.10^8$	$2.89.10^8$	$3.79.10^8$
Sabourard agar (yeast and moulds)	$3.57.10^7$	$1.91.10^6$	$8.16.10^7$

The regression of the modified Gompertz equation modeled efficiently the biogas production during biodigestion, providing the parameters of the equation and coefficients of determination (r^2) and contingency (Chi^2), as shown in Table 6.

Table 6. Parameters of the non-linear regression *B*(t).

Parameters	Trial		
	0.5%	3.0%	5.5%
B (mL)	37.88 ± 1.01	34.97 ± 1.05	39.24 ± 1.71
Rb (mL day^{-1})	12.49 ± 0.58	10.58 ± 0.53	11.10 ± 0.80
λ (day)	12.31 ± 0.13	11.75 ± 0.16	11.18 ± 0.24
r^2 = 0.9958			
Chi^2 = 0.7994			

The coefficient of determination (r^2) was close to 1, indicating a good regression of the process, and the contingency coefficient (Chi^2), was 0.8, suggesting dependence between the studied variables in the regression, that is, biogas production (*B*) and time (*t*). The maximum production predicted by the model was not reached by the experimental data, which was between 7 and 8 mL day^{-1}.

Also, the parameters of biogas production did not differ significantly between the three tests, as well as in the physicochemical parameters for the biofertilizers and for the microbiological analyses of the sludge formed, indicating that under the three conditions studied, there is no advantage in using a larger amount of manure, although the use of inoculum anticipates the biogas production peak and increases the effective biomass potential. The highest amount of inoculum in this case did not cause an increase in efficiency of the process, negatively influencing the biogas production, since it was not elevated, and occupied a volume that could be filled by the substrate to be treated.

Our findings indicated the use of bovine manure as an inoculum for the anaerobic biodigestion of vinasse where, in low amounts (0.5 to 3%), it was possible to treat around 80% of the chemical oxygen demand, producing biogas, which can be used to generate energy, in addition to producing a biofertilizer with excellent characteristics for the nutrients nitrogen and phosphorus (NP) and with very low amount of coliforms.

It is important to mention that the use of anaerobic biodigestion in sugarcane biorefineries is of utmost importance because, besides the environmental factor associated with the removal of organic matter and its mineralization, facilitating the absorption by the plants when the biofertilizer is applied, it also has the energy factor of biogas generation, which increases the process self-sufficiency (Moraes et al., 2014, Silva & Abud, 2016). Many types of inoculum can be used, where each has a specific microbial population that can influence the process. Because of this, the study of each source is necessary and, as can be seen with bovine manure, the same desired characteristics were obtained in an anaerobic biodigestion process. This efficiency is usually measured by the removal of chemical oxygen demand (COD), between 60-80%, and by the percentage of methane production, 60-70% (Moraes et al., 2015), range of values found in this research. The biofertilizer obtained from anaerobic biodigestion has excellent properties for vegetable fertilization, drastically minimizing the polluting impact caused when fresh vinasse is used (Silva & Abud, 2014).

Conclusion

Biodigestion occurred with an HRT of 20 days, reducing the content of chemical oxygen demand and total solids by 67 and 40%, respectively. The nitrogen and total phosphorus contents were preserved and were concentrated because there is little or no gas adsorption of these components, making the biofertilizer suitable for use. The acidogenic phase was verified between 5 and 7 days, with a drop in pH, followed by the production and collection of biogas, which provided an average of 70% methane in its composition. After analyzing all the parameters and comparing the obtained means, we noticed no increase in the efficiency of biogas production caused by the amount of inoculum added. The results allow to recommend the use of the lowest values of inoculum [0.5 to 3.0% (m v^{-1})], which can afford a greater amount of effluent to be treated within the biodigester. The anaerobic biodigestor, operating in batch mode, stored about 50 to 60% of the effluent COD in the form of sludge, probably due to microbial proliferation.

References

Amaral, C. M. C., Amaral, L. A., Lucas Júnior, J., Nascimento, A. A., Ferreira, D. S., & Machado, M. R. F. (2004). Biodigestão anaeróbia de dejetos bovinos leiteiros submetidos a diferentes tempos de retenção hidráulica. *Ciência Rural, 6*(6), 1897-1902.

American Public Health Association. (1995). *Standard methods for the examination of water and wastewater* (19th ed.). Washington, DC: American Public Health Association.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guvy, A. J., ... Van Lier, J. B. (2009). Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Science and Technology, 59*(5), 927-934.

Arruda, M., Amaral, L., Pires, O., & Barufi, C. (2002). Dimensionamento de biodigestor para geração de energia alternativa. *Revista Científica Eletrônica de Agronomia, 1*(2), 1-8.

Barbosa, E. A. A., Arruda, F. B., Pires, R. C. M., Silva, T. J. A., & Sakai, E. (2012). Cana-de-açúcar fertirrigada com vinhaça e adubos minerais via irrigação por gotejamento subsuperficial: ciclo da cana-planta. *Revista Brasileira de Engenharia Agrícola e Ambiental, 16*(9), 952-958.

Barros, R. M., Tiago Filho, G. L., Nascimento, Y. D. S., Gushiken, E., Calheiros, H. C., Silva, F. G. B., & Stano Junior, A. (2009). Estudo da produção de biogás da digestão anaeróbica de esterco bovino em um biodigestor. *Revista Brasileira de Energia, 15*(2), 95-116.

Bebé, F. V., Rolim, M. M., Pedrosa, E. M. R., Silva, G. B., & Oliveira, V. S. (2009). Avaliação de solos sob diferentes períodos de aplicação com vinhaça. *Revista Brasileira de Engenharia Agrícola e Ambiental, 13*(6), 781-787.

Brasil. (2004). *Decreto de 21 de outubro de 2004*. Cria o Grupo de Trabalho Interministerial do Setor Sucroalcooleiro da Região Nordeste. Retrieved from https://www.camara.gov.br/sileg/integras/409753.pdf

Brito, F. L., Rolim, M. M., & Pedrosa, E. M. R. (2005). Teores de potássio e sódio no lixiviado e em solos após a aplicação de vinhaça. *Revista Brasileira de Engenharia Agrícola e Ambiental, 9*(Suplemento), 52-56.

Chernicharo, C. L. A. (1997). *Reatores anaeróbios: princípios do tratamento biológico de águas residuárias*. Belo Horizonte, MG: UFMG.

Cortez, L. A. B., Silva, A., Lucas Junior, J., Jordan, R. A., & Castro, L. R. (2007). Biodigestão de efluentes. In L. A. B. Cortez, & E. S. Lora (Coords.), *Biomassa para energia* (p. 493-529). Campinas, SP: Unicamp.

Costa, E. S., Barbosa Filho, O., & Giordano, G. (2014). *Reatores anaeróbios de manta de lodo (UASB): uma abordagem concisa*. Rio de Janeiro, RJ: FEN/Uerj.

De Vrieze, J., Raport, L., Willems, B., Verbrugge, S., Volcke, E., Meers, E., … Boon, N. (2015). Inoculum selection influences the biochemical methane potential of agro-industrial substrates. *Microbial Biotechnology, 8*(5), 776-786.

Diesel, R., Miranda, C. R., & Perdomo, C. C. (2002). Coletâneas de tecnologias sobre dejetos suínos. *Boletim Informativo BIPERS*. Retrieved from https://docsagencia.cnptia.embrapa.br/suino/bipers/bipers14.pdf

Environmental Protection Agency. (1985). *Land application of municipal sludge*. Cincinati, OH: Center for Environmental, Research Information.

Esposito, G., Frunzo, L., Giordano, A., Liotta, F., Panico, A., & Pirozzi, F. (2012). Anaerobic co-digestion of organic wastes. *Reviews in Environmental Science and Bio/Technology, 11*(4), 325-341.

Freire, W. J., & Cortez, L. A. B. (2000). *Vinhaça de cana-de-açúcar*. Guaíba, RS: Agropecuária.

Galbiatti, J. A., Caramelo, A. D., Silva, F. G., Gerardi, E. A. B., & Chiconato, D. A. (2010). Estudo qualiquantitativo do biogás produzido por substratos em biodigestores tipo batelada. *Revista Brasileira de Engenharia Agrícola e Ambiental, 14*(4), 432-437.

Lamo, P. (1991). *Sistema produtor de gás metano através de tratamento de efluentes industriais.* Piracicaba, SP: Methax/Biopaq - Codistil.

Lora, E. S., & Andrade, R. V. (2009). Biomass as energy source in Brazil. *Renewable and Sustainable Energy Reviews, 13*, 777-788.

Moraes, B. S., Junqueira, T. L., Pavanello, L. G., Cavalett, O., Mantelatto, P. E., Bonomi, A., & Zaiat, M. (2014). Anaerobic digestion of vinasse from sugarcane biorefineries in Brazil from energy, environmental and economic perspectives. *Applied Energy, 113*, 825-835.

Moraes, B. S., Zaiat, M., & Bonomi, A. (2015). Anaerobic digestion of vinasse from sugarcane ethanol production in Brazil: Challenges and perspectives. *Renewable and Sustainable Energy Reviews, 44*, 888-903.

Oliver, A. P. M., Neto, A. A. S., Quadros, D. G., & Valladares, R. E. (2008). *Manual de treinamento em biodigestão*. Salvador, BA: Instituto Internacional Winrock Brasil.

Orrico Junior, M. A. P., Amorim, A. C., & Lucas Junior, J. (2009). Biodigestão anaeróbia de dejetos suínos com e sem separação da fração sólida em diferentes tempos de retenção hidráulica. *Engenharia Agrícola, 29*(3), 474-482.

Patil, J. H., Raj, M. A., Muralidhara, P. L., Desai, S. M., Raju, & Mahadeva Raju, G. K. (2012). Kinetics of anaerobic digestion of water hyacinth using poultry litter as inoculum. *International Journal of Enviromental Science and Development, 3*(2), 94-98.

Paula, M. B., Holanda, F. S. R., Mesquita, H. A., & Carvalho, V. D. (1999). Uso da vinhaça no abacaxizeiro em solo de baixo potencial de produção. *Pesquisa Agropecuária Brasileira, 34*(7), 1217-1222.

Quadros, D. G., Oliver, A. P. M., Regis, U., Valladares, R. E., Souza, P. H. F., & Ferreira, E. J. (2010). Biodigestão anaeróbia de dejetos de caprinos e ovinos em reator contínuo de PVC flexível. *Revista Brasileira de Engenharia Agrícola e Ambiental, 14*(3), 326-332.

Rajendran, K., Aslanzadeh, S., & Taherzadeh, M. J. (2012). Household biogas digesters: a review. *Energies, 5*(8), 2911-2942.

Raposo, F., De La Rubia, M. A., Fernandez-Cegri, V., & Borja, R. (2012). Anaerobic digestion of solid organic substrates in batch mode: an overview relating to methane yields and experimental procedures. *Renewable and Sustainable Energy Reviews, 16*(1), 861-877.

Salomon, K. R., & Lora, E. E. S. (2009). Estimate of the electric energy generating potential for different sources of biogas in Brazil. *Biomass and Bioenergy, 33*(9),1101-1107.

Sganzerla, E. (1983). *Biodigestores: uma solução*. Porto Alegre, RS: Agropecuária.

Silva, C. E. F., & Abud, A. K. S. (2014). Acompanhamento do tempo de retenção hidráulico (TRH) na biodigestão da vinhaça e utilização de seu biofertilizante em sementes de feijão. *Scientia Plena, 10*(7), 1-7.

Silva, C. E. F., & Abud, A. K. S. (2016). Anaerobic biodigestion of sugarcane vinasse under mesophilic conditions using manure as inoculum. *Ambiente & Agua – An interdisciplinary Journal of Applied Science, 11(4)*, 763-777.

Silva, C. E. F., & Bertucco, A. (2016). Bioethanol from microalgae and cyanobacteria: a review and technological outlook. *Process Biochemistry, 51*(11), 1833-1842.

União dos Produtores de Bioenergia. (2016). SAPCANA – Sistema de Acompanhamento da Produção Canavieira. Safra 2015/2016. Retrieved from http://www.udop.com.br/download/estatistica/producao_estados/2015a2016_acompanhamento_producao_estados.pdf

Von Sperling, M. (2007). *Basic principles of wastewater treatment*. London, UK: IWA Publishing.

Yusuf, M. O. L., Debora, A., & Ogheneruona, D. E. (2011). Ambient temperature kinetic assessment of biogas production from co-digestion of horse and cow dung. *Research in Agricultural Engineering, 57*(3), 97-104.

Received on December 28, 2016.
Accepted on February 6, 2017.