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Stability of an anaerobic single reactor filled with dolomitic limestone with increased organic load of sugarcane

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ABSTRACT

The anaerobic single-stage reactor was evaluated to treat vinasse and to evaluate its stability. This bench reactor was filled with dolomitic limestone with a horizontal plug flow to simulate a drainage channel. The experiment lasted 129 days while the reactor was submitted to different applied organic concentrations (chronologically applied: 3.0; 5.0; 12.0; 9.0 and 7.5 g L^{-1} as COD, chemical oxygen demand). COD removals were 50% and 9% with 3.0 and 7.5 g L^{-1}, respectively. With 12.0 g L^{-1}, reactor efficiency increased to 33%, with an abrupt drop to 3% on the 84th day. Therefore, in order to avoid reactor collapse, a remedial measure was necessary. The system remained in batch without feeding for 19 days (from the 85th to the 104th day) with 9.0 g L^{-1}. Afterwards, it was observed that the performance of the system tended to stabilize, reaching 47% with 7.5 g L^{-1} in the 118th day. At the end of the experiment, the potassium content of the wastewater decreased from 800 mg L^{-1} to 594 mg L^{-1} (on an average 25%) and calcium and magnesium increased within the reactor liquor. The dissolution of the limestone inside the liquor reactor probably caused this result. After the treatment with limestone, the average pH value of the effluent increased from 4.9 to over 6.0 in all organic concentrations. It could be concluded that the reactor filled with dolomitic limestone in these operational conditions assured a low efficiency in COD removal, potassium reduction, increasing values of pH, alkalinity, calcium and magnesium. The instability was observed when there was increase in organic load to 12 g L^{-1} with subsequent recovery.

Keywords: ethanol distillery, agro-industrial wastewater, biological load, vinasse, anaerobic treatment.

Estabilidade de um reator anaeróbio de único estágio preenchido com pedras de calcário dolomítico com aumento da carga orgânica de vinhaça de cana de açúcar

RESUMO

O reator anaeróbico de estágio único foi avaliado para tratar a vinhaça e avaliar sua estabilidade. Este reator de bancada foi preenchido com calcário dolomítico com fluxo contínuo horizontal simulando um canal de drenagem da vinhaça. O experimento durou 129 dias em que o reator foi submetido a diferentes concentrações orgânicas cronologicamente aplicadas (3,0;

5,0; 12,0; 9,0 e 7,5 g L^{-1} como DQO, demanda química de oxigênio). As remoções de DQO foram de 50% e 9% com 3,0 e 7,5 g L^{-1}, respectivamente. Com 12,0 g L^{-1} a eficiência do reator aumentou para 33%. Mas, houve uma queda abrupta para 3% aos 84 dias. Então, a fim de evitar o colapso do reator, uma medida corretiva foi necessária. O sistema permaneceu em regime batelada sem alimentação durante 19 dias (do 85º ao 104º dia) com 9,0 g de L^{-1}. Depois, observou-se que o desempenho do sistema tendeu à estabilidade, atingindo 47% com 7,5 g L^{-1} no 118º dia. No final do experimento, o teor de potássio do efluente diminuiu de 800 mg L^{-1} para 594 mg L^{-1} (em média 25%) e cálcio e magnésio aumentaram dentro do licor do reator. Provavelmente, isto pôde ser observado devido a dissolução do calcário dentro do reator. Após o sistema de tratamento com calcário, o valor médio do pH do efluente aumentou de 4,9 para mais de 6,0 em todas as concentrações orgânicas. Pôde-se concluir que o reator preenchido com rochas de calcário dolomítico nestas condições operacionais assegurou baixa eficiência na remoção de DQO, redução de potássio, valores crescentes de pH, alcalinidade, cálcio e magnésio. A instabilidade foi observada quando houve os aumentos da carga orgânica para 12 g L^{-1} com recuperação subsequente.

Palavras-chave: carga biológica, destilaria de etanol, efluente agroindustrial, vinhaça, tratamento anaeróbio.

1. INTRODUCTION

Brazilian production for the harvest year 2015/2016 was 30.562.284 m^3 of ethanol according to Ministry of Agriculture, Livestock and Food Supply (Brazil, 2017), and Brazil has 383 ethanol distilleries in operation (ANP, 2016).

Vinasse is the final by-product of biomass distillation of sugar crops (beet and sugarcane), starch crops (corn, wheat, rice, and cassava), or cellulosic material (harvesting crop residues, sugarcane bagasse, and wood), mainly for the production of ethanol (Christofoletti et al., 2013). The wastewater is generated at a high temperature (90°C) (Wilkie et al., 2000), and in many plants it is held in a tank until it reaches ambient temperature before it is used in soil.

The volume of vinasse wastewater generated depends on the production of alcohol. The final disposition of the vinasse presents a business and environmental challenge, as it generated in great volume, about 12 to 14 liters for each liter of ethanol produced (Wilkie et al., 2000). Due to the large quantities of vinasse produced, alternative treatments and uses have been developed, such as recycling of vinasse in fermentation, fertirrigation, concentration by evaporation, and yeast and energy production (Christofoletti et al., 2013).

In order to optimize the energy potential and sustainability of bioethanol production, the liquid streams should not be considered as residues of the process (Moraes et al., 2015). Also, the effluent contains significant amounts of nutrients and organic matter because the vinasse is a liquid byproduct of the distillation of the fermented broth of the sugarcane. It is rich in potassium, calcium, magnesium, sulfur and micronutrients (Guagnoni et al., 2003). Ribas (2006) observed that the potassium content of vinasse was wide-ranging, from 1.7 to 12.5 g L^{-1}. The same was observed by Moraes et al. (2015) in a wide review of literature, and the content ranges from 0.6 to 6.5 g L^{-1} with different feedstocks of sugarcane.

The most widely used alternative is the application of this liquid to cultivated soils with sugarcane as biofertilizer. However, the successive application of the residue in the same cultivation areas has resulted in alterations in soil and groundwater quality. Brazilian Resolution CONAMA n. 357 (CONAMA, 2005) establishes dispersement patterns that may only be used after some type of treatment.

In this context, anaerobic digestion is becoming a great environmental and economic asset for generating methane and for being marketed as carbon credits or as an energy resource. Anaerobic treatment reduces the organic matter, but even the minerals can be concentrated.

Vinasse is considered a highly saline organic residue, containing approximately 75% biodegradable material and 20% mineral salts, with 8% potassium (Wilkie et al., 2000). It is assumed that its repeated disposition in all types of soils can create an imbalance among the soil bases (Bataglia et al., 1986; Silva et al., 2007), with adverse effects on agricultural soils and biota in general (Christofoletti et al., 2013). In the proposed system, the use of limestone rocks in the anaerobic reactor provides slow solubilization of calcium to the wastewater in treatment, and can lead to more-balanced levels between calcium and potassium.

The integration of anaerobic digestion in a sugarcane bio-refinery to treat and recover the energy or organic matter contained in the vinasse can be a great alternative (Wilkie et al., 2000; Tonello and Ribas, 2009; Moraes et al., 2015).

Anaerobic digestion consists of complex and sequential metabolic processes that occur in the absence of molecular oxygen and depend on the activity of at least three distinct groups of microorganisms (acidogenic bacteria, acetogenic bacteria and methanogenic archaea) to promote the stable and self-regulating fermentation of organic matter, resulting mainly in methane and carbon dioxide gases (Mosey, 1982). Each group has distinct optimum growth conditions for the development of the global process when there is stability inside the reactor.

The stability of an anaerobic reactor depends on several parameters; for example pH, alkalinity and concentration of organic acids, etc (Pohland and Ghosh, 1971; Speece, 1996). However, all microorganism groups involved in anaerobic digestion can develop in different lengths of a single reactor.

Several configurations of anaerobic reactors have been used in the anaerobic digestion of vinasse. The anaerobic reactor plug flow has been used widely in the treatment of several types of wastewater with good performance and satisfactory reduction of organic load (Ribas and Barana, 2003). In this reactor configuration, material supports for the microorganisms should be used.

Due to its advantages (low cost, non-toxic, non-reactive with carbon dioxide to generate vacuum inside the reactor, contains macronutrients that will enrich the biofertilizer) compared to other alkalizing substances, dolomitic limestone has been used as an anaerobic reactor filling to neutralize generated acids in the process. In Brazil, research with dolomitic limestone as material support was initiated in an acidic reactor for the cassava wastewater treatment (Ribas and Cereda, 2003) and later for sugarcane vinasse (Tonello and Ribas, 2009).

Regarding phase separation, anaerobic digestion can occur in a single reactor or with phase separation, i.e., uncoupling acidogenesis from methanogenesis.

The up-flow sludge blanket reactor (UASB) is the most-used configuration for vinasse anaerobic treatment due its better performance (Wiegant et al., 1986, Souza et al., 1992, Vlissidis and Zouboulis, 1993, Driessen et al., 1994 and Harada et al., 1996). However, other configurations of bench scale reactors have already been studied as alternatives to vinasse treatment: fluidized reactor filled with pumice stone (Balaguer et al., 1997) or with activated granular carbon (Fdz-Fernandez et al. 2001), structured-bed reactor (Aquino et al., 2017) and horizontal anaerobic fixed-bed reactor with single stage (Telh, 2001) and two stages (Tonello and Ribas, 2009).

A similar configuration of the reactor to the present work was used for vinasse treatment in anaerobic reactors of two phases (Tonello and Ribas, 2009). However, in this study the reactor was singlestage filled with limestone. This configuration works as a reactor plug flow that is a simplified digester built with cheap materials and simpler operation than other systems that require agitation (Kubiac and Dubuis, 1985).

Limestone rocks have already been used for alkaline support of reactors of various sizes. Silva et al. (2012) evaluated mine water treatment with limestone for sulfate removal with particle sizes between 0.42 mm and 0.59 mm as fixed-bed experiments. Zhou et al. (2011) used an average diameter of 3–15 mm of sulfur-limestone as media in a lab-scale upflow biofilter. Tonello and Ribas (2009) filled the reactor with limestone sizes between 19 mm and 38 mm. Ribas and Cereda (2003) assessed two sizes of dolomitic limestone to stabilize cassava wastewater during the acidogenic phase in anaerobic reactor, one of 7.93 mm - 25.4 mm and the other of 25.4 mm 38.1 mm. As can be observed, the size of the material used as a fixed bed for anaerobic reactors is highly variable.

Anaerobic reactors presented stability and good performance, with COD removal of 64% in the highest volumetric organic load applied, 3.3 g L^{-1} d^{-1}. The pH, AI/AP ratio, bicarbonate alkalinity and total volatile acids also had optimum results of 7.8, 0.9, 0.63 $gHCO_3^-$ L^{-1} and 1.42 gHAc L^{-1}, respectively. The highest biogas production was on average 1.4 L d^{-1} with a yield of 0.112 $L_{biogas}.L_{reactor}.d^{-1}$ and 0.271 $L._{biogas}.gDQO_{rem}.d^{-1}$ (Tonello and Ribas, 2009).

Therefore, the objective of the present study was to evaluate the stability of a horizontal anaerobic single reactor with increased organic load, filled with dolomitic limestone rocks, the with goals of organic matter removal, conversion from organic matter to biogas and the alteration of the chemical characteristics of the vinasse regarding the levels of calcium, magnesium and potassium.

2. MATERIALS AND METHODS

2.1. Wastewater

The wastewater used was collected in the vinasse storage tank and stored at 4ºC. The characteristics of the vinasse used in the experiment were: pH 4.78, total solids 16617 mg L^{-1}, biological oxygen demand 3430 mg L^{-1}, chemical oxygen demand 28217 mg L^{-1}, calcium hardness 2000 mg Ca L^{-1}, magnesium hardness 1680 mg Mg L^{-1}, electric conductivity 10050 µS cm^{-1}, total nitrogen 560 mg L^{-1}, nitrate (NO_3^-) 45.00 mg L^{-1}, nitrite (NO_2^-) 2.00 mg L^{-1}, potassium 440.0 mg L^{-1} and sulfate (SO_4^{2-}) 250.0 mg L^{-1}.

2.2. Anaerobic single reactor

The system was comprised of a tube of PVC (polyvinyl chloride) 1.0 meter in length and 0.1 m in diameter, with a total volume of 6.0 L, 50% filled with limestone rocks (p/v) with a useful volume of 3.0 L.

The reactor was arranged horizontally simulating the normal piping conduction of vinasse to the field, as shown in the Figure 1.

The system was fed daily, and the effluent was fed to the reactor by gravity. The feeding container was located one meter above the reactor, suspended by a wooden support. The affluent was put in the upper container, which was connected to the 19 mm-pipe to feed the reactor. In the superior part of the reactor, three transparent 10 mm plastic tubes were coupled with the external tips immersed in water, where the biogas escaped. The reactor was operated at room temperature. The inflow pH was not corrected in order to verify the dolomitic limestone action in the buffering and system operation.

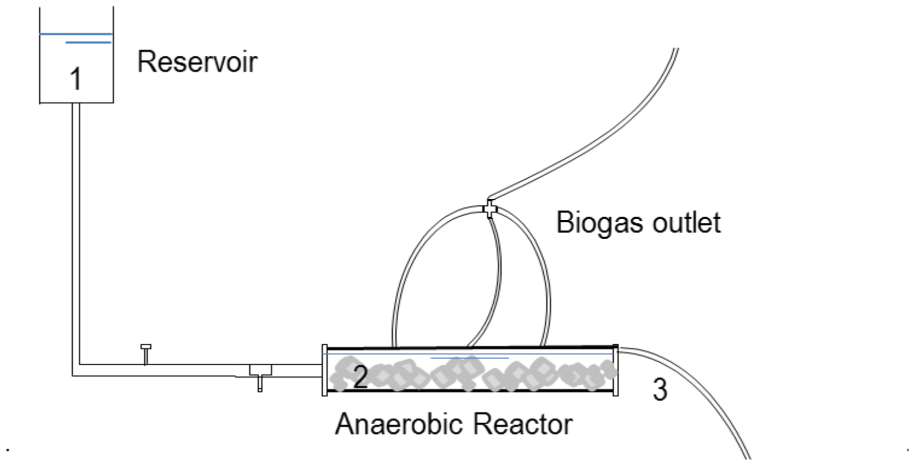

Figure 1. Schematic diagram of the experimental apparatus: 1 - elevated inflow reservoir; 2 - limestone rocks packed inside the reactor; 3 - treated effluent outlet.

2.3. Experiment

The pH of the affluent was measured daily and was not corrected. The flow of the reactor was adjusted to be on average 2.52 L d^{-1} to maintain the HRT (hydraulic retention time) close to 1.0 day. The variation of the organic load over time was arranged with the vinasse diluted in water to concentrations of 3.0; 5.0; 12.0; 9.0 and 7.5 g COD L^{-1}. The goal was to adapt the inoculum and detected reactor instability to the highest organic concentration using a concentration of up to 10.0 g COD L^{-1} as a reference applied in a two-phase reactor (Tonello and Ribas, 2009). Between 9.0 and 7.5 g COD L^{-1}, the system stayed in batch without feeding for 19 days (from the 90th to the 108th day) with an influent of 9.0 g COD/L to restore reactor performance and acid consumption (Pohland and Ghosh, 1971). Table 1 presents the operation parameters.

Table 1. Experimental conditions submitted to the anaerobic reactor in each experimental phase.

Conditions	Organic concentration (g COD L^{-1})				
	3.0	5.0	12.0	9.0	7.5
Q_{in} (L d^{-1})	2.96	2.67	2.47	2.48	2.25
OT (d)	17	18	43	11	21
Period (d)	1-17	18-35	36-78	79-89	109-129
HRT (d)	1.00	1.02	0.93	1.05	1.00
OLR (g L^{-1} d^{-1})	3.0	4.9	12.9	8.6	7.5
VOL (g L^{-1} d^{-1})	2.96	4.45	9.88	7.44	5.62

Q_{in} – inflow rate; OT - operation time; HRT hydraulic retention time; OLR–organic loading rate; VOL – volumetric organic loading.

Organic loading rate (OLR) was calculated by (Equation 1):

$$ORL\ (g_{COD}\ L^{-1}d^{-1}) = \frac{C_{COD}}{HDT} \quad (1)$$

In which C_{COD} is the COD concentration (g L^{-1}) in the affluent and *HDT* is the hydraulic retention time (days). And the volumetric organic loading rate was calculated by (Equation 2):

$$VOL(g_{COD}\ L^{-1}_{reactor}\ d^{-1}) = \frac{Q_{in}\ x\ C_{COD}}{V_{reactor}} \quad (2)$$

In which Q_{in} is the inflow rate (L d^{-1}) and $V_{reactor}$ is the useful volume (L).

The experiment lasted for 129 days, including the period without feeding after the fourth phase.

2.4. Inoculum

The inoculum originated from an anaerobic batch reactor that treated swine manure for 30 days and was introduced to the effluent in the fifth day of operation. The inoculum was composed of 7.4 g TS/L_{sludge}, 5.5 g TS/ L_{sludge} and 1.9 g TS/ L_{sludge}.

2.5. Limestone Rocks

The reactor was filled with dolomitic limestone previously selected to be sized between 4.75 and 9.52 mm in mesh sieve ABNT (in English, Brazilian Association of Technical Standards) 4 and 3/8", respectively. That is the commonly used grading size of commercial coarse aggregate (ASTM C 33, 2016); in Brazil the size is equivalent to grade zero, since the literature presents a wide range of sizes, as already discussed briefly. The limestone used was 30% calcium oxide and 20% magnesium oxide.

2.6. Analytical methodology

The monitoring of the adaptation phase of the reactor was accompanied by the analyses of pH (APHA, 2012), alkalinity to bicarbonate (partial), intermediary and total, total volatile acidity (Dillalo and Albertson, 1961; Ripley et al., 1986) and chemical demand of oxygen (APHA, 2012). The levels of solids (APHA, 2012) were determined in the inoculum used.

3. RESULTS AND DISCUSSION

The effluent entered without pH correction, averaging 4.84. Daily, the pH-treated effluent was measured an increase in pH of between 5.5 and 7.0 occurred naturally made by the action of calcareous in contact with the wastewater.

In general, the analyses to monitor the performance of the anaerobic system presented satisfactory results for the adaptation phase of the system. The average values of the effluent pH in the study of load variation were above 6.0, which is inside of the acceptable range of 6.0 to 7.4 (Speece, 1996). Only the last organic load (7.5 g L^{-1}), had treated effluent that was characterized with high acidity.

Notice that the limestone rocks not just had great importance in the system for supplying carbonates for the elevation of the pH (Figure 2), but it was also important to supply a physical fixed bed for the microorganisms. Studies show that part of the bacteria lives loose inside the reactor and the other part needs a base to which it can adhere (Speece, 1996).

Regarding pH, limestone use in the reactor will cause a rise in pH with the removal of the H^+ ions, alkalinity and hardness as H^+ and CO_2 react away to form calcium bicarbonate as in the stoichiometry below, for example (Watten et al., 2017):

$$CaCO_3 + H^+ \leftrightarrow HCO_3^- + Ca^{2+} \quad (1)$$

$$HCO_3^- + H^+ \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2 \quad (2)$$

$$CaCO_3 + H_2CO_3 \leftrightarrow Ca^{2+} + 2\ HCO_3^- \quad (3)$$

$$CaCO_3 + H_2O \leftrightarrow Ca^{2+} + HCO_3^- + OH^- \quad (4)$$

In the COD concentration of 3.0 g L^{-1}, the effluent pH was in the range of 4.3 to 5.22, and after being treated in the system, the effluent pH ranged between 6.33 and 7.31. According to Speece (1986), for the methanogens archaea the pH should be between 6.8 and 7.4. Around 7.2, the pH is considered slightly alkaline.

In the startup of the reactor with 3.0 g L^{-1}, the values of the effluent pH crossed the value of 7.0, reaching 7.31, but with the increase of the organic load to 5.0 g L^{-1} the pH values oscillated between 5.55 and 7.27.

As soon as the load was increased to 5.0 g COD L^{-1}, the system presented a small drop in the pH value of 6.5. The same behavior was observed with the 12.0 g COD L^{-1}, when the pH had a wide oscillation; more time therefore was required in order to stabilize the reactor, with 43 days in the same concentration. The pH average in this phase was 6.14. Since vinasse does not have a high protein content, as can be inferred from the work of Wilkie et al. (2002), Moraes et al. (2015), Ferraz Junior et al. (2016), Aquino et al. (2017), it has a low-buffer capacity that causes slow system recovery and unstable conditions for microorganisms.

The ability to avoid large variations in pH and to neutralize acids is called alkalinity. In anaerobic digestion, this property naturally originates with the methanogenesis and decomposition of proteins and other substrates (Silva and Boncz, 2012).

A smaller load still was therefore applied, 9.0 g COD L^{-1}, in order to recover the system, because the reactor was presenting a high concentration of volatile acids (1923 mg L^{-1} on average) with 12.0 g COD L^{-1}. Therefore, after this phase, the system remained in batch without feeding for 19 days, so that the methanogenic archaeas had time to consume the produced organic acid. Afterwards, the organic concentration was decreased to 7.5 g COD L^{-1}.

It is noticed that after the period in batch, between 9.0 and 7.5 g COD L^{-1}, corresponding to 19 days without feeding interruption (as shown in the graph, Figures 2 and 3), there was the expected recovery with an increase of the effluent pH up to 7.0.

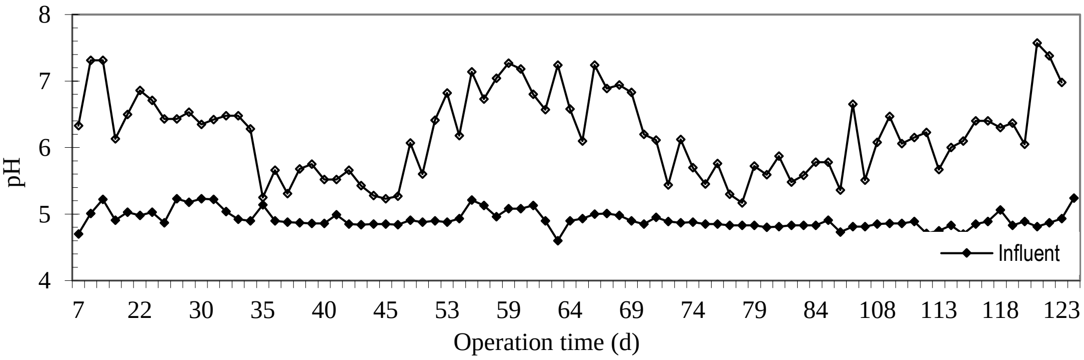

Figure 2. Values of pH of the effluent and influent measured during all of the experimental phases.

Regarding COD, it was observed that the reactor in the concentration of 3.0 g COD L^{-1} presented a 50% removal of COD, according to the graph in Figure 3.

The pH stability (on average pH 7.0) began with 5.0 g COD L^{-1} of organic load. However, it was observed that the efficiency dropped to 9% of COD removal with 21 days of operation.

In the graph of Figure 3, great variations in the removal according to the increase of organic load and no removal were observed. The variations can be observed by the standard deviation with respect to the average COD in each applied load.

The non-removal was observed in the third phase, with a concentration of 12.0 g COD L^{-1}, which might have been caused by a great increase of organic load from flies of the order Diptera, more specifically of the Calliphoridae family, suddenly appearing inside the system. The system was cleaned and a screen was placed on the reservoir and the non-removal did not reoccur.

An increase the retention time with the applied organic load of 12.0 g COD L^{-1} was necessary in this stage so that there was time for a higher removal. The system tended to obtain a reduction of 33% in the same phase of the experiment, until the 77th day.

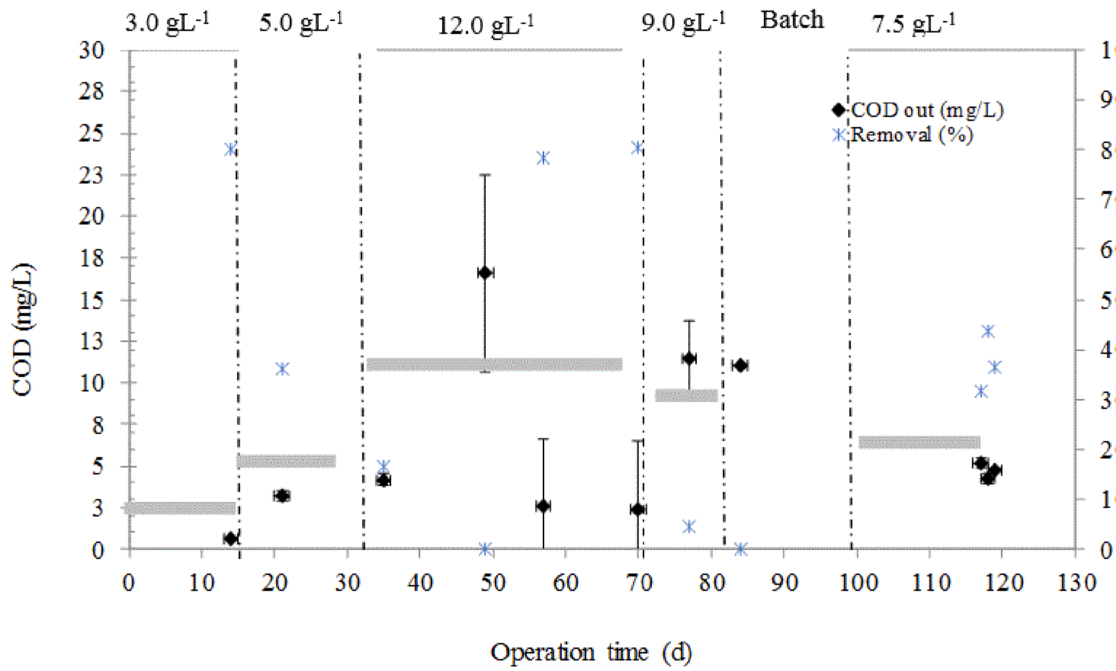

Figure 3. Monitoring and removal of COD during all of the organic loadings.
Note: Horizontal bars delimit the applied load.

As the organic load increased in the system, COD removal slightly decreased, probably due to the organic "shock" suffered by the microorganisms causing yield reduction.

After the feeding interruption period in batch of the system for 19 days, between the 79th and 89th day, the reactor gradually presented recovery reaching 47% of removal with 7.5 g COD L^{-1}.

The bicarbonate alkalinity showed in Figure 4 is a parameter used to indirectly evaluate the performance of anaerobic systems. It was observed with 3.0 and 5.0 g COD L^{-1}, the system presented alkalinity production to bicarbonate.

In 12.0 g COD L^{-1}, an accentuated drop in alkalinity and an increase of the total volatile acidity was measured, probably due to high applied organic load which stopped the operation of the methanogen microorganisms that are known be sensitive to abrupt changes of affluent concentration. But the system recovered after the 49th day in the same organic concentration, again generating bicarbonate alkalinity of approximately 1500 mg$CaCO_3$ L^{-1}. From this point on, the system presented variations in the values of the effluent.

Viana (2006) studied the thermophilic treatment (at 55°C) of cane vinasse by an UASB reactor with progressive increase of organic load. The operation reached the organic limit loading in 6.5 g COD $L^{-1}d^{-1}$ (1 day of hydraulic detention), limited by the excessive production of total volatile acids beyond 1200 mg L^{-1}, too toxic a methanogen biomass.

Tonello and Ribas (2009) operated under environmental conditions and a reactor model similar to this work. When the methanogen reactor was submitted to 10.0 g COD L^{-1} of cane vinasse, the COD removal was 64% and pH 7.8.

The instability of the anaerobic digestion process happens when the speed of volatile acids production is larger than its consumption, which can result in the fall of the pH and in the inhibition of the methanogenic archaeas activity. Thus, the alkalinity measures, mainly the bicarbonate alkalinity and volatile acids salts, supply fundamental data for the monitoring of anaerobic systems (Foresti, 1994).

Figure 4 shows the behavior of the bicarbonate alkalinity (BA) along every experimental phase with duration of 124 days. It is observed that the affluent presented insignificant amounts of BA, because no alkalizing agent was added and the vinasse has low buffer capacity.

It can be inferred that the higher amount of BA in the effluent indicates greater stability in the anaerobic system on the part of the methanogenic microorganisms, since bicarbonate is a product of the methanogenic metabolism.

In Figure 4 it is possible to observe that total volatile acidity (TVA) was produced during of the experimental phases in the effluent, which demonstrates that the acidogenic phase that is the precursory phase of methane production occurred satisfactorily. This could have harmed the methanogenic stage, because in high concentrations of acidity, methanogenic archaeas cannot degrade organic acids and convert to methane, because it does not consume the substrate as quickly.

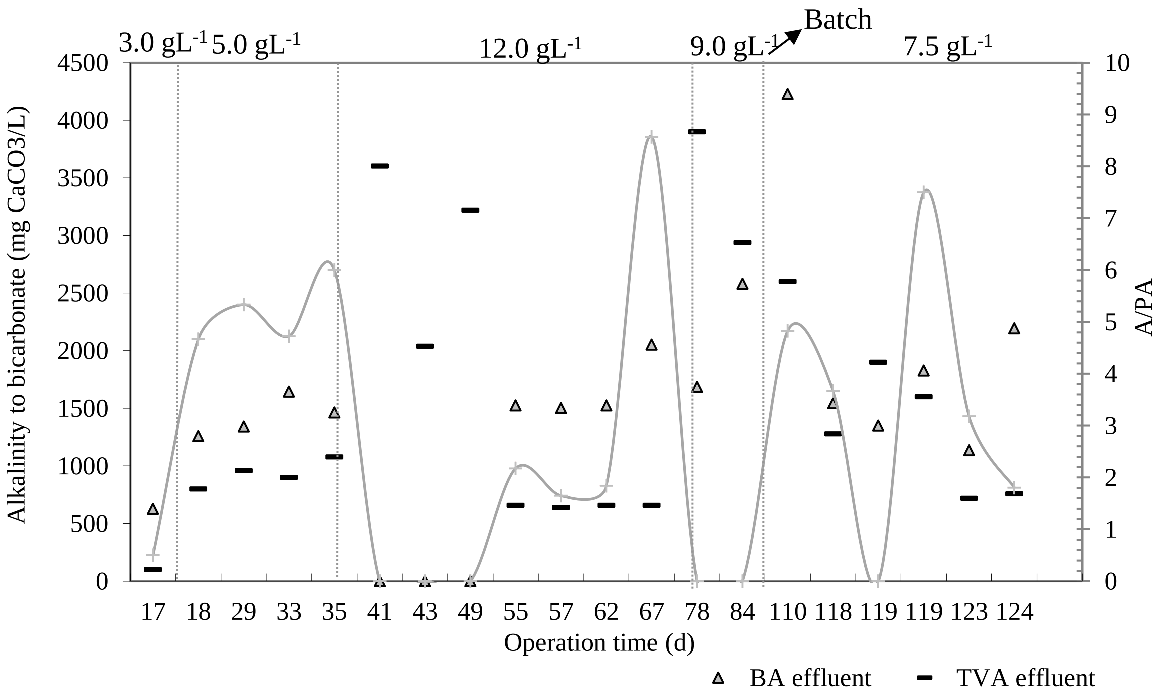

Figure 4. Bicarbonate alkalinity and total volatile acidity of the effluent.

The ratio between intermediate alkalinity (IA) and partial alkalinity (PA) presented represents a balance between intermediary alkalinity (corresponding to bicarbonate) and partial alkalinity (corresponding to organic acids) that is measured in the output of the anaerobic reactor.

Tonello and Ribas (2009) observed average values with the organic load of 10.0 g COD L^{-1} an IA/PA ratio of 0.9, bicarbonate alkalinity of 0.63 $gHCO_3^{-}L^{-1}$ and total volatile acidity of 1.42 g acetic acid.L^{-1}.

Some authors, as in the well known scientific paper of Ripley et al. (1986), consider that this relationship should be close to 0.3 to indicate the stability of the system. In this case, the relationship IA/PA varied during the whole experimental phase from 0 to 8.5, indicating the instability of the adaptation phase in all organic loads evaluated.

Among the elements calcium, magnesium and potassium, interesting results were observed, considering the treated effluent as bio fertilizer, because one of the limitations of the soils in the humid tropical area is the low fertility in depth. This fact is reflected in the small volume explored by the sugar cane root and, consequently, lower productivity.

Table 2 displays the values of the analyzed elements of four removed samplings of the last experimental phase and the relationship between K:Ca:Mg of both influent and effluent.
It was observed that the relationship among the elements Mg:Ca:K, calculated by dividing the largest values for the smallest among them (Mg), decreased. The Mg values in the effluent decreased due an increase of the Ca and Mg from the dolomitic limestone. In spite of the amount that those two elements increased, there was no variation in the relationship between the effluent and influent, except in the sampling of the 119th day.

The treated effluent can be used as biofertilizer with larger the element amounts and more-balanced levels among them, which are better for soil fertility and for nutrient absorption by the sugar cane crop. Notice in Table 2 that Ca and Mg increased in the effluent and that K decreased in all of the samples with regard to the effluent, which possibly characterizes a biofertilizer of better quality. But to prove such hypothesis, it would be necessary to test its application in soil to observe the effects on the crop.

Table 2. Analyses of calcium, potassium and magnesium when the reactor was submitted to the organic load of 7.5 g COD L^{-1} of both influent and effluent.

Collection Day	Influent				Effluent			
	Ca	Mg mg L^{-1}	K	Mg:Ca:K	Ca	Mg mg L^{-1}	K	Mg:Ca:K
116	166.04	72.96	792	1:2:10	314.92	178.36	528	1:2:3
117	166.04	72.96	792	1:2:10	274.52	155.66	409,2	1:2:3
118	204.53	107.49	924	1:2:9	380.47	197.13	858	1:2:4
119	141.20	52.66	686	1:3:13	201.09	91.81	514.8	1:2:5

Certainly, the increase of Ca and Mg is due to the solubilization of the limestone rocks that reacted with the vinasse that has an acidic pH.

The K decrease is probably due the reaction of this element with the soluble materials inside the reactor. For instance, the water used in the dilution of the vinasse was from the water supply of the city with chlorine added. The chlorine could have reacted with the potassium, forming potassium chloride, what reduces the levels of this element in the ionic form, which would not be detected in the method used to quantify it.

The biogas was variable with the application of 3.0 g COD L^{-1} and 5.0 g COD L^{-1}, respectively. In the other phases the biogas was not measured.

The biogas production was near 0.50 L/d (standard coefficient ± 0.27 L/d) in the standard conditions, normal temperature and pressure (NTP), and the maximum value achieved was 1.37 $L.d^{-1}$ with 3.0 g COD L^{-1}. It is estimated that the conversion to biogas (CH_4 and CO_2) was 47% and 30% to methane (considering that the biogas was about 65% CH_4).

Theoretically, if all organic matter applied is anaerobically biodegraded and converted to methane, then 0.35 L methane per g COD added in the standard conditions are expected to be converted. Then, with 5.0 g COD L^{-1}, the biogas was on average 0.38 ± 0.18 $L.d^{-1}$ (NTP), 0.21 L methane per day and the conversion to biogas (CH_4 and CO_2) was 22% and 12% to COD:CH_4.

Therefore, in these two phases where the biogas results were analyzed, it was possible to suppose that the average biogas production during the experimental period could be under the maximum in the conversion of organic matter in biogas or methane.

The wear on the limestone was determined at the end of the experimental period by determining the difference in volume. It was determined that 1.3 L of dolomitic limestone (2.87 g cm^{-3}, regarding Sampaio and Almeida, 2008) was eroded during the 129 days of the experiment. It is estimated that one kilogram of calcareous limestone is needed to treat 89 liters of vinasse in the evaluated concentrations. This amount shows the applicability of limestone's use in wastewater anaerobic treatment.

According to the results obtained, the reactor treatment system filled with dolomitic limestone is promising. However, the conditions of organic loading and phase separation are not conclusive.

Phase separation is economically feasible when scaling up anaerobic digestion plants in biorefineries. Despite the higher capital and operating costs in such schemes, the estimated biogas and electricity production costs reached equivalent or lower values compared with those of single-phase anaerobic digestion layouts (Fuess et al., 2017).

Finally, the use of limestone in this single reactor was an interesting pH conditioner and balancer of macro elements (Ca, Mg and K).

4. CONCLUSION

The results obtained in this research demonstrated that the proposed system in a single physical compartment reduced organic matter and potassium, which are the largest inconveniences of the vinasse when disposed in soil without treatment.

Despite the difficult adaptation of the reactor to higher organic loads and instability with 3.0 to 12.0 g COD L^{-1}, effluent anaerobically treated by the reactor filled with limestone presented an increase in levels of calcium, magnesium, pH and alkalinity to 71%, 74%, 6.3, 2048 mg$CaCO_3$ L^{-1} in the intermediate organic load of 7.5 g COD L^{-1}, respectively. Also, the treated wastewater can benefit soil, and may motivate the use of this type of reactor to be placed in channels through which the vinasse flows until it reaches crops, thus eliminating the need for a larger area for this purpose.

More studies are necessary to achieve greater COD removal efficiency and conversion to methane. The same system in two physical phases (acidogenic and methanogen) and other support materials inside the reactors to adhere to biomass should be evaluated.

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