Sewage Sludge, Goat Rumen and Cattle Rumen as Inoculum for the Anaerobic Degradation of *Manipueira*

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Abstract

*Manipueira*is the effluent of starch and flour production from cassava.High organic matter content is one of its main characteristics. The biodegradability of *manipueira*was assessedusing anaerobic sludge, cattle rumen and goat rumen as inoculum. The following parameters were evaluated: the removal efficiency of the chemical oxygen demand (COD);the constant rate of decay of COD (K); the production of biogas and the balance of COD.The essayswere conducted using batch tests at reactor bottles with a useful volume of 0.104 L and0.026 L of headspace. Three different concentrations of biomass were used (2, 3 and 4.0 $g.L^{-1}$) per inoculum,witha total of nine treatments. The stabilization time was 264 hours for the reactors with sludge and cattle rumen and 196 hours for those with goat rumen. The greatest COD removal efficiency was recorded for sludge (>80%) and cattle rumen (89%), which were also the greatest producers of biogas.The Kvalues were similar in all treatments (0.1 day^{-1}). In general, treatments with sludge and cattle rumen (2 $g.L^{-1}$)provided the best methane production rates(0.20, 0.23, 0.24 and 0.27 L CH_4 $g^{-1}COD_{removed}$) and can be used as inoculum for anaerobic reactors.

Keywords: Biogas; cassava; COD; *manipueira*; rumen; sludge

INTRODUCTION

In the semi-arid region of Brazil the main economic and subsistence activities are cassavacultivation, goat breeding and cattle breeding. Brazil and Indonesia are the greatest producers of cassava worldwide. Cassava is usedfor consumption and the production of starch and flour. Aeffluent of cassava is*manipueira*, which has high organic COD content,ranging from 12to 20g of O_2. L^{-1}. Due to its pollutant potential and the fact that itcan be a source of carbohydrates, *manipueira*has been studied by several researchers looking for treatment techniques to be used in the biodegradation of organic material, in association with the production of renewable biofuels (Budiyono and Kusworo, 2011; Sun et al., 2012, Li et al., 2013). Operational parameters have been tested to increase the production of methane and to reduce the organic load, including temperature, hydraulic retention time, inhibitor compounds, reactors configuration, and inoculum(Fongsatitkul et al., 2012).

The inoculum used in processes of anaerobic biodegradability is usually anaerobic sludge obtained from the digesters of sewage treatment plants and agroindustries,using concentrations that range from 2to 5 $g.L^{-1}$(Bertolino et al., 2007). One alternative to sludge that has demonstrated significant increases in the anaerobic conversion of lignocellulosic materials is the use of ruminal microorganisms.These microorganisms have been studied greatly in relation to the anaerobic digestion of lignocellulosic biomass, including agricultural waste and organic fractions of solid waste from aquatic plants and municipalities (Zhao et al 2009;Yue et al., 2012; Baba et al., 2013).

The main factors that affect the production of methane in ruminal ecosystems are pH and volatile fatty acids (VFA), which are one of the greatest products in systems that inhibit methanogenesis(Kumar et al., 2009).However, studies using rumen as a source of microorganisms have found high values of VFAduring the process of anaerobic digestion:1,600 and 2,400 $mg.L^{-1}$acetic acid (Wang et al., 2009); 7,140 $mg.L^{-1}$acetic acid (Baba et al., 2013). These VFA inhibit the activity of methanogenic bacteria.

By studying the ideal environmental conditions for the bacteria of inoculum, such as pH, which is neutral for sludge and rumen, and temperature (close to 30ºC for sludge and 39º C for rumen), it is possible to obtain satisfactory results in the process of anaerobic digestion. Luo et al (2009) and Sun et al. (2012) used sludge as inoculum and performed anaerobic treatment on waste from the cassava industry using a sequential batch reactor and multistage UASB. The authors reported removal efficiency rates of between 80% and 87.9% for organic material.Barana (2000) obtained values ranging from 0.22 to 0.24 L CH_4 $g^{-1}COD_{removed}$on a laboratory scale and from 0.19 to 0.21 on a pilot scale, both of which used *manipueira*as substrate and sludge as inoculum. Zhang et al. (2013) treated*manipueira*with sludge as the inoculum in a single phase and obtained a methane production value of 0.125 L CH_4 $g^{-1}COD_{removed}$.

Therefore, considering the availability of rumen and the need to treat *manipueira*in the Brazilian semi-arid region, the aim of the present study was to assess its biodegradability under the influence of ruminal inoculum and to compare the results with those obtained using sewage sludge. The following assessment parameters were adopted: the efficiency of COD removal; the constant rate of decay of COD(K), as a kinetic parameter; the production of biogas and the balance of CODin terms of biodegradation.

METHODS

Substratesand Inoculum

The *manipueira*came from a tapioca starch factory in the city of Araripina in the state of Pernambuco (PE), Brazil. The first inoculum was sludge from an Upflow anaerobic sludge blanket reactor, which treats domestic sewage from an area with a low-income population in Recife-PE. Cattle and goat rumen were the other inoculum. These were collected from the pre-stomach of the animals and filtered through cheesecloth. Table 1 displays the characteristics of the wastewater from the tapioca starch factory and the inoculum.

Table 1 – Characteristics of the wastewater from the tapioca starch factory and ruminal fluids

Parameters	Substrate	Sludge	Goat Rumen	Cattle Rumen
COD (mg.L^{-1})	37,700	424	4,316	3,774
pH	5.80		7.21	7.5
Phosphorus (mg.L^{-1})	182.69	....	4123	5733
Nitrogen (mg.L^{-1})	28.53	....	162	174
Alkalinity (mg.L^{-1} de $CaCO_3$)	71.50	....	4283	5733
Volatile Acids (mg de HAc.L^{-1})	25.50	....	8824	9883
Cyanide (mg.L^{-1})	30.23	.....	....	....
SSV (mg.L^{-1})	4,332.50	37,000	9,400	16,000

Experimental Setup

The anaerobic biodegradability of*manipueira*, under different inoculumand concentrations,was assessed based on the following measurements: the efficiency of removing filtered COD (COD_F);the constant rate of decay of COD(K),as a kinetic parameter;and the production of biogas. The constant Kwas determinedbased on the following equation:

$$(-r) = -\frac{dS}{dt} = k_d\, S$$

Where *r*is the velocity of the reaction (Mass/Volume.Time), *S*is the concentration of the limiting reagent (Mass/Volume), *T*is time (days) and K is the constant velocity for the first-order reaction (day^{-1}).

The tests were conducted in reactor vials of 0.130 L, using 0.104 L as the useful volume and 0.026 L as the headspace. Three concentrations of biomass (2, 3 and 4 g.L) were tested for each source of inoculum, thus characterizing 9 treatments (Table 2), which were essayed in triplicate.

Table 2 – Experimental Conditions

Treatment	Concentration of the biomass of the inoculum (g VSS.L^{-1})	Inoculum	Substrate (2 g COD.L^{-1})
1	2	Sludge	*Manipueira*
2	3	Sludge	*Manipueira*
3	4	Sludge	*Manipueira*
4	2	Goat rumen	*Manipueira*
5	3	Goat rumen	*Manipueira*
6	4	Goat rumen	*Manipueira*
7	2	Cattle rumen	*Manipueira*
8	3	Cattle rumen	*Manipueira*
9	4	Cattle rumen	*Manipueira*

Macro and micronutrients supplemented nutritional requirements. The concentrations in each reactor bottle (per liter) were as follows: NH_4Cl (56.00 mg), K_2HPO_4 (50.40 mg), $MgSO_4.7H_2O$ (20.00 mg), $CaCL_2$ (1.40 mg), $NaHCO_3$ (80.00 mg), $FeCl_2.4H_2O$ (400.00 mg), $ZnCl_2$ (0.010 mg), $MnCl_2$ (0.100 mg), $NiCl_2$ (0.028 mg), H_3BO_3 (0.010 mg), $CuCl_2.2\ H_2O$ (0.0076 mg), $CoCl_2.6\ H_2O$ (400.00 mg), $ALCl_3.6\ H_2O$ (0.018 mg), $(NH_4)_6Mo_7O_{24}.4\ H_2O$ (0.010 mg), EDTA (200.00 mg) andHCl (200µL.L^{-1}), bearing in mind the nutritional requirements of the microorganisms of sludge (Florêncio, 1994) and rumen (Baba et al., 2013).

Reactor vials were prepared in triplicate at the same time as control vials, which did not receive *manipueira*. The reactors were closed with rubber septa and aluminum seals. Adaptedsyringes (0.015 L) were used to collect and measure the biogas. The reactors with sludge were incubated at 30 ± 2º C, whereas those with rumen were incubated at 39 ± 2º C (optimal temperature for ruminal bacteria - Hook et al., 2010). Every 48h, one set of triplicates was analyzedand the vials were disposed. The following parameters were analyzed: pH; COD and volatile fatty acids (VFA) in mg of acetic acid L^{-1}(APHA,1998).Analysis of variance (ANOVA) at1% of probability was used to investigate significant differences between the treatment groups.

RESULTS AND DISCUSSION

Environmental Parameters

The results obtained for pH, and volatile fatty acids (VFA) did not differ statistically from the normal distribution. Therefore, they were applied to analysis of variance with 1% of probability. Their mean values are displayed in Table 3. The digestion time was 264 hours for reactors with sludge and cattle rumen and 196 hoursfor goat rumen, when they did not exhibit biogas formation for three consecutive days.

Table 3 –Mean values for pH and VFAon day zero (influent) and on the final day of testing (effluent)

Assay		T1	T2	T3	T4	T5	T6	T7	T8	T9
pH	Infl	8.11	7.98	7.99	7.82	7.93	7.91	8.22	7.33	7.84
	Effl	6.82	6.92	6.94	6.89	6.94	6.91	6.93	6.99	6.99
VFA	Infl	320	336	432	384	456	480	304	480	528
(mg HAc.L^{-1})	Effl	240	265	240	1248	1344	1464	816	984	1560

The pH values of the influent ranged from7.33to 8.11and the pH of the effluent ranged from 6.82 to 6.99 (close to neutrality). Thus, there was a decrease in the pH during the biodegradability period. However, the decrease was not sufficient to take it out of the ideal range for methanogenic microorganisms. During the entire period, the dataremained within the ideal interval (from 6.50 to 7.00) for methanogenesis(Speece, 1996).

Ruminal pH is not only determined by the quantity of acids produced. Other factors are involved, including the buffering capacity of the rumen and the velocity of degradation of non-fibrous carbohydrates (Kumar et al., 2009), which was the case of the substrate used in the present study (*manipueira*). Sun et al. (2012)investigated the anaerobic treatment of cassava starch wastewater using an up-flow multi-stage anaerobic reactor and reported that the pH in the effluent ranged from 6.8 to 7.28. For rumen, Kumar et al. (2009) reported that these pH values ranged from 6.00 to 7.60. Poulsenet al (2012) noted that pH values from 6.00to 6.50did not have a negative effect on the production of methane with ruminal fluid.

All VFAvalues decreased in the reactors with sludge. The greatest decrease was recorded for the biomass concentration of 3 g.L^{-1} (T2), which reduced by 25% (T1), 28.6% (T2)and22% (T3), with a statistical difference based on a probability level of 1%.

Although VFAare already present in rumen theirvalues in the effluent increased due to the increased concentration of biomass, the percentage of the increases were similar in all treatments except T8: the values doubled in T8 and tripled in all other treatments. Previous studies have used rumen as a source of microorganisms and reported elevated values of VFA during the process of anaerobic digestion. Wang et al. (2009) detected high concentrations of acetic acid (1,600mg/L and 2,400 mg/L). Baba et al. (2013) stated that rumen was an effective catalyst in the conversion of waste to VFA, with values of 7,140 mg.L^{-1}for acetic acid and a pH of 6.07. The VFAresults found in the present study corroborated those found by Yue et al. (2012), who compared sludge and rumen as inoculum in anaerobic reactors and found that the amount of VFA´s in reactors with rumen was twice as high as in reactors inoculated with sludge. The results for the rumen reactor (1,990 mg/L)were very similar to those found in the present study for both goat rumen and cattle rumen.

Biodegradability of *Manipueira*

Table 4 displays the mean and standard deviation values of total COD (COD_T)andfiltered COD (COD_F). Figures 1a and 1bdisplay the mean values for the efficiency of COD_TandCOD_Fremoval, respectively.

Table 4 –Mean and standard deviation (SD) values of COD_T and COD_Fobtained in the influent and effluent of the reactor bottles at the end of the testing

COD (mg.L^{-1})	T1	T2	T3	T4	T5	T6	T7	T8	T9
$COD_{TInfluent}$	8748	7217	7761	7432	7711	6256	8356	8305	8913
DP	2117	539	1118	195	618	882	1278	253	418
$COD_{TEffluent}$	1304	763	1042	4225	4042	3926	2772	2812	2997
DP	265	233	118	1113	404	1067	676	221	424
$COD_{FInfluente}$	1591	1484	1337	2925	2723	2123	1990	1682	1883
DP	150	25	15	766	267	243	96	419	81
$COD_{FEffluent}$	318	186	153	950	930	1189	205	550	1314
DP	10	6	22	96	224	0	102	140	110

Figure 1a shows that the greatest efficiency of COD_Tremoval occurred for sludge with 85%, 89%and 86% for T1, T2 and T3, respectively. For goat rumen, the removal values were 43%, 48%and 37% and the greatest efficiency was recorded for the 3 g.L^{-1} treatment, similar to sludge, although with half of the efficiency observed for sludge treatment. Cattle rumen did not exhibit differences between the concentrations for efficiency, maintaining a mean value of 66% for removal. Metcalf and Eddy (2003) reported that the removal of organic material in anaerobic systems can vary between 75% and 85%.

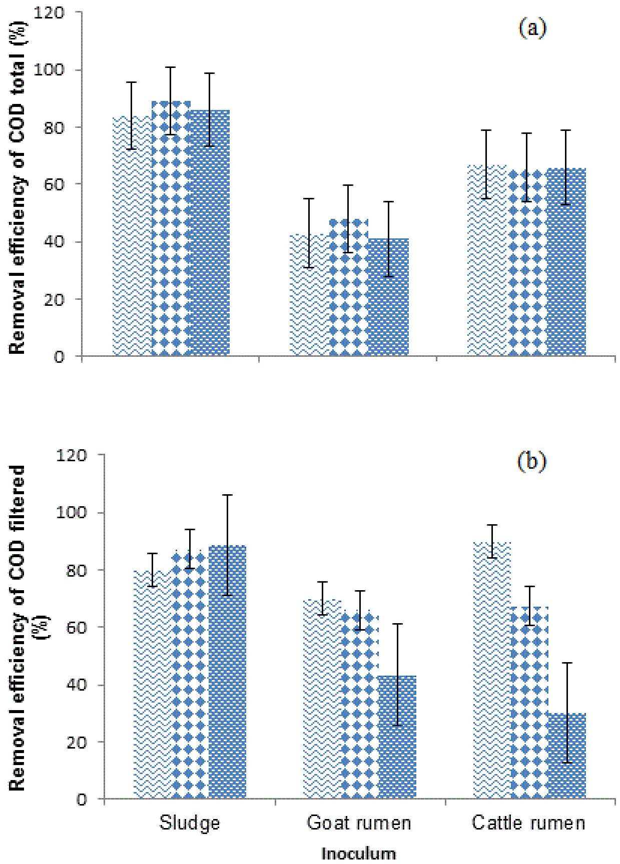

Figure1 – Removal efficiency of COD_T (a) and COD_F (b) for experiments using inoculum at concentrations (in g VSS.L^{-1}) of 2, 3, 4

For COD_F, the increase in the concentration of the biomass of sludge caused an increase in the removal efficiency, with80% (T1), 87.47% (T2) and 88.57% (T3).The opposite effect was recorded for the goatand cattle inoculum. Between the treatments using ruminal inoculum, the greatest efficiency of COD_Fremoval occurred for cattle rumen at a concentration of 2 g.L^{-1} (89.88%), which is very similar to the sludge value at 4 g.L^{-1}.The cattle rumen also exhibited the greatest removal efficiency (30%)with 4g.$L^{-1}$of biomass. The lowest rates of COD_Fremoval efficiency were associated with the treatments that exhibited the greatest values for non-degraded VFAin effluent (T4, T5, T6, T8 and T9). Statistically, the removal efficiency of T2 and T3 (2 and 3g/L of sludge) did not differ from T7 (2 g/L of cattle rumen) at a probability level of 1%.Luo et al (2009) used sludge as inoculum and conducted anaerobic treatment of industrial cassava waste in a sequential batch reactor with a TDH of 5 days (120 hours) and aorganic loadingrate of 11.3g COD.L^{-1}.d^{-1}. The authors reported that the efficiency of organic material removal was80%.

Table 5 confirms that the highest values for the constant rate of decay of COD (K) occurred with COD_T, only in the treatment with sludge, for the three concentrationsof biomass. The opposite effect was recorded for the treatments with rumen. In other words, the K values for COD_Twere lower.This suggests that $COD_T$was digested quicker than COD_Fduring treatment with sludge. According to Costa et al. (2007), this could be related to the characteristics of the material to be degraded. *Manipueira*is more easily assimilated by ruminal microorganisms, which act earlier than sludge microorganisms in soluble material.

Table 5 - Constant rate of decay (K_d) ofCOD_T and $COD_F$for all treatments

K_d (day^{-1})	T1	T2	T3	T4	T5	T6	T7	T8	T9
K_dCOD_T	0.245	0.299	0.288	0.063	0.072	0.057	0.100	0.099	0.099
K_dCOD_F	0.140	0.141	0.101	0.125	0.119	0.064	0.207	0.102	0.097

With the exception of T6 K (0.064 day^{-1}) and T7 K (0.207 day^{-1}), the K_{CODF}values were close to 0.1 1.d^{-1}for the three inoculum. Thus, the type of inoculum and the concentration of biomass did not affect the rate of decay of COD_F when comparing sludge and goat rumen. However, for cattle rumen with a lower concentration of biomass, the rate of decay of $COD_F$was double the rate for sludge and goat rumen. In absolute values, there is a tendency to decrease $K_d$due to the increase in the concentrations of biomass. Metcalf & Eddy (2003) indicated a value of 0.1 for domestic sewage treated in anaerobic systems. The treatments that exhibited the lowest K values (T6, T9, T5 and T4)also exhibited the lowest removal efficiency (43.99%, 30.20%, 65.80%and 67.50%, respectively). This suggests that the accumulation of VFAmay have occurred due to kinetic parameters, as previously suggested by Aquino and Chernicharo (2005) andPoulsen et al. (2012).

Volume of Biogas

The values for the volume of biogas (V_{biogas})were submitted to the Shapiro-Wilk test, with the level of significance set at 5%. A p-value of less than 0.01 was obtained, indicating that the data exhibited normal distribution.

Figures 2a, 2b and2c display the accumulated volume of biogas during the experimental period. The production of biogas can be confirmed in the first 24 hoursof all treatments, tending to stabilize around 192 h of degradation for sludge and 165 h for rumen, when the COD_Fvalues also stabilized in the effluent. The greatest V_{biogas}(mL) values were recorded for T2 (42.07±1.09), T3 (41.93±1.99)and T8 (40.93±0.66). The lowest values were recorded for T4 (27.47±1.33), T5 (29.27±0.24) and T6 (26.93±0.35). Therefore, the greatest production of biogas occurred for sludge, followed by cattle rumen and then goat rumen. Cattle rumen with 2 g.L^{-1}(T8)did not differ statistically (1% probability)from T2 and T3. The volumes produced for goat rumen did not differ between each other.

According to Suzuki et al. (2012),*manipueira* commonly acidifies in single-phase reactors, leading to the collapse of biogas production. This could have occurred in T4, T6 and T9, which exhibited high levels of VFA accumulation. However, Wang et al. (2009) detected high concentrations of acetic acid (2,400 mg/L) that did not significantly inhibit the activity of methanogenic bacteria, obtaining the maximal yield of accumulated methane with values of 1,600 mg.L^{-1}acetic acid. This value is lower than the mean VFAvalues in the effluent of the present study (1,236 mg.L^{-1}acetic acid).

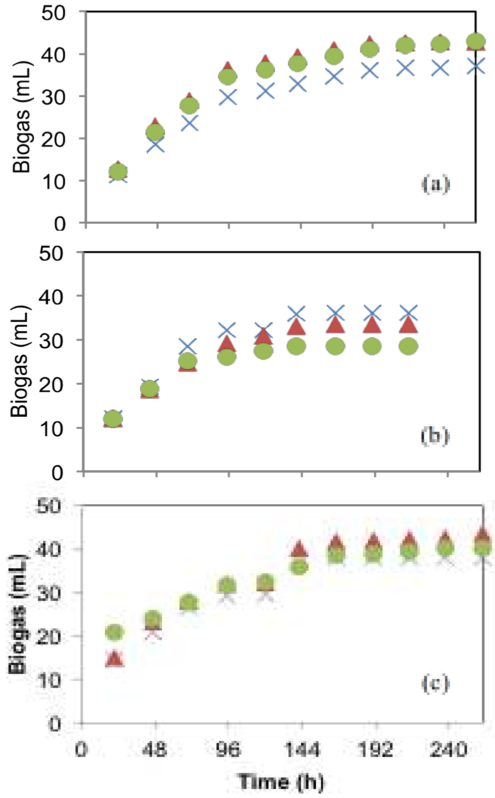

Figure2 – Accumulated volume of biogas experiments using sludge (a), goat rumen (b) and cattle rumen (c) at concentrations (in g VSS.L^{-1}) of ×2, ▲ 3,and ● 4

Table 6 displays the volume of methane (V_{*CH4}), the methane yield in relation to theoretical methane(E),the rate of methane production (*CH_4),and the COD balance.The volume of methane (V_{*CH4})was estimated at 75% of the$V_{Biogas}$of the experiment, as described by Shahet al. (2014). Thus, the greatest production of methane occurred for T2 (31.55 mL), T3 (31.3 mL) and T8 (30.7 mL), which is in agreement with the greatest efficiency for COD_Fremoval.

By applying the coefficients of cellular production as Y_{acid}= 0.15 and$Y_{methane}$= 0.03 dCOD g^{-1}$COD_{removed}$ (Metcalf & Eddy, 2003) and adjusting to the incubation temperature of the trials, it was possible to calculate the theoretical volume of methane (V_{TCH4}) as 0.0462 L forthe trials with sludge and 0.0480 L for those with rumen.

The greatest yields of methane, in relation to theoretical methane (E), occurred for sludge with 3 g.L^{-1} (68.29%) and 4 g.L^{-1} (67.75%),and for cattle rumen with 3 g.L^{-1} (63.96%).These results are similar to those obtained by Baba et al. (2013), who pre-treated paper waste with ruminal fluid and reported CH_4yields of 60.85%, 73.4% and 64.2%. Yue et al. (2012) compared ruminal microorganisms and sludge in the anaerobic digestion process of aquatic plants and reported a greater methane yield (expressed in COD) for the reactor inoculated with sludge (2321.6 mg COD.L^{-1})when compared with the reactor inoculated with rumen (1814.1 mg COD.L^{-1}). In the present study, the removal efficiency of COD of the treatment using sludge did not differ statistically from that of cattle rumen, although statistically different from goat rumen.

The inhibition of methanogens observed in the present study with goat rumenprobably did not occur as a result of nutrients lack or toxic compounds. The microorganisms received nutrient supplements and although cyanide is considered toxic, the cyanide values for *manipueira*in the present study (30.2 mg.L^{-1}) were well below that which is considered toxic for the anaerobic bacteria of sludge (125mg.L^{-1}) (Gijzen et al. 2000).

Based on stoichiometry of anaerobic digestion, the rate of production of theoretical methane was calculated at 0.394 L CH_4 $g^{-1}COD_{removed}$for the trial with sludge at 30ºC and at0.407 L CH_4 $g^{-1}COD_{removed}$for the trial with rumen at 40ºC. As displayed in Table 6, the production rate of methane (*CH_4) was calculated based on the V_{biogas}values.

Table 6 – Production of methane and COD balance

Parameters	T1	T2	T3	T4	T5	T6	T7	T8	T9
V_{*CH4}(L)	0.025 ± 2.45	0.032±0.82	0.031±1.64	0.021±1.00	0.022±0.36	0.020±0.26	0.026±0.18	0.031±0.49	0.026±2.87
E (%)	53.57±5.30	68.29±1.77	67.75± 3.55	42.92±2.08	45.35±0.75	42.08±0.55	54.17±0.36	63.96±1.03	53.60±5.98
*CH_4	0.20±0.02	0.23±0.01	0.25±0.01	0.11±0.02	0.12±0.01	0.22±0.01	0.14±0.00	0.27±0.04	0.14±0.01
%COD_{CH4}	65.96	72.12	73.03	54.83	54.52	35.87	74.11	55.52	56.58
%COD_{Biom}	13.89	15.35	15.25	11.67	11.42	7.63	15.77	11.82	11.39
%COD_{VFA}	21.51	11.01	11.38	33.50	33.12	56.50	10.2	32.36	13.78

Note that the methane production rates (*CH_4) for sludge (Table 6) were the most similar to the theoretical methane values (0.394 L CH_4 $g^{-1}COD_{removed}$), with 50.76% (T1), 58.38% (T2) and 63.45% (T3), which were similar to the theoretical values in terms of the proportion of increase in the concentrationof biomass. However, themethane production rates (*CH_4) of T8 (0.27 L CH_4 $g^{-1}COD_{removed}$)was the most similar value to the theoretical value: only 33.66% less.

In general, the sludge treatment and T8 with cattle rumen exhibited the best results for *CH_4 (0.20, 0.23, 0.24 and 0.27 L CH_4 $g^{-1}COD_{removed}$), which were very similar to values found by Barana (2000), who reported results of 0.22 and 0.24 L CH_4 $g^{-1}COD_{removed}$on a laboratory scale and 0.19 and 0.21 on a pilot scale, both of which used *manipueira*as substrate and sludge as inoculum.Zhang et al. (2013) treated *manipueira*with sludge inoculum in a single phase and obtained methane production rates of 0.125 L CH_4 $g^{-1}COD_{removed.}$ Yue et al. (2012) obtainedinthe reactor inoculatedwith digester sludge showed a higher product yield than thatinoculated with rumen fluid.However, the maximum product formation rate in the reactorinoculated with rumen fluid(207.2 mg COD.L^{-1}.d^{-1}) was much higher than that inoculatedwith digester sludge(120.4 mg COD.L^{-1} d^{-1}) with methane, wereexpressed as chemical oxygen demand (COD).

The products of methane fermentation,biomass and volatile fatty acid, were expressed in %COD_{CH4}, %COD_{Biom} and %COD_{VFA} respectively(Table 6) using the equations described by Chernicharo (2007)for COD balance. Sewage sludge and cattle rumen treatments performed best in terms of removing biodegradable compounds and transforming them into methane (%COD_{CH4}).The greatest plotswere found in T2 and T3with sludge and in T7with cattle rumen. The same behavior was observed for the percentage of the influent COD converted into biomass (%COD_{Biom}). The lowest quotas of influent CODconverted into methane and biomass were found for the goat rumen treatment. Consequently, the greatest quotas of influent CODstill present in the effluent as VFA´s occurred for T4, T5 and T6, similar to the lowest quotas of influent CODconverted into methane. Treatment with ruminal inoculum exhibited VFA accumulation.

The rumen behavior recorded could be attributed to the fact that many of the archaea species present in rumen are hydrogenotrophic (Hook et al., 2010) and practically all ruminal methane is produced by reductions in hydrogen and carbon dioxide, given that VFAare not commonly used as substrate for methanogenesis. Consequently, conversion to hydrogen and carbon dioxide is a slow process that is inhibited by the ruminal ecosystem itself.

CONCLUSIONS

The biodegradability of *manipueira*depended on the inoculum source and concentration. Sewage sludge and cattle rumen performed best in terms of removing biodegradable compounds and transforming them into methane. Treatments with ruminal inoculum exhibited VFA accumulation, although not leading to significant decreases in pH.For cattle rumen, the COD removal efficiency and biogas production increased when decreasing the concentrations of biomass. This may have occurred probably due to accumulation of VFAin treatments with high biomass concentration. Cattle rumen could potentially replace sewage sludge as an alternative inoculum to treat *manipueira*.

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