Article

# Anaerobic biodegradation of cassava wastewater under different temperatures and inoculums

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# Abstract

The production of starch generates, as a by-product, the cassava wastewater (manipueira), which can be treated by anaerobic digestion to provide biogas and minimize its polluting potential. The most commonly utilized biomass in the anaerobic digestion is the anaerobic sludge. The literature presents, as an alternative to sludge, bovine manure and ruminal fluids, being scarce the studies with the cassava wastewater. This research evaluated the influence of temperature on the microbial ability of cattle and goat rumen in anaerobically biodegrading the manipueira in substitution to the anaerobic sludge. The cattle and goat rumen specific methanogenic activities (SMA) were compared with that of the anaerobic sludge. Subsequently, by using the inoculum which had the best SMA results, cassava wastewater biodegradability tests were performed, investigating the kinetics of the organic matter removal and methane production at 32 ° C and 39 ° C. The bovine rumen presented better results in the SMA (0,315 g COD-CH, g VSS.d<sup>-1</sup>) and methane production (1,026 mL). The temperature of 32 °C did not influence the activity of bovine ruminal inoculum as the kinetics of the biodegradation of the manipueira did not differ for the evaluated temperatures (0.1799 d<sup>-1</sup> at 32°C and 0.1781 d<sup>-1</sup> at 39°C). Bovine rumen achieved glucose reduction of 76% and 80% and methane yield of 77% and 79% for the tests at 32°C and 39°C, respectively. It is inferred that this type of inoculum might be used in reactors of anaerobic digestion processes for the treatment of the cassava wastewater at the ambient temperature of the semiarid region.

Keywords: Biodegradability, methane, rumen, cassava wastewater

## Introduction

The cultivation of cassava and the farming of goats and cattle are economic and subsistence activities in the Brazilian semiarid region. Brazil and Indonesia are the largest producers of cassava for consumption and production of flour and starch, whose main by-product is the manipueira (cassava wastewater), besides shavings and leaves (Ubalua 2007; Zhang et al., 2016), which, treated through anaerobic microbial digestion, generate the biogas that might be used as a source of renewable energy (Kuczman et al., 2014), minimizing its polluting potential (Okudoh et al., 2014; Sanches et al., 2017).

Anaerobic digestion requires an interaction of fermentative and methanogenic microorganisms (microbial biomass), being particularly dependent on a strict control of environmental conditions (temperature, pH, alkalinity) (Chernicharo, 2007).

The most commonly used biomass in the processes of anaerobic digestion is the anaerobic sludge of digesters in sewage treatment plants or agroindustries, in concentrations from 2 to 5 g of volatile suspended solids (VSS).L<sup>-1</sup> (Anyanwu et al. 2015). Considering that there are places where there are no anaerobic treatments which can provide such inoculums, literature presents as an alternative to anaerobic sludge: cattle

manure (Sen and Suttar, 2012), swine manure (Panichnumsin *et al.*, 2012), chicken manure (Luo *et al.*, 2010), microalgae (Budiyono and Kusworo, 2011) and stomach fluids of ruminants (Ward et al., 2008; Budiyono et al., 2009) with comparison studies within inoculums (Elbeshabishy *et al.*, 2010; Astals *et al.*, 2013), being scarce the studies with cassava wastewater.

The specific methanogenic activity (SMA) measures the maximum rate of methane production under specific conditions, evaluating the biomass ability to convert an organic substrate into methane (Souto et al., 2010). Consequently, this biomass must possess a high SMA in order to contribute to the success of anaerobic digestion.

The literature indicates that most of the anaerobic reactors are projected in the mesophilic range (Kaparaju et al., 2010; Sun et al., 2012; Intanoo et al., 2016), being the predominant methane-forming microorganisms, in anaerobic reactors operated in the range of 30 - 35°C, those of the genera Methanobacterium, Methanobrevibacter (hydrogenotrophs) and Methanospirillum, and the genera Methanosarcina and Methanosaeta (acetoclastic) (Tchobanoglous et al., 2003; Gerardi, 2006). However, observing the ideal environmental conditions for the bacteria of the ruminal ecosystem, the optimum temperature is 39°C (Hook et al., 2010).

In this sense, this research evaluated the influence of temperature on goat and cattle microbial ruminal ability in aerobically biodegrading the cassava wastewater in replacement to the anaerobic sludge. In order to perform it, were compared the specific methanogenic activities (SMA) of the rumens and of an anaerobic sludge, the reduction efficiency of organic matter and the methane production thorough the removal kinetics of each inoculum.

## Material and methods:

## Substrate and inoculums

The cassava wastewater came from a flour house in the city of Araripina, Pernambuco state, Brazil. The goat and cattle ruminal fluids utilized as inoculums were directly collected from the pre-stomach of the animals and filtered through cotton cloth. The anaerobic sludge was collected from an upflow anaerobic sludge blanket (UASB) reactor utilized in the treatment of domestic sewage in the city of Recife, PE. Table 1 shows the characteristics of the cassava

 Table 1. Characteristics of the cassava wastewater and ruminal fluids

	Cassava	Bovine	Goat	Angerobic
Parameters	wastewater	rumen	rumen	sludge
COD (mg.L <sup>-1</sup> )	37700	3774	4316	420
рН	5,80	7,5	7,21	7,03
Total phosphorus (mg.L-1)	183	5733	4123	5,01
Ammoniacal nitrogen (mg.L-1)	28	174	162	120
Alkalinity (mg CaCO <sub>3</sub> L <sup>-1</sup> )	72	5733	4283	460
Volatile acids (mg HAc.L <sup>-1</sup> )	25	9883	8824	3540
Volatile suspended solids (mg VSS.L-1)	4332	16000	9400	37710

wastewater and of the three inoculums. Specific methanogenic activity (SMA)

For the SMA essays, the methodologies of Field et al. (1988) and Florêncio et al. (1993) were adopted, utilizing as substrate a mixture of acetic, propionic and butyric acids in a concentration of 4 g COD.L<sup>-1</sup>, a biomass concentration of 2 gSSV.L<sup>-</sup> <sup>1</sup>of the inoculum and manual agitation every 12 hours in all incubated bottles, according to Souto et al. (2010), with an average duration of 30 days.

For each essay, 1.3 L reactor bottles were used in triplicates, with a headspace volume of

20% (Aquino et al., 2007) and sealed with rubber septa, connected to a 15 mL surgical syringe through a glass hose to transport the biogas. The reactor bottles, along with control bottles, inoculated without the introduction of the substrate, were incubated in a room maintained at  $32 \pm 2^{\circ}$ C through a 1500-W ambient heater, and manually and intermittently shaken (Souto et al., 2010) every 12 hours.

The biogas measurement was performed through the volumetric method of direct measurement of methane volume by washing the biogas from the reactor bottle through the glass hose in a solution of 3,0% (m/v) sodium hydroxide for absorption of CO2 (Aquino et al., 2007). The produced methane volume was gauged by measuring the volume of the sodium hydroxide solution displaced by the washed gas (Aquino et al., 2007).

The value of the maximum specific methanogenic activity of each inoculum (g  $COD_{CH4}/gVSS.d$ ) was calculated from the maximum methane productions in 24 hours, according to Equation 1:

$$SMA = \frac{V_{CH_4/t}}{f.VSS.V_u} \tag{1}$$

Where is the maximum methane volume produced in the considered time interval (mL); t is the considered time interval (days); f is the stoichiometric conversion factor (the Temperature

of 30 °C 1 g DQO is equivalent to 390 ml of CH4 produced); VSS is the biomass concentration (g/L);  $V_{u}$  is the useful volume of the flask.

Effect of incubation temperature on ruminal microbial activity

In order to evaluate the effect of incubation temperature on ruminal microbial activity in the biodegradability of the cassava wastewater, only the ruminal content which presented best SMA performance was used as inoculum. The essays were conducted in 0,130 L reactor bottles, utilizing 0,104 L of usable volume at 0,026 L headspace. The ruminal biomass concentration was 2 g VSSL<sup>-1</sup>. The temperatures of 32 and  $39 \pm 2^{\circ}$ C, were tested in a Q316M model incubator, thus characterizing two treatments (Table 2).

able 2. Experimental configurations for biodegradation evaluation

Treatments	Inoculum biomass (g VSS.L-1)	Inoculums	Substrate (2 g DQO.L <sup>-1</sup> )	Temperature (°C)
1	2	Bovine rumen	Cassava wastewater	32
2	2	Bovine rumen	Cassava wastewater	39

The cassava wastewater was added to the reactor bottles in the concentration of 2 g COD.L<sup>-1</sup>24 hours after the addition of the inoculum and nutrients, in order to provide adaptation of the microorganisms to the environment. The concentrations of macro (N-NH<sub>4</sub><sup>+</sup>, P-PO<sub>4</sub><sup>+</sup>, Mg, Ca) and micro nutrients (Fe, Ni, Zn, Co...), besides alkalinity (NaHCO<sub>3</sub>), were maintained in each reactor bottle (per liter) considering the nutritional requirements of the sludge microorganisms recommended by Florêncio et al. (1993) and the rumen microorganisms, as recommended by Hu et al. (2007) and Baba et al., 2013).

On day 0 all triplicates were prepared at the same time, sealing the reactor bottles with rubber septa and aluminum seals, and adapting 15 ml to the septa for the collection and measurement of the generated methane gas. Three sets were prepared for analysis on days 0, 1, 3, 5, 7, 9 and 11. The method followed the methodology of Amorim et al. (2013), which consists in removing a triplicate set at every established time interval for sampling and analysis of the reaction content. Every 48 hours a triplicate set had its reaction content analyzed separately, and after the sampling the three reactor bottles were discarded. Control bottles were prepared, although without substrate addition. The maximum digestion time of 264 hours (11 days) was adopted.

The methane measurement was daily, and performed through the volumetric method of Direct Measurement of Methane Volume, by washing the biogas from the reactor bottle through a glass hose in a solution of 3.0% caustic soda to absorb CO<sub>2</sub> (Aquino et al., 2007).

The agitation was manual (Souto et al., 2010) in all incubated bottles.

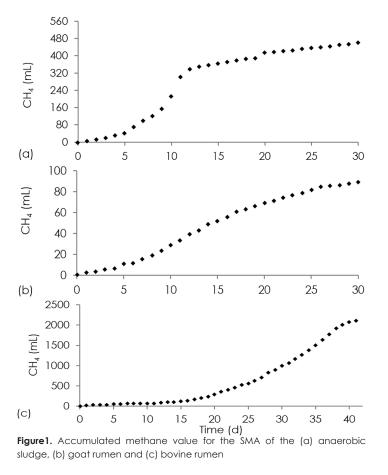
The following parameters were analyzed: pH, total and filtered COD in 0,45 µm membrane, partial alkalinity (PA) and volatile fatty acids (VFA) in mg Acetic acid.L<sup>-1</sup>. The anaerobic biodegradability of the cassava wastewater, under different temperatures, was evaluated through the following parameters: filtered COD removal efficiency (COD), Total Solids (TS), Total Volatile Solids (TVS), Volatile Suspended Solids of the biomass (VSS) and glucose (APHA, 2012). The methane rate of production or yield ( $T_{CH4}$ ) was determined through the relation between the volume of effectively produced and measured methane and the applied COD mass of the system (mL CH<sub>4</sub>·g<sup>-1</sup>COD<sub>applied</sub>), and the methane yield in relation to the theoretical methane ( $R_{CH4}$ ).

According to Bertolino et al. (2008), the knowledge of the substrate utilization kinetics is an important parameter for the verification of the microorganisms' ability in stabilizing the organic matter present in the sewage, that is, to determine the treatability of the effluent. In order to evaluate the rate of organic matter consumption during the biodegradability tests in the reactor bottles, the K constant was determined through Equation (2). Teixeira et al.(2008), obtained that the firstorder kinetics was the one which best fit the obtained results for COD removal.

$$(-r) = -\frac{dS}{dt} = k_d S \quad (2)$$

Where: *r* is the reaction rate (mass/volume. time), *S* is the concentration of the limiting reactant (mass/volume), *T* is the time (days) and  $K_d$  is the rate constant for first-order reaction (day<sup>-1</sup>).

The results were statistically evaluated in the ASSISTAT® software (Beta Version 7.7), through descriptive statistics and Analysis of Variance (ANOVA) applying the Shapiro-Wilk homoscedasticity test. Confirmed a significant difference (p-value<0,05) to compare the means, a significance level of 5% was adopted. Tukey's test was applied for the parametric data series, and Kruskal-Wallis test for non-parametric data, which compares three or more independent samples, indicating if there is a difference within at least two of them.



#### **Results and discussion:**

Specific Methanogenic Activity

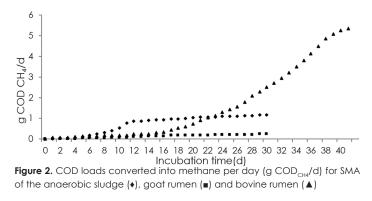
The cumulative productions of methane in the SMA essays were highly varied, as might be

observed in Figures 1a, 1b and 1c, as well as the accumulated methane production in terms of g  $COD_{CH4}/d$  (Figure 2).

Whilst for the anaerobic sludge (Figure 1ª)

there was a production of circa 350 mL until the 15° day, in the same period the production was of 50 mL for the goat rumen (Figure 1b) and of 150 mL for the bovine rumen (Figure 1c). However, at the end of the 30° day, the highest methane production was for the SMA of the bovine rumen with 1.026 mL of methane, followed by the SMA of the anaerobic sludge with 460 mL and by the goat rumen with 91 mL. It was observed that either for the cumulative methane production (Figures

1a, 1b and 1c) as for the COD loads converted into methane (Figure 2), the bovine rumen, even after the 30 days, did not present a tendency for the stabilization of methane production. As for sludge and goat rumen, from the twelfth day, there was a tendency for stabilization. The volumes are only related to the conversion of the substrate into methane, since that in the control serum bottles there was no methane production.



The maximum rates of methane production for the anaerobic sludge and for goat rumen occurred between the seventh and the ninth day of the test. As for the bovine rumen, they occurred between the twenty-third and twenty-fifth days. Table 3 presents the SMA values for the three inoculums in the stretch of highest activity, expressed in accumulated volume of methane (mLCH<sub>4</sub>/d) and as mass of COD converted into methane (g  $COD_{CH4}/d$ ) per quantity of biomass in g VSS.

Table 3. Obtained SMA of the three evaluated inoculums	

Inoculums	S	бМА
Anaerobic sludge	48 mL CH₄/gVSS.d	0,210 g COD <sub>CH4</sub> g <sup>-1</sup> VSS.d <sup>-1</sup>
Goat rumen	16 mL CH₄/gVSS.d	0,014 g COD <sub>CH4</sub> g <sup>-1</sup> VSS.d <sup>-1</sup>
Bovine rumen	113 mL CH₄/gVSS.d	0,315 g COD <sub>CH4</sub> g <sup>-1</sup> VSS.d <sup>-1</sup>

Although for the anaerobic sludge and for the goat rumen the maximum rates of methane production have occurred in the same period, the SMA value for the goat rumen was quite inferior to that of the anaerobic sludge, with this same value being found by Lucena et al. (2001) for this type of sludge, as well as by Sun et al. (2012) who, characterizing the SMA of the anaerobic sludge treated with cassava wastewater, obtained values of 0,31 and 0,73 gCOD CH<sub>4</sub>/g VSS.d, corresponding to 134,23 and 316,09 mL CH $_4$ /(g VSS.d). The SMA of the bovine rumen presented the highest value, but a longer test time was necessary. The SMA values, either for the anaerobic sludge as for the bovine rumen, were superior to the values of 0,10 and 0,17 g

 $COD_{CH4}$  g<sup>-1</sup>. VSS d<sup>-1</sup> for the food sludge, found by Schneiders et al. (2013).

Considering that the influence of the VSS concentration on biomass and the concentration of substrate, described by Souto et al. (2010) were eliminated, since the same concentration of VSS was utilized for all inoculums and the same concentration of substrate in the three SMA, the differences within the SMA values might be attributed to the different types of microbial mass.

The SMA of the goat ruminal inoculum might have been inhibited by the temperature in which the test was performed ( $32 \pm 2^{\circ}$ C), seen that, according with Hook et al. (2010), the optimal temperature for the ruminal microorganisms is 39°C. It is also a fact which justifies an adaptation

of the cattle ruminal microorganisms at the temperature of 32°C, that after the 25° test day the methane production was increased, tending to stabilize around the 38° day.

Effect of the incubation temperature on the bovine ruminal microbial activity during the biodigestion of cassava wastewater

Behavior of the Environmental Parameters

The normality test indicated an abnormal distribution for the pH data (p-value 0,00875) and normality of the data of PA (p-valor 0,70347) and VFAs (p-value 0,23117, with average values and standard deviations presented in Table 4.

Tukey's test with 5% of significance level identified that the pH values in the effluent are statistically different, that is, the temperature did influence in the pH value. The affluent pH values were close to 6,80. The pH of the effluents were close to neutrality at 32°C and presented alkaline character (8,00) in case of the biomass at 39°C.

Tukey's test with 5% of significance level identified that the PA and VFAs values in the effluent are statistically equal, that is, the temperature did not influence in the results of the alkalinity and the VFAs during the biodegradation of the cassava wastewater with bovine rumen.

Table 4. Means and standard deviation of the pH, PA and VFAs in the beginning and in the last day

	Begir	nning	Last day		
	32°C 39°C		32°C	39°C	
рН	6,75 ± 0,08	6,83 ± 0,08	7,00 ± 0,00 b	8,00 ± 0,00 a	
PA (mgCaCO <sub>3.</sub> L <sup>-1</sup> )	198,67 ± 8,33	225,00 ± 44,19	150,60 ± 8,33 a	141,33 ± 5,66 a	
VFAs (mgHAc.L <sup>-1</sup> )	650,45 ± 33,40	650,39 ± 5,11	627,65 ± 21,39 a	626,23 ± 3,17 a	

\*Means followed by the same letter do not statistically differ within each other (p>0,05) through Tukey's test. Subtitle: PA = Partial alkalinity; VFAs = Volatile fatty acids.

## Removal of biodegradable organic compounds

Table 5 presents the descriptive statistic of the results of the removal efficiencies (RE) during the biodegradability of cassava wastewater, of  $COD_T$  and  $COD_F$ , of glucose, and the TS and TVS at 32°C and 39°C.

The normality test indicated a normal distribution for the values of removal efficiency of the COD,  $COD_F$ , carbohydrate, total solids of the liquid phase and total volatile solids (p-value 0,33266). The statistic results of comparison within the removal efficiency means of  $COD_T$ ,  $COD_F$  and

glucose were not significant. Nevertheless, by analyzing the data in Table 5, it might be verified that the comparison of the removal means of TS and TVS presented significant differences within each other, although not significant when compared for the temperatures of 32°C and 39°C. In this manner the statistical analysis puts in evidence that the evaluated temperatures did not affect the activity of the ruminal inoculum during the biodegradation of the cassava wastewater.

RE (%) at 32°C						RE (%)	at 39°C			
	COD	COD <sub>F</sub>	Glucose	TS	TVS	COD	COD <sub>F</sub>	Glucose	TS	TVS
Mean	74 a	71,7 a	76,2 a	33,3b	46 C	73,2 a	70,5 a	75,7 a	36 b	41,3c
SD	1,582	3,185	2,320	5,068	1,330	1,124	4,109	3,882	6,940	2,89
CV (%)	2,14	4,44	3,04	15,23	2,90	1,54	5,8336	5,13	19,28	7,01
Var	2,504	10,153	5,382	25,684	1,775	1,263	16,890	15,072	19,280	8,386

Table 5. Descriptive statistic of the results at 32°C and 39°C

\*Means followed by the same letter do not statistically differ within each other (p>0,05) by Tukey's test. Subtitle: SD: Standard Deviation; CV: coefficient of variation; Var: Variation

Figures 3 and 4 demonstrate the removal efficiencies of the  $COD_{T}$ ,  $COD_{F}$  and carbohydrates in terms of glucose. It was observed that at the end of the biodegradation period, the behavior

of the  $COD_{T}$ ,  $COD_{F}$  and glucose were similar, either at 32° C as at 39°C, what did not occur with the TS of the liquid phase, which, although having been reduced, its percentage did not follow the cited parameters. This was also observed in Table 5 and Figure 5, whose TS reductions were of 33,3% (32°C) and 36% (39°C).

the concentration of the volatile suspended solids in the biomass, either for the biodegradation at 32° C as at 39°C, what was expected, mainly because the essays were performed in batch reactor, in the absence of biomass drag, common in continuous flow systems.

According with the data of Table 5, a decrease in the total volatile solids of the liquid phase was observed, as well as the increase in

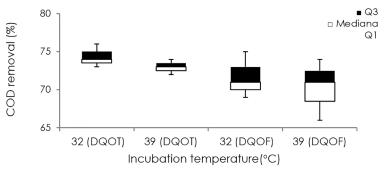
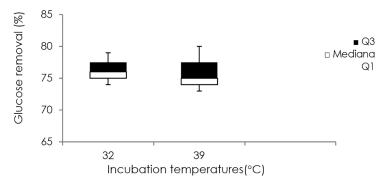
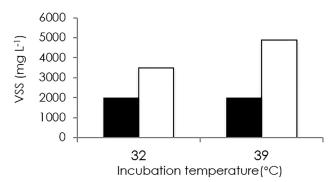
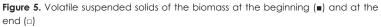


Figure 3. Removal efficiency of the  $\text{COD}_{_{T}}(\text{DQO}_{_{T}})$  and  $\text{COD}_{_{F}}(\text{DQO}_{_{F}})$  at the end of the biodegradation period









By statistically comparing the reduction means of the TVS values of the liquid phase at 32°C and at 39°C there was no significance (p-value 0,64756), with the same occurring for the increase in the VSS values of the biomass for both temperatures (p-value 0,87554). The temperature did not statistically affect the reduction of the TVS nor the increase in biomass, although, in absolute values, the highest increase percentage was observed for the biomass incubated at 39° C, according with Figure 5.

In this manner it is worth noting that, statistically, there were no significant differences within the obtained results for the removal of organic matter of the effluent at different temperatures, inferring that it might be possible to utilize bovine rumen as inoculum in anaerobic biodegradation processes at the same temperature utilized for the sludge of anaerobic sewages, that is, at 32°C. Coefficient of kinetic decay of the COD,  $K_i(d^{-1})$ 

The kinetic data of the biodegradation and the descriptive statistic are presented in Table 6 for COD and glucose, and, in Table 7, for the total solids and total volatile solids. The statistical evaluation indicated normality for the average values of the decay rate  $K_i(d^{-1})$  constants for the COD and glucose (p-value 0,77034), and for TS and TVS (p-value 0,58527).

The means comparison through Tukey's test at the level of 5% probability of  $K_i$  for removal of COD and glucose presented F value 1,6551, not being, therefore, significant (p>=0,05). As for the

TS and TVS, the F value of the ANOVA was 4,8758, being significant at the level 5% probability, as might be verified in Table 7, highlighting that the difference was significant only within the K<sub>i</sub> values for the TS and TVS 32°C. That is, the degradation rate of the TVS was higher than the degradation rate of the TS at the temperature of 32°C, not being significant when compared the average K<sub>i</sub> values with the temperature of 39°C, indicating the potential of the microbial mass of the bovine rumen for the biodegradation of organic compounds, and that the temperature did not interfere in this ability of the inoculum.

Table 6. Coefficient of kinetic decay (K<sub>i</sub>) for COD and glucose

	k	(d-1) at 32°	С	K	(d-1) at 39°	С
	COD	COD	Glucose	COD	COD <sub>F</sub>	Glucose
Mean	0,169	0,158	0,180	0,164	0,153	0,178
SD	0,008	0,014	0,012	0,005	0,017	0,021
CV (%)	4,540	9,050	6,883	3,160	11,334	11,702
Variance	5,89E-05	0,00021	0,00015	2,71E-05	0,0003	0,00043

Table7. Coefficient of kinetic decay (K<sub>i</sub>) for TS and TVS

	K <sub>i</sub> (d <sup>-1</sup> ) at 32	2°C	K <sub>i</sub> (d <sup>-1</sup> ) at 39°C		
	TS	TVS	TS	TVS	
Mean	0,0508b	0,0770a	0,0563ab	0,0668ab	
SD	0,009	0,003	0,014	0,006	
CV (%)	18,35	4,02	24,88	9,28	
Variance	8,7E-05	9,6E-06	1,96E-04	3,83E-05	

\*Averages followed by the same letter do not statistically differ within each other (p>0,05) by Tukey's test.

## Methane volume and yields

The absolute volume values of produced methane ( $V_{CH4}$ ) were subjected to the Shapiro-Wilk test at the level of 5% of significance, (p-value 0,27242; F 3,1555) consequently obtaining the normality of the data. The averages of the total volumes produced at 32°C and 39°C did not present statistic differences when subjected to Tukey's test (F 0,1093; p 0,7574).

Figure 7 presents the volume of the daily produced methane throughout the degradation period, and Figure 8 presents the accumulated methane volume during the biodegradation period. Methane production could be verified in the first 24 hours for both treatments. It was observed that the apex of production occurs in the third day, occurring an abrupt decline in the fourth day, and thence assuming a decline tendency.

Of the produced value at the end of the 8° day, the volume regarding the endogen

decay was discarded, corresponding to 9 mL for the control bottles incubated at 32°C, and 10 mL for the control bottles at 39°C, resulting in a total volume of effectively produced methane of 49 mL and 52 mL, respectively. By applying the coefficients of cell production Y<sub>acidogenic</sub> of 0,15 and Y<sub>methanogenic</sub> 0,03 gCOD.g<sup>-</sup> <sup>1</sup>COD<sub>removed</sub> (Tchobanoglous et al., 2003) plus adjusting the incubation temperature of the essays, the theoretical volume of methane  $(V_{TCH4})$ was calculated, corresponding to 0,0632 L for the experiments at 32°C, and 0,0653 L for the experiments at 39°C with rumen. Based on the effectively produced volume, the methane yield, in relation to the theoretical methane ( $\rm R_{{}_{CH4}}$ ) was 77% and 79% for the experiments with rumen at 32°C and 39°C, respectively.

In the temperature of 32°C the rate of methane production was 249mL  $CH_4/gCOD_{applyedb}$  with removal of 72% of  $COD_F$ . As for the temperature of 39°C, the rate was 251mL

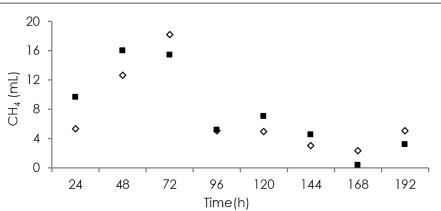


Figure 7. Volume of daily produced methane throughout the degradation period at temperatures (°C) of 32 ( $\diamond$ ) and 39 ( $\blacksquare$ ).

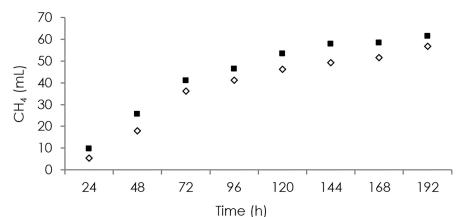


Figure 8. Accumulated methane value for the biodegradation of cassava wastewater with bovine rumen at temperatures (°C) of  $\diamond$  32 and  $\blacksquare$  39.

 $CH_4/gCOD_{applyed}$  with removal of 71% of  $COD_F$ . Glucose removal was 76% for both temperatures. Intanoo et al. (2014) obtained values of 164,87 mL CH<sub>4</sub>/g COD<sub>applied</sub> and COD removal of 72%, utilizing cassava wastewater in a two-phase UASB reactor, although under thermophilic temperature (55°C). According to the authors the specific methane production rate is also utilized to indicate the ability of the microbial mass to produce methane from organic compounds per reactor unit or per unit of biomass dry weight. In this work, the specific methane production rates at the temperatures of 32°C and 39°C were 371mL CH<sub>4</sub>/gVSS.d and 190 mLCH<sub>4</sub>/gVSS.d, while Intanoo et al. (2014) obtained the value of 356 mL CH,/gVSS.d, and Intanoo et al. (2016) obtained a methane yield of 650 mL CH,/gVSS.d and methane yield of 115 mL CH<sub>4</sub>/gCOD<sub>removed</sub>.

Intanoo et al. (2014) did also calculate the methane yield in mL  $CH_4/gVSS$  applied, obtaining the value of 840 mL  $CH_4/g.TVS$  applied. In this research the methane yields at the temperatures of 32°C and 39°C were 0,217 and 0,226 mL CH $_{\!_4}/g.VSS$  of applied biomass.

Therefore, with no significant differences within the results obtained in the biodegradation utilizing bovine ruminal inoculum at different temperatures, it might be inferred that the biomass activity was not statistically influenced by the evaluated temperatures.

#### Conclusions

In the SMA essays, the obtained results demonstrated that the existing microorganisms in the bovine rumen presented a higher potential in converting the organic matter of the acid substrate into methane, when compared to those of the anaerobic sludge and goat rumen. Considering the high specific methanogenic activity of the bovine rumen, this inoculum presents potential for being utilized in substitution to the anaerobic sludge in treatment stations of sanitary swage.

The anaerobic biodegradability of the cassava wastewater utilizing bovine rumen as microbial mass revealed to be efficient. This fact was highlighted by the percentages of organic matter reduction in terms of glucose, which variated from 76% to 80%, and methane yields of 77% and 7% at temperatures of 32°C and 39°C, respectively.

It might be finally concluded that the temperature of 32°C did not influence the biomass activity of the bovine ruminal inoculum, seen that the kinetic of the cassava wastewater biodegradation did not differ for the evaluated temperatures (32 and 39°C). Therefore, it might be inferred that this type of inoculum might be alternatively utilized in reactors of anaerobic digestion processes for the treatment of cassava wastewater in the ambient temperature of the semiarid region, being a viable implantation option for the small and average scales of the local industries, with significant socioenvironmental gains.

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