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Evaluation of the Production of Methane from the Vinasse of the Rum Distillery through the Anaerobic Digestion Process

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ABSTRACT : *Vinasse is a residual substance produced in the distillation of alcohol; it represents a major environmental problem for the alcohol industry which causes damages to the aquatic life especially when large volumes are dumped in rivers, streams and landfills. The use of the vinasse and its anaerobic treatment for the production of biogas to be used as energy and the residual sludge that could be used as bio fertilizer or animal feed.*

The Biochemical Methane Potential (BMP) is a discontinues test which is mainly used to determine the performance of methane from a raw material. The test even provides information about the anaerobic degradability of a substrate, including its rate of degradation.

The general objective of this study was to determine methane production in the laboratory by testing the Biochemical Methane Potential (BMP) from the rum distillery´s vinasse deriving from the Agrarian Agricultural Company Andahuasi S.A.A. located in the Huaura-Sayan valley, in the province of Huacho, 190 Km from Lima.

In the completely randomized experimental design (CRD) at a 95% confidence there is enough statistical evidence to affirm that treatment B produces a greater Biochemical Methane Potential than treatment A. Being constituted both treatments by vinasse of cane rum as substrate and cow manure as inoculum, however, treatment B was developed through a two-phase anaerobic digestion.

A Biochemical Methane Potential (BMP) of 153.0 ml CH4 /g SV substrate (253.2 ml CH_4 / g COD substrate) was determined for treatment A and a BMP of 19.5 ml CH4 /g SV substrate (320.2 ml CH_4 / g COD substrate) for treatment B. The ratio of 0.3 g SV substrate / g SV inoculum is effective for determining the methane production potential of cane rum vinasse as methane production was underestimated. A maximum energy potential of 0.118 Kw-h was calculated for each liter of cane rum vinasse. A two-stage anaerobic digestion process is recommended from treatment of cane rum vinasse due to its high organic matter concentration and its acid character

KEYWORDS: *Vinasse, anaerobic digestion, biogas, Biochemical Methane Potential*

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I. INTRODUCTION

Vinasse is a residual substance produced in the distillation of alcohol, it represents a major environmental problem for the alcohol industry. No convenient and economical solution has been found for the disposal of this reddish-black color, viscous and with high Biochemical Oxygen Demand (BOD) residue which causes damage to aquatic life especially when large volumes are poured into rivers, streams and landfills [11]

The word vinasse is derived from the Latin word "*vinacaeus"*, originally known as yeast wine, its use as a food supplement in ruminants and non-ruminants is reported in many tropical countries and in Europe, furthermore its use as a fertilizer has been reported since the beginning of the 20th century. Vinasse is classified as a class II waste, it is not inert and not dangerous. The chemical composition of ethanol vinasses are variable and depends on the raw materials used in the fermentation to obtain the wine. The characteristics of the wine also depend on the must, fermentation system, type of yeast, and distillation and separation process [3]

A direct use of vinasse is for the feeding of growing and finishing pigs by its concentration at 60 ° Brix. The use of vinasses and their anaerobic treatment for the production of biogas to be harnessed as energy has also been investigated and the sludge residual could be used as bio fertilizer or animal feed. The summary of the process and treatment of vinasse is summarized in Figure 1.
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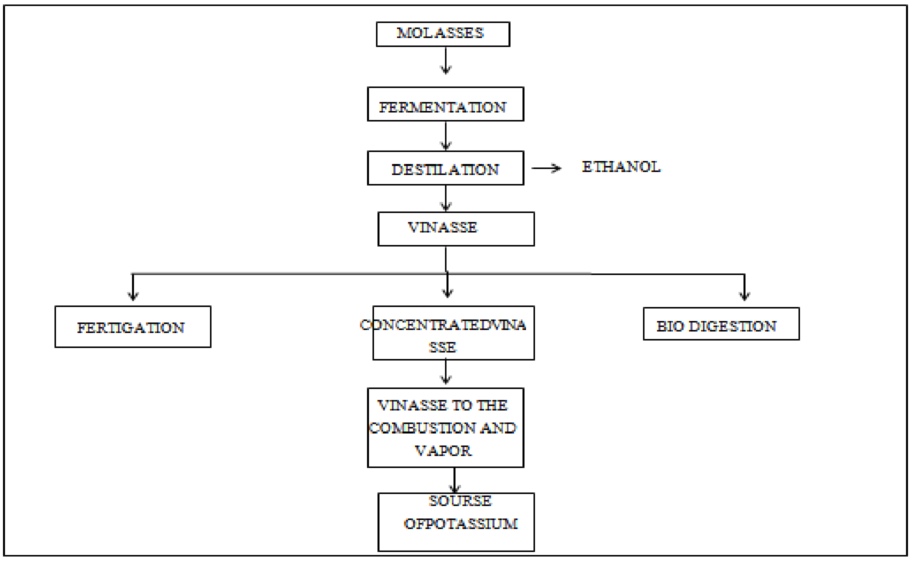


Figure 1 Vinasse processing and treatment flow diagram [10]

The biochemical methane potential (BMP) is a discontinuous assay which is mainly used to determine the performance of raw methane. The test even provides information on anaerobic degradability of a substrate, including its rate of degradation. A general evaluation of inhibitor components may also be performed [8]. Standardized anaerobic degradation tests are described in standards on 11734, DIN 38 414-S8 or VDI (2006).

The general objective of this study is to determine methane production in the laboratory by testing the Biochemical Potential of Methane (PBM) from the rum distillery's vinasse from Agricultural Sugar Company Andahuasi S.A.A. (Andahuasi Sugar Company). It located in the Huaura - Sayán valley, in the province of Huacho, at 190 Km. of Lima.

II. MATERIALS AND METHODS

The sample of vinasse from the rum distillery comes from the Agricultural Sugar Company Andahuasi S.A.A. located in the Huaura - Sayán valley, in the province of Huacho, at 190 Km. of Lima. The company has 6,000 hectares of its own productive land and 3,000 additional hectares of its associated farmers. It is an agro-industrial company dedicated to the cultivation of sugar cane (yield of 160 MT per hectare); production and marketing of sugar and its derivatives, with a processing plant with a maximum milling capacity of 2000 tons of cane per day.

The Methane Biochemical Potential (BMP) test took place at the facilities of the Environmental Engineering Laboratory of the National Agrarian University La Molina (UNALM).The following equipment and materials were used for the BMP test: OxiTop® Control measuring equipment, NaOH in beads control, buffer ($NaHCO_3$), thermostatic bath equipment and CH_4 volumetric measuring equipment.

The physicochemical properties of the vinasse were determined according to standard methods and official international methods of analysis (AOAC). These methodologies are summarized in Table 1.

Table 1. Methodologies used for the physical-chemical characteristics of vinasse

Parameters	Analysis method
Total Solids (%)	Standard Methods 20th Edition 2-80
Total ashes (%)	AOAC 19th Edition 2012.923.03
Raw Fat (%)	AOAC 19th Edition 2012.922.06
Total Nitrogen (mg / L)	Standard Methods 20th Edition 4-125
Raw Fiber (%)	AOAC 19th Edition 2012.920.86
pH	Standard Methods 20th Edition 4500-H

For the evaluation of the potential methane production of the cane rum vinasse by anaerobic digestion, the Methane Biochemical Potential (BMP) test was used. Two different procedures were developed, including in one of them the pretreatment of the substrate (vinasse) before carrying out the BMP test, while in the other there was no pretreatment.

The pretreatment consisted of spontaneous and anaerobic fermentation of the substrate during 4 days to increase the efficiency of the hydrolytic phase of anaerobic digestion and to take advantage of the acid character of the vinasse. This pre-treatment is also known as pre-acidification and the whole process is called two-stage anaerobic digestion. For this, a container was filled with the vinasse and placed in a thermostatic bath equipment at 30°C, it was hermetically sealed leaving only an outlet pipe to a water trap to release the CO_2, then the CO_2 was taken to another container, also hermetically sealed, which contained a dilution of NaOH in order to capture it, the latter container also had a conduit to a test tube, so that it can verify that no methane is produced, otherwise a volume of water would move towards the test tube. In other words, volumetric methane measurement equipment was used in pre-acidification to verify that this gas was not produced during this stage.

The inoculum selected to BMP test was constituted by cattle manure from the farm of the National Agrarian University la Molina.

The Biochemical Methane Potential (BMP) test was performed using a manometric method. The OxiTop® Control measuring equipment was used for that purpose (Figure 2), which is a team of monitoring of pressure consisting of a reactor 1L with a measuring head which is inserted in the "mouth" of the reactors and a control that uses an infrared interface to transferred dates.

Mixtures of inoculum and substrate in a ratio of 0.3 grams of volatile solids of substrate for each gram of volatile solids of inoculum were placed within each reactor. 3 replicates were performed for each of the two proposed treatments and also controls or targets were made for correction due to endogenous methane from manure. Targets were made in a volumetric taking into account that according to research carried out by Souto *et al* in which three CH_4 measurement methods were evaluated: volumetric with characterization of the biogas volume using one alkaline solution and manometric (Oxitop® system), it was found that the production of CH_4 was similar in these methods [20].

Figure 2. Oxitop® Control measuring equipment

The chosen proportions were determined using the criteria indicated by Drosg *et al*, which recommends that g SV substrate / g SV sludge must be less than or equal to 0.5.[8]

No nutrients were added to the reactors, because in the study conducted by Bermúdez *et al* no incidence was observed in the anaerobic digestion of the vinasse by adding or not adding nutrients. They point out that it is important to bear in mind that vinasses comes from molasses, which is reported as a byproduct rich in micronutrients[5].

Each bottle was filled to 0.8 L, leaving 20 percent free space for the biogas. It should be noted that Ortiz obtained the best biogas production values with small headspace volumes (20 to 25 percent of the total volume) [16].

To evaluate only the production of methane, 4 NaOH beads were placed (Parra, 2014) in the compartment with each reactor in the mouth, so that all the CO_2 is absorbed. It is necessary to point out that the highest percentage of biogas is composed of CH_4 and CO_2, so it will be assumed that the pressure generated within each reactor will only be generated by CH_4. Souto *et al* tested the efficiency of the NaOH solution in the volumetric method and the NaOH beads used in the Oxitop® System for CO_2 capture, as the analysis of the biogas samples by chromatography revealed the absence of this gas [20].

On the other hand, before closing the reactors, the solution was neutralized (due to the acid character of the vinasse); it was using sodium bicarbonate ($NaHCO_3$) until it reaches a pH of 7 units, because the methanogenic bacteria require a neutral pH.

The reactors were maintained at a controlled temperature of 30 ° C in order to obtain a high growth rate and activity of the microorganisms (Figure 3).
A daily monitoring of the pressure variation in the reactors was performed and the BMP test was stopped when the daily volume of gas production was less than 1 percent of the total gas production. [8]

Figure 3. Implementation of the monitoring system

Because the OxiTop ® Control measuring system is a pressure monitoring system, it was necessary to transform this pressure to the volume of methane produced per gram of volatile solid of the substrate under standard conditions, so that it is comparable with other investigations. To do this, the equations provided by Cárdenas *et al* were taken into accounts, which are presented below: [6]

$$n_{CH_4} = \frac{\Delta P * V_1}{R * T_e}$$

$$V_{CH_4}\mathrm{SC} = \frac{n_{CH_4} * R * T_{\mathrm{SC}}}{P_{\mathrm{SC}}}$$

$$H_{CH_4} = 10\left(\frac{-673.74}{T_e} + 6.88\right)$$

$$X_{CH_4 dissolved} = \frac{\Delta P}{H_{CH_4}}$$

$$M_{CH_4 dissolved} = \frac{M_{H_2O} * X_{CH_4 dissolved}}{1 - X_{CH_4 dissolved}}$$

$$n_{CH_4 displayed} = M_{CH_4 dissolved} * V_u$$

$$V_{CH_4 dissolved}\,\mathrm{SC} = \frac{n_{CH_4 dissolved} * R * T_e}{\Delta P}$$

$$V_{TCH_4}\mathrm{SC} = V_{CH_4}\mathrm{SC} + V_{CH_4 dissolved}\,\mathrm{SC} - V_{CH_4 control}$$

$$BMP = \frac{V_{TCH_4}\mathrm{SC}}{gSV}$$

- nCH4: Moles of CH4 (mol).
- ΔP: Increase in pressure (atm).
- V_l: Free volume (L).
- V_{CH4}SC: Volume of CH4 standard conditions (L).
- T_{SC}: Temperature at standard conditions (K).
- P_{SC}: Pressure at standard conditions (atm).
- H_{CH4}: Henry constant for CH4 (atm).
- $X_{CH4dissolved}$: Molar fraction of CH4 dissolved.
- $M_{CH4dissolved}$: Molar concentration of dissolved CH4 (mol / L).
- M_{H2O}: Molar concentration of water (mol / L).
- R: Constant of ideal gases (atm*L / K * mol).
- Te: Temperature of the experiment (K).
- $n_{CH4displayed}$: Moles of CH4 dissolved (mol)
- V_u: Useful reactor volume (L)
- $V_{CH4dissolved}$CE: Volume of dissolved CH4 (L).
- V_{TCH4}SC: Total volume of CH4 standard conditions (L).
- V_{CH4} control: Volume of methane produced by the inoculum without substrate (L).
- PBM: Methane Biochemical Potential (L /gSV).
- gSV: Initial volatile solids (SV) of the substrate (gSV).

In order to statistically determine whether there is a significant difference between the means of biochemical methane potential of each of the two treatments, a completely randomized experimental design was performed and the statistical analysis of variance (ANOVA) test was applied at 95 percent confidence where the independent variable was biochemical potential, the factor was treatment and anaerobic reactors constituted the experimental units.

Model:
$Y_{ij} = u + T_i + E_{ij}$
Y_{ij} = Biochemical Methane Potential obtained by applying the i^{th} treatment in the j^{th} reactor
u = Biochemical Methane Potential
T_i = Effect of the i^{th} treatment
E_{ij} = Experimental error obtained by applying the i^{th} treatment in the j^{th} reactor
Variance analysis:
H_0: the PBM averages of each treatment are the same
H_1: At least an average of one from the treatments is different.

Finally the comparison was done between the averages using the "T" test

III. ANALYSIS AND DISCUSSION OF RESULTS

3.1 Physicochemical characterization

For this research a primary characterization of vinasse was determined according to the standard methods and international official methods of analysis (AOAC). The above mentioned properties summarize in the Table 2.

Table 2. Physicochemical characteristics of vinasse

Parameters	Value
Total Solids (%)	8.2
Total ashes (%)	3.99
Raw Fat (%)	1.33
Total Nitrogen (mg / L)	2.35
Raw Fiber (%)	1.97
pH	4.92
Electrical conductivity (mS / cm)	36.7
Brix degrees (° Bx)	10

The vinasse analyzed as shown in Table 2 is 10°Brix diluted vinasse. There is also an appreciable amount of nutrients such as nitrogen that comes from the yeasts used which, due to their value, can be used as an animal supplement, minerals that are represented by the ash content of 3. 99% coming from the sap of the plant composed mainly of potassium and magnesium that can be used as amendments and fertilizers in the soil of sugarcane crops that, in spite of obtaining good yields, are limited due to the low pH value. The low solids content of 8.2% also turns out to be an impediment when transporting the vinasse for its use.
A new characterization was made for the BMP test. The physicochemical properties of both the substrate (sugarcane rum vinasse) and the inoculum (cow manure) were determined, the results of which are presented in Tables 3 and 4.

Table 3. Physical-chemical characterization of the substrate (cane rum vinasse)

Parameters	Units	Value
Hydrogen potential	--	4,85
Electric conductivity	dS/m	30,30
Total solids	g/L	89,98
Volatile solids	g/L	55,34
Fixed solids	g/L	34,64
Chemical Oxygen Demand	mg/L	33450,00
C / N ratio	--	4,49
C Total	mg/L	3209,00
N Total	mg/L	714,00
N Amoniacal	mg/L	644,00
P Total	mg/L	553,69
K Total	mg/L	5175,00
Ca Total	mg/L	5250,00
Mg Total	mg/L	2700,00
Na Total	mg/L	735,00
Pb Total	mg/L	1,05
Cd Total	mg/L	N.D.
Cr Total	mg/L	N.D.
Fe Total	mg/L	81,90
Cu Total	mg/L	1,50
Zn Total	mg/L	7,15
Mn Total	mg/L	6,00
B Total	mg/L	2,96

Note: N.D. (Not detectable)

Table 4. Physicochemical characterization of inoculum (cow manure)

Parameters	Units	Value
Hydrogen potential	--	8,22
Electric conductivity	dS/m	3,39
Humidity	%	84,99
Total solids	%	15,01
Volatile solids	%	12,22
Fixed solids	%	2,79
C / N ratio	--	24,60
Total Alkalinity	mg $CaCO_3$/L	3520,00
C	%	46,74
N	%	1,90
P2O5	%	1,44
K2O	%	0,83
CaO	%	2,22
MgO	%	1,32
Na	%	0,10
Fe	ppm	2020,00
Cu	ppm	58,00
Zn	ppm	220,00
Mn	ppm	188,00
B	ppm	16,00
Pb	ppm	6,00
Cd	ppm	N.D.
Cr	ppm	N.D.

Note: N.D. (Not detectable)

3.1.1 Hydrogen Potential (pH) and Total Alkalinity

According to Bautista, the pH represents the degree of acidity present in the bio digester; its optimum value is between 6.6 and 7.6 [4]. With pH values below 5 and above 8 there is a risk of inhibiting or even stopping the process. It is important to keep this parameter constant because the activity of the methanogenic population is highly vulnerable to pH changes compared to the other populations present in the sludge [Quintero and Rondón, 2012]. Martí points out that if the pH were less than 6 it would produce a biogas poor in methane, which has lower energy qualities [12]. As can be seen in the results, vinasse has a pH of 4.85 units while cow manure has a pH of 8.22 units. It is important to note that the acid character of the vinasse is balanced with the basic character of the manure, reaching a pH close to 6 during mixing in the proportions determined for the treatments proposed.

Many authors claim that anaerobic degradation is more efficient at pH values close to neutrality, however, different studies on the influence of pH indicate that it can´t be generalized, due to aspects such as the physicochemical characteristics of the substrate that can provide buffer capacity since each microbial group involved in anaerobic degradation has a specific optimum pH range[17].

On the other hand, the total alkalinity presented by the manure was 3520 mg $CaCO_3$ / L. Previous studies have shown that values of bicarbonate alkalinity greater than 2500 mg / L ensure good pH control [12].

3.1.2 Electrical Conductivity (EC)

The vinasse presented a value of electrical conductivity of 30.3 dS/m, whereas the manure presented a value of 3.39 dS/m. The conductivity of the vinasse is high in comparison to that of the manure, which suggests a high salts contained in the vinasse. It is important bear in mind that the free ions are going to influence the osmotic pressure of the medium; the most saline medium can provoke the dehydration and later death of the bacteria [16].

3.1.3 Total solids (TS) and volatile solids (VS)

In the case of vinasse, there is a very high concentration of TS (89.98 g/L), likewise VS (55.34 g/L) indicate that approximately 60% of this effluent contains organic matter (VS/ TS = 0.61). For the case of manure is 84.99% moisture, 15.01% TS and 12.22% VS, i.e. approximately 80% of dry matter is organic (VS/ TS = 0.81).

A high VS/TS relationship confirms the high content of biodegradable organic matter and a low relationship is related to the plant content of difficult degradation, lignin, so it is considered that the vinasse is suitable for biodegradation [17].

3.1.4 Chemical Oxygen Demand (COD)

The COD of the vinasse was 33 450 mg / L. When comparing the concentration in organic matter of the vinasse with that of the urban black waters, between 500 to 1000 mg / L, it turns out to be thirty times greater. It should be noted that the high organic load of an effluent indicates it as a potential substrate for anaerobic degradation.

3.1.5 Carbon-Nitrogen Ratio (C / N)

According to Soria *et al*,the optimal relationship of C/N is 30:1 for the growth of microorganisms; when the relationship is very narrow (10:1) there are losses of assimilatable nitrogen, which reduces the quality of the material digested. If the relationship is very broad (40:1) the growth is inhibited due to lack of nitrogen [19].

Al Seadi *et al* recommend a relationship C: N: P: S of 600:15:5:1. Vinasse reported a C/N ratio of 4.49 while manure a ratio of 24.6, this indicates that there is a high amount of nitrogen in the vinasse[Seadi *et al* (2008)]. The total nitrogen concentration in the vinasse was 714 mg/ L; also a concentration of ammoniacal nitrogen of 644 mg/L was detected. Experience indicates that inhibition of the anaerobic digestion process can occur at a total concentration of ammoniacal nitrogen from 3000 to 3500 mg/L, so that no problem is expected to occur depending on the amount of nitrogen [9]. On the other hand, it is also stated that the concentration range of ammonia that causes inhibition depends on the different substrates, inoculums, environmental conditions (example: temperature and PH) and acclimatization periods [21]. Likewise, certain ions such as sodium, calcium and magnesium were found antagonists of inhibition by ammonia the latter were found in high concentrations in the vinasse [7].

3.1.6 Metals

According to Chen *et al*, various thresholds toxicity have been established for metals such as calcium, magnesium, sodium and potassium. Likewise, there are antagonistic and synergistic relationships between them, so that these interrelations reach different equilibriums for each particular case [7].

In the case of heavy metals, they have an inhibitory effect when they interfere with the enzymatic system of organisms; however, low concentrations of certain heavy metals are necessary for microbial activity. Cobalt, molybdenum and nickel are heavy metals important for the activity of the methane producers and their enzymes [18]. It is difficult to say that heavy metal concentrations cause inhibition and which metals are toxic because many of the results reported in the literature vary considerably. The range is broad in terms of threshold concentrations for several of the heavy metals, but is in order of 100 mg/L. Some metals such as iron, however, are relatively non-toxic and may appear in the process at concentrations of hundreds of grams per liter without causing any problems. A good explanation for this is that metals bind to different organic compounds (chelates) in the process or form precipitates such as sulphides. The level at which inhibition occurs is even affected by the fact that different combined metals can have antagonistic and synergistic effects between the two [18].

Vinasse presented medium to high concentrations of calcium, magnesium, potassium and sodium. On the other hand, metals such as copper, zinc, boron and lead were detected at very low concentrations. In the case of cadmium and chromium, these were not detected in the samples.

In the case of bovine manure similar patterns were presented in the concentrations of metals in the samples.

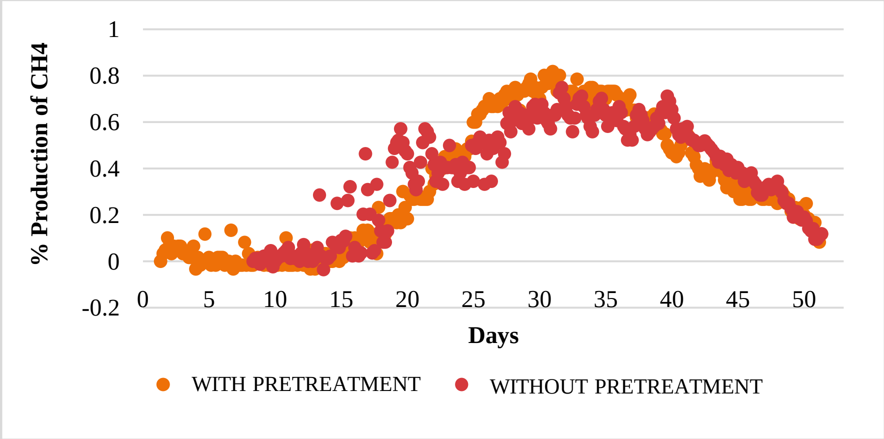
3.1.7 Biochemical potential of methane (PBM)

The monitoring began on October 5, 2017 and stopped on the 24th of November of 2017, that is to say it lasted 51 days. The Oxitop ® Control system performed automatic measurements each 4 hours at 9:30, 13:30, 17:50, 21:30, 01:30 and 05:30 hours.

The results of the average volume of methane generated every 4 hours (in percentage) are shown in Figure 4. It is observed that the highest percentage of methane (more than 70%) occurred between the days 20 to 45, this is due to the microorganisms first require a period of adaptation to the substrate (between days 1 and 20 approximately), after this stage is carried out the Biodegradation of the majority of the substrate (for approximately 20 days) and finally a decline in methane production begins (from the day 45) because the amount of substrate with which the microorganisms had been consumed considerably. It should be noted that the time of adaptation of the inoculum was relatively prolonged because it was prepared from fresh bovine manure. According to the revised bibliography, fresh manure does not show a very high specific methanogenic activity (SMA) compared to other inoculums such as sludge from wastewater treatment plants, or UASB reactor sludge, which they can reach AME of up to 0.2 or 0.5 g cod/g SSV * day this is due to the latter are already specialized in anaerobic degradation of organic matter [15].

It should also be mentioned that the temperature at which the assay was carried out (30 °c) was chosen because the microorganisms that perform anaerobic digestion achieve optimal methane yields at mesophilics temperatures, approximately between 37 to 43 °c, and thermophilic, approximately between 50 to 60 °C at lower temperatures it declines the rate of growth and activity of the methanogens [9].

Figure 4 Average variation of methane production each 4 hours



The results of accumulated volume of methane in milliliters per gram of volatile solid both for the experimental units that went through a pretreatment and for those that did not pass through this were evaluated statistically using a Completely Randomly using the Minitab software. Before performing the analysis of variance (ANOVA), the assumptions of normality of errors and the homogeneity of variances were verified, being fulfilled both in the two treatments. The analysis of variance at 95% confidence verified that the means of the two proposed treatments are not the same. Finally, the Student's "T" test was used to conclude that at 95% of confidence there is enough statistical evidence to affirm that treatment B produces a greater biochemical potential of methane than treatment A. Being treatment B the one that went through A pre-fermentation phase during 4 days.

Before presenting the results it is important to note that the targets generated an average volume of 200 ml of CH_4/g SV of inoculum, this gas is the product of residual organic matter content in the inoculum, such “endogenous” methane was subtracted from the results of each one of the reactors for the calculation of the BMP in order to evaluate the methane production potential only from the substrate.

Likewise, it stands out that statistically significant difference between the two treatments shows that the ratio of 0.3 g SV substrate/g SV inoculum is effective for the determination of the methane production potential of the vinasse of cane rum as the production of biogas was not underestimated, in the opposite case, i.e. when using a higher substrate-inoculum ratio, it was possible not to provide enough microorganisms to degrade the amount of substrate used and both treatments would have presented probably similar results.

Figure 5 shows the accumulated methane volume in milliliters per gram of average volatile solid for the two treatments proposed. Also, table 5 shows the BMP calculated in ml CH_4/g SV substrate and ml CH_4/g COD substrate depending on these results.

Figure 5. Biochemical Methane Potential (BMP) calculated for each treatment.

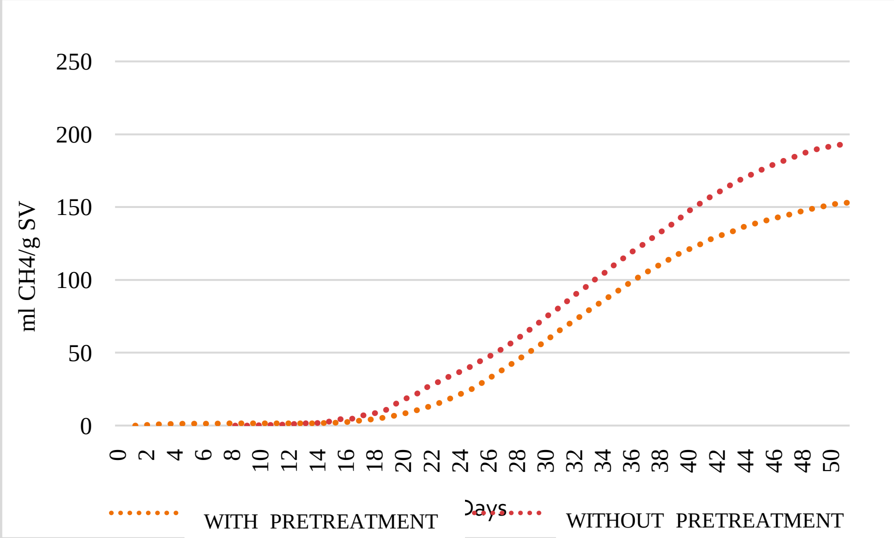


Table 5. Results of Biochemical Methane Potential

Treatment	BMP (ml CH4 / g VS substrate)	BMP (ml CH4 / g COD substrate)
A (one phase)	153,0	253,2
B (two phases)	193,5	320,2

According to Figure 5 and as it was statistically verified, the PBM, i.e. the cumulative volume of methane in milliliters per gram of volatile solid substrate (ml CH_4/g SV substrate) for the experimental units that passed through the pretreatment produced a greater methane volume than those that did not pass by the pre-acidification or anaerobic digestion of two phases. This behavior can respond to several factors, as detailed below.

According to Parra anaerobic digestion involves several species of symbiotic microorganisms that can be divided into two groups: Acidogenics and Methanogenics [17]. These two groups differ considerably in their physiology, kinetics and growth requirement. Operations in two separate stages would allow the optimization of conditions for each of the two groups of microorganisms and an increase in efficiency in the processes. Two-stage anaerobic treatment is the most available for wastewater containing high levels of organic solids. COD elimination rates and methane production in two-stage reactors was 116 and 43 percent (respectively) higher than those in a single-stage unit [Hassan & Nelson, 2012].

According to Montgomery and Bochmann enzymes that degrade cellulose, hemicellulose and starch work better at PH between 4 and 6 at temperatures between 30 and 50 °C, therefore, a pre-acidification stage increases the rate of degradation creating a suitable environment for Enzymes. Another positive effect occurs because in addition to H_2 and volatile fatty acids, CO_2 is formed during the pre-acidification stage. CO_2 can be presented in three forms: a high pH values is in the form of ion carbonate CO_3^{-2}, pH neutral as HCO_3-and pH acid as CO_2, which is volatile and released in the gas produced in hydrolysis. This means that there will be less CO_2 in the gas produced in the methanogenic stage and therefore higher CH_4 concentration. Finally, another advantage is that the microorganisms of the hydrolytic phase are less sensitive to many chemicals (such as phenols, ammonium, etc.) than the microorganisms of the methanogenic stage, in this way many chemical inhibitors can be destroyed in the first stage [13].

IV. CONCLUSIONS

- The vinasse product of the cane rum distillery is a substrate susceptible to degradation through the anaerobic digestion process.
- In a completely randomized experimental design (CRD) to a 95% confidence there is sufficient statistical evidence to affirm that treatment B produces a greater biochemical potential of methane than treatment a. Being both treatments for vinasse of cane rum as a substrate and bovine manure as inoculum, however, treatment B was developed through a two-stage anaerobic digestion.
- A biochemical potential of methane (PBM) of 153.0 ml CH4/g SV substrate (253.2 ml CH4/g COD substrate) was determined for treatment A and PBM of 193.5 ml CH4/g SV substrate (320.2 ml CH4/g cod substrate) for treatment B.

- The ratio of 0.3 g SV substrate/ g SV Inoculum is effective for the determination of the methane production potential of the vinasse of cane rum since methane production was not underestimated.
- Optimized conditions for each of the two groups of microorganisms responsible for anaerobic degradation (hydrolytic-Acidogenics and Methanogenics), for the treatment of cane rum vinasse in a two-phase process.
- A maximum energy potential of 0.118 Kw-h was calculated for each liter of vinasse of cane rum.
- The anaerobic digestion process for the case study is expected to be more efficient over time as the microbial populations specialize in the degradation of the substrate.
- It is recommended to use a two-stage anaerobic digestion process for the treatment of vinasse of cane rum due to its high concentration of organic matter and its acid character.
- It is recommended to use the ratio equal to 0.3 g SV substrate/ g SV Inoculum for the start of an anaerobic reactor that uses vinasse of cane rum and bovine manure as inoculum.
- It is recommended to test the process of anaerobic digestion of the vinasseof cane rum at a real scale.

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