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## Biogas Production Potential from Native Beverage Vinasse of Mexico

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**Abstract** - This work presents the results of the physicochemical characterization and evaluates the potential of vinasse (a wastewater obtained from local beverage of Chiapas, Mexico) as nutrient source for biogas production by anaerobic digestion in an Upflow Anaerobic Sludge Blanket (UASB) bioreactor. This wastewater contains high level of organic matter,  $120.2 \pm 18.4 \text{ g} O_2/\text{L}$  of chemical organic demand (COD), 0.77 biodegradability index (BI), and a ratio 21:1 of C/N. The UASB bioreactor had a working volume of 4 L, at 32 °C, 10 days of hydraulic retention time (HRT). The COD removal was of 91 %, the production of methane was 1860 mL/day and 264.83 mL CH<sub>4</sub>/gCOD of biochemical methane potential (BMP).As such, the UASB bioreactor fed with vinasse can be sustainable alternative for the bioenergy production from wastewater, shifting the paradigm of wastewater management from 'treatment and disposal' to 'beneficial utilization' as well as 'profitable endeavor'.

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## 1. Introduction

The wastewater of ethanol distillation (also called vinasse) are produced and discharged without treatment directly in surface water (rivers, streams or lakes) or even for the direct irrigation of crops, however vinasse disposal into the environment is hazardous and has a considerable pollution potential. Its high COD of approximately 24,500-120,000 mg/l [1] and a high quantity of dissolved organic carbon (10,000 - 14,000 mg/l approx.) [2], means that its disposal into natural water bodies can cause their eutrophication (a gradual increase in phosphorus, nitrogen and other nutrient concentration in an aquatic ecosystem). which induces a great increase in the concentrations of algae and microorganisms at the surface, avoiding the solar light passage and decreasing the oxygen dissolved in the water bodys until causing anoxia and death of aquatic life. However, due to their origin they contain a great amounts of biomass, organic matter, nitrogen, sugars, among other nutrients[3,4], able to produce biogas by anaerobic digestion in bioreactors UASB, which they have a low cost of implementation and maintenance, reduced excess sludge production, low energy consumption and simplicity in operation [5,6].In Comitan-Chiapas, Mexico, typical alcoholic beverage called "Comiteco" was produced by *Agaveamericana L.* distilled [7], the process of elaboration consists of four stages, i.e., Incision of agave hearts, mead collection of the incision of living plant, must fermentation and distillation/rectification. In the distillation/rectification stage, between 15 and 23 L of distillery slops of vinasse are generated for each liter of Comiteco distilled. The principal object of this work is evaluation of vinasse from "Comiteco" and to establish if this residue has potential energy to produce biogas through UASB reactor.

## 2. Materials and Methods

## 2.1 Vinasse characterization

Vinasse was provided by the company Balun-Canan S.A. de C.V. located in Comitan, Chiapas, México, which stored this vinasse at temperatures of 4 °C until its use.Vinasse was analyzed accordingto the Standard Methods for Examination of Water and Wastewater [8]. The parameters measured were: pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD), suspended solids (SS), total solids (TS), total volatile solids (TVS), total

suspended solids (TSS), total suspended solids volatile (TSSV), Total dissolved solids (TDS), density, conductivity, acidity, nitrogen, phosphorus and sulfates. The total organic carbon (TOC) was determined by the method of Walkley and Black [9], total phenols were determined by spectral analysis using the Folin-Ciocalteau reagent [10], reducing sugars were determined by 3,5dinitrosalicylic acid method (DNS) [11]. High Performance Liquid Chromatography (HPLC) Perkin Elmer 200, a refractive index detector and column Hi-Pex H<sup>+</sup> was used to quantify sucrose, glucose, acetic acid and ethanol. Water was used as a mobile phase, and a flux of 0.3 mL/min at 30 °C. Quantitation was performed using an external standard. All the analysis were made by triplicate

### 2.2 UASB Bioreactor

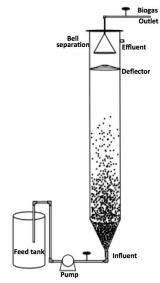


Fig. 1. UASB bioreactor

UASB bioreactor (Figure 1) had volume of 4L operation with cylindrical shape, a height-diameter ratio  $(H/\phi)$  of 5 and HRT of 10 days. The bioreactor with 1L of anaerobic microbial complex was inoculated 2.52 gTS/mL (from a wastewater plant, San Cristobal de las Casas-Chiapas, Mexico) previously adapted. During this period the following parameters were monitored in the bioreactor: pH (potentiometer HACH model SenSion 3), COD (Standard Methods, [8])and volume of methane production.

## 2.3. Methane production and Biochemical methane potential

Theoretical production of methane should be calculated with conditions of temperature, under atmospheric pressure which SMA (specific methanogenic activity) mounts are made, you must determine the correction factor [12].

Correction factor for temperature and pressure is calculated using Equation (1) [13]:

$$K(T) = \frac{P * K}{R * (273 + T)}$$
 Eq.(1)

Where K (T) is the correction factor (g COD/ L), P is atmospheric pressure (atm), R is the gas constant (0.08206 atm\*L /mol\* K), K is the corresponding organic charge to a mol CH<sub>4</sub> (64 g COD/mol, that is, 350 mL of CH<sub>4</sub> per gram of COD removed) and T is the temperature of the assembly (°C). Theoretical volume of methane was calculated with Eq. (2):

$$V_T = \frac{COD_{CH_4}}{K(T)} \qquad Eq.(2)$$

Where K (T) is the correction factor (g COD/L), the  $COD_{CH_4}$  is COD charge removed (gCOD), the  $V_T$  is theoretical volume produced (L). Considering methane (CH<sub>4</sub>) production, SMA calculation was made [14]:

$$SMA\left(\frac{gCOD}{gTVS*day}\right) = \frac{m*24}{V_T*M}$$
 Eq. (3)

Where m is the maximum gradient in methane production curve (vol. accumulated  $CH_4$  vs time), M is the mass of sludge (g), the theoretical volume  $V_T$  is produced (L) and 24 is the correction factor for a day.

The technique used for measured of methane production was adapted Raposo et al. [15], this consisted in preparing a solution of 5 % NaOH and phenolphthalein, then added in a test tube and the solution was poured to the crystallizer, where the graduate cylinder was placed inverted.

Technique reported by Harris [16] was used for analyzing the composition of biogas quantifies  $CO_2$  and  $CH_4$ . The BMP was determined from the equation 4 [17]. The accumulated volume of methane was converted to standard conditions for temperature and pressure. The accumulated methane was plotted versus time kinetics until the speed was constant. On this last point constant speed is where the value of BMP (mL CH<sub>4</sub>/gCOD) took place.

$$BMP = \frac{V_{CH_4}}{OM} \qquad Eq. (4)$$

Where BMP (mL CH<sub>4</sub>/gCOD) is the biochemical methane potential,  $V_{CH_4}$  is the methane-accumulated volume (mL CH<sub>4</sub>) during the experiment in standard conditions for temperature and pressure, OM is the organic matter (gCOD).

The percentage of biodegradability is obtained from equation 5[17].

$$BI(\%) = \frac{BMP}{350 \, mL \, CH_4/g \, COD} x \, 100 \qquad Eq. (5)$$

Where 350 is the theoretical volume of methane per gram of COD removed at normal temperature and pressure (T =  $273^{\circ}$ K; P = 1 atm).

## 3. Results and discussion 3.1 Physical characterization

The biochemical characteristics of Comiteco vinasse are important to highlight because provide a better growth and microbial activity of anaerobic system The physicochemical characterization of vinasse of Comiteco was done in accordance with the requirements of NOM-001-ECOL-1996 Mexican official standards are shown in Table 1.

Table 1. Physicochemical characterization of vinasse of Comiteco

Parameter	Comiteco's Vinasse
рН	3.895 ± 0.013
Acidity (mg CaCO $_3$ /L)	79.33 ± 5
Density (g/mL)	$1.0086 \pm 0.02$
Conductivity (mS/cm)	$4.2 \pm 0.05$
COD (gO <sub>2</sub> /L)	120.221±18.447
BOD(gO <sub>2</sub> /L) Total solids (g/l) Total volatile solids (g/L) Total suspended solids (g/l)	102.180 71.69 ± 0.2 62.9 ± 0.17 9.2 ± 0.1
Total suspended solids volatile (g/L)	$8.8 \pm 0.08$
Total dissolved solids (g/l) TOC (mg/L) Total phosphorus (mg/l) Total nitrogen (mg/l) Brix (°Brix)	$62.5 \pm 93.1$ $32,809 \pm 1500$ $723 \pm 50$ $1600 \pm 150$ $6 \pm 1$
Reduction sugar (mg/L)	8.243± 0.96
Sucrose (g/L)	7.44± 1.14
Glucose (g/L)	$3.02 \pm 0.25$
Acetic acid (g/L)	15.14 ± 3.2
Ethanol (g/L) Total sulfates (g/L) Competitiveness index Total phenols (mg Gallic acid/L)	6.44 ± 0.95 3499.14 ± 344.3 30 476.6 ± 84

Microbiological process requires mainly of carbon and nitrogen sources and mineral salts (phosphorus and sulfur), all organic material are able to produce biogas by an anaerobic digestion [18]. The stillage of Comiteco possess of a lot of organic material of BOD (102,000  $gO_2/L$ ) and has a high biodegradability level (BI), it indicates the percentage of organic material able to be biodegradable [19]. It can be calculated according to the formula BOD/COD or BMP/350 [20], thus has a BI of 0.79 (BOD/COD) and 0.75 (BMP/350), hence 77% in vinasse is organic material biodegradable and can be produced biogas by anaerobic digestion.

The quality and quantity of produced biogas depend on the composition (C / N and S) and the nature of the residue used [21].In the feedstock, optimum ratio of these elements are in rate of C/N 30:1 to 15:1 [22] so vinasse of Comiteco had a ratio 21:1 of C/N being a suitable substrate for methanogenic archaea and efficiently to carry out anaerobic digestion.Archaea Microbial consume more carbon than nitrogen, these elements are the principal sources of energy of methanogenic archaea [23].

Another factor that we need to consider is competitiveness index, is calculated according the  $COD/SO_{4}$ -<sup>2</sup> formula. If index are more than 10 there are not competition between the sulfate-reducing bacteria and methanogens [24]. In spite of sulfuric acid or ammonium sulfate is used in the process of fermentation broth, Comiteco's vinasse has a ratio of 30 so there is inhibition by the sulfate-reducing bacteria, by providing methanogenesis this substrate.

A quite considerable amount of reducing sugars in vinasse of Comiteco was determined (8.24 g/L) because this vinasse was originated by fermentation of must by added honey mash and honey brown sugar as a substrate for yeast. Glucose 3.02 g/L and sucrose was found in a concentration of 7.44 g/L by HPLC analysis. This feature provides a high source of carbon to be used by microorganisms in anaerobic digestion and carry out the production of biogas. Sucrose, glucose, fructose, ethanol (6.44 g/L) and acetic acid (15.14 g/L) have not adverse impact on anaerobic digestion. Because they are easily assimilated by microbial consortium [25], as it is shown in Table 2.

Table 2. Biochemical reactions in anaerobic digestion of organic matter at 25°C.

Reactions	$\Delta G^0$ (kJ/mol)	Eq.
$C_{12}H_{22}O_{11} + 9H_2O \rightarrow 4CH_3COO^- + 4HCO_3^- + 8H^+ + 8H_2$	- 475.5	(3.1.1)
$C_{12}H_{22}O_{11} + 5H_2O \rightarrow 2CH_3CH_2CH_2COO^- + 4HCO_3^- + 6H^+ + 4H_2$	- 554.1	(3.1.2)
$C_{12}H_{22}O_{11} + 3H_2O \rightarrow 2CH_3COO^- + 2CH_3CH_2COO^- + 2HCO_3^- + 6H^+ + 2H_2$	-610.5	(3.1.3)
$C_6H_{12}O_6 + 4H_2O \rightarrow CH_3COO^- + 4H^+ + 4H_2$	-207	(3.1.4)
$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2CH_2COO^- + 2HCO_3^- + 3H^+ + 2H_2$	-135	(3.1.5)
$CH_3CH_2COOH + 2H_2O \rightarrow 2CH_3COO^- + H^+ + H_2$	+ 48.2	(3.1.6)
$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$	+ 9.6	(3.1.7)
$CH_3COOH + H_2O \rightarrow CH_4 + HCO_3^- + H^+$	-31	(3.1.9)

Adapted from van Lier [28].

The anaerobic process is affected by pH. The optimal pH is between 5.5 and 6.5 for acidogenic [26] and between 7.8 and 8.2 for methanogenic. The optimal pH for mixed

cultures is range of 6.5 - 8.2 [27]. Stillage of Comiteco pH has 3.9 which does not favor to the process of anaerobic digestion, it was necessary neutralize with NaOH,Ca(OH)<sub>2</sub>or NaHCO<sub>3</sub> before feeding the bioreactor.

However according to the physicochemical profile vinasse of Comiteco has an excellent BI and nutrient content able to be assimilated by anaerobic microorganisms, it just the removal of organic matter, the COD would decrease it would increase the pH and a bioenergetic called biogas is generated.

### 3.2 COD removal

Figure 2 shows the behavior of the UASB bioreactor during the evaluation period of 56 days, where it can be observed that the bioreactor operated stably throughout the evaluation period, reaching a percentage of removal greater than 90%. However, even when the removal rate was high, COD effluents with 6500 mg/L were generated, This effluent still does not meet the permitted limit requested by the world health organization (WHO)[29], so it is recommended because of that a second anaerobic treatment is recommended to meet the allowable limit.

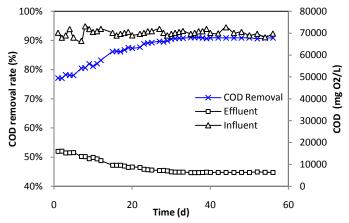


Fig. 2. UASBbioreactor performance.

## 3.3 Methane production

Biogas is a mixture of gases produced in the anaerobic reactor. The only economic value gas with biogas is methane. Methane can be used as a fuel, is a natural flammable gas is odorless and burns cleanly (3.31).

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$$
 (3.31)

The reactor UASB was feed with Comiteco vinasseand obtained 1860 mL of (CH<sub>4</sub>) methane per day, the results are shown in Figure 3.Using eq. 1 the value of K (t) is 2.39 gCOD/L, this value was substituted in eq. 2, to correct the theoretical volume of methane (V<sub>T</sub>), which was 2.6714 L of CH<sub>4</sub> was obtained, this value is considering that all COD removal was converted to methane, however the UASB bioreactor generated biogas with 67 % methane composition, so that multiplying  $V_T$  by the methane production ( $V_T * (\%_{CH_4}) \cong V_{CH_4}$ ) 1790  $\cong$ 1860 mL CH<sub>4</sub>.

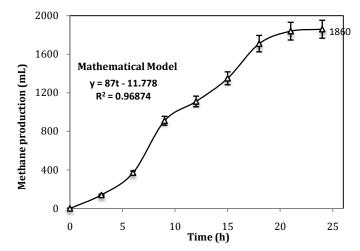


Fig 3. Kinetic methane production

To calculate the SMA, the first step was calculated the slope of the mathematical model of methane production (Figure 3), which was obtained by differentiating the function.

Then the values of m,  $V_t$  and M were replaced in eq. 3 to calculate SMA. The amount of SMA was 0.31 (g COD)/(grVSS\*day) for the inoculum used, the range 0.1 to 1.5 (g COD)/(grVSS\*day) is reported by [30-32]. The value of SMA was determined as reported by Torres-Lozada and Pérez [14].

Figure 4 shows the profile of methane production from comiteco vinasse. The curve obtained possible to determine the BMP and biodegradability of these.

The numerical value of BMP vinasse is determined from equation 4. The value obtained was 264.83 mL CH<sub>4</sub>/gCOD. This value is similar to the reporter byJanke et al. [33] and Leite et al. [34] (220-302 mL CH<sub>4</sub>/gCOD). The anaerobic biodegradability was determined from equation 5, In the mesophilic range the percentage of biodegradability of the comiteco vinasse was 75%, this value corroborates the biodegradability index calculated by the BOD/COD.This value is greater than reported by Robles-González et al. [3] and Moraes et al. [35] (40-50 %). Based in BMP and biodegradability of comiteco vinasse, this residue showed for satisfactory biogas potential for energy production.

Pure methane has a calorific value of  $37,259 \text{ kJ/m}^3$  (1000 Btu/ft<sup>3</sup>). When methane is mixed with carbon dioxide and other gases produced in the digestion, the calorific value decreases significantly. Biogas with greater concentration than 45% methane is flammable.Methane production by UASB reactor contained 67% of methane concentration (CH<sub>4</sub>) and 33% of dioxide of carbon (CO<sub>2</sub>); these yields are similar described by Deubleinn and Steinhauser [36], (55-70 of CH<sub>4</sub>% and 30-45% of CO<sub>2</sub>).

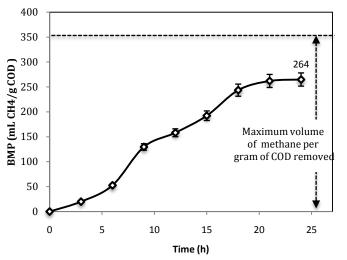


Fig. 4. Kinetic biochemical methane potential

#### 4. Conclusions

Vinasse of Comiteco can be considered as biodegradable effluents (BI of 77%) with a high potential to be used as a nutrient source (substrate) has a high competitiveness index, does not present inhibition by the sulfate-reducing bacteria, andit has an excellent nutrient content able of being assimilated by microorganisms, for biogas generation by anaerobic digestion in an upflow anaerobic sludge blanket reactor (UASB). The COD removal was 91 % and biogas produced of 1860 mL/day, is [13] flammable and the composition of biogas in the reactor is 67% of  $CH_4$  and 33% of  $CO_2$ , so it can be considered that [14] biogas obtained had a close energy power to 6,000 kcal/m<sup>3</sup> and biochemical methane potential of 264.83 mL CH<sub>4</sub> per gram of COD removed. As such, the UASB bioreactor fed with vinasse can be sustainable alternative for the [15] bioenergy production from wastewater, shifting the paradigm of wastewater management from 'treatment and disposal' to 'beneficial utilization' as well as 'profitable endeavor'.

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