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Research Article

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Anaerobic Treatment of Vinasse from Sugarcane Ethanol Production in Expanded Granular Sludge Bed Bioreactor

Florencia Ramos-Vaquerizo[1], Abumalé Cruz-Salomón[1,2*], Edna Ríos-Valdovinos[1], Francisco Pola-Albores[1], Selene Lagunas-Rivera[3], Rocío Meza-Gordillo[2], Víctor M. Ruíz-Valdiviezo[2], Roel Simuta-Champo[4] and Joel Moreira-Acosta[1]

[1]*Institute for Research and Innovation in Renewal Energies, University of Science and Arts of Chiapas, Chiapas, México*
[2]*Department of Chemical and Biochemistry Engineering, National Institute of Technology of Mexico, Chiapas, México*
[3]*Professor CONACYT, Department of Chemical and Biochemistry Engineering, National Institute of Technology of Mexico, Chiapas, México*
[4]*Faculty of Engineering, University of Science and Arts of Chiapas, Chiapas, México*

Abstract

The sugarcane vinasse cause an environmental problem, due they do not a previous treatment and are discharged directly into the environment. For this reason, the main aim of this project was to evaluate the anaerobic digestion in expanded granular sludge bed (EGSB) bioreactor as a sustainable alternative for the treatment of sugarcane vinasse. In addition, the production of biogas and biochemical methane potential (BMP) were determined. Vinasse was characterized physico-chemically and its parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), pH, metal ions, among others were evaluated. Simultaneously, an anaerobic inoculum was acclimated in a batch bioreactor to assimilate the vinasse during 75 days. After that, the EGSB bioreactor was started-up with the previously acclimated inoculum and evaluated to determinate the BMP, biogas production and COD removal. The EGSB bioreactor was operated with a hydraulic retention time (HRT) of 7 days under stable conditions (i.e., alkaline factor of 0.24, pH 7.2 and temperature 26°C) for 140 days, generating a maximum chemical oxygen demand (COD) removal efficiency close to 75%, biogas production contained 76% methane concentration which generates a calorific power of 6507 kcal/m^3 with an average methane production of 4.2 L of CH_4/day and BMP of 244.64 mL CH_4/gCOD. According to the obtained results, the anaerobic EGSB bioreactor can be a solution to the environmental challenges that this agro-industry confronts, since could be a sustainable alternative to simultaneously solve the environmental pollution caused by vinasse and produce bioenergy for the operation in the same agro-industry.

Keywords: Anaerobic digestion; Anaerobic EGSB bioreactor; Microbial adaptation; Methane; BMP

Introduction

The sugar and alcohol industries in Mexico have a long historical tradition and they have become one of the most important agro-industries in the country. Currently, they cultivate approximately 650 thousand hectares of sugarcane, which are used for the production of sugar and alcohol ethyl. Alcohol is obtained from the transformation of final honeys (molasses) as raw material; the annual production reaches more than 50 million liters of alcohol per year with yields up to 250 liters of alcohol/ton of molasses [1].

Nowadays, alcohol industries are facing a real problem with the generation of their effluents [2], which also called vinasses (the main liquid stream from the first-generation ethanol production process). The major environmental problem is because in the sugarcane processing plants generally generate from 8 to 15 L of vinasse per liter of produced ethanol [3,4] (Figure 1) so presently in Mexico between 400-750 million liters of vinasse per year are produced

Vinasses are characterized as an effluent with a high pollution potential, containing high levels of organic compounds and nutrients (mainly potassium but also nitrogen and phosphorous) [5]. They are derived from the ethanol distillation step, leaving the columns at a temperature in the range of 85-90°C. Generally, they contain high concentrations of dissolved solids; up to 50% of this parameter can be reducing sugars [5,6], non volatile compounds coming from the fermentation broth, phenolic and polyphenolic compounds, relatively excessive concentrations of mineral salts and ash [3]. Vinasses are acid with a pH that usually ranges from 3.5 to 5.0, dark colored (brownish, attributed to the presence of melanoids) and they present high electrolytic conductivity (250-300 dS/m). The organic load is very high with extremely elevated values of biochemical oxygen demand (35,000-50,000 mg O_2/L) and chemical oxygen demand (70,000-150,000 mg/L) [3,4,6]. Biodegradability indices in the range 0.2–0.7 mg BOD/mg COD are very common [3,5,7].

Vinasses are very aggressive and recalcitrant effluents, whose they are direct discharge to water bodies and soil may cause severe environmental impact (Figure 2) like salinity, sodicity, phytotoxicity, anoxia, eutrophication, death of aquatic life, and many severe health problems [8].

In addition, these agro-industry to consuming large amounts of water, which is a limited resource in the country, contaminate the rivers, streams or lakes, which is where they usually discharge their effluents without treatment [8]. For example, in Mexico there is not specifically standard that regulates the discharge of sugarcane vinasses or alcohol industry wastewater in general. So far, the applicable environmental regulation is the Mexican Official Standard NOM-001-SEMARNAT-1996 [9]. which sets the maximum limits of pollutants in wastewater discharges waste in water bodies, soil, wetlands, etc., and

***Corresponding author:** Abumalé Cruz-Salomón, Institute for Research and Innovation in Renewal Energies, University of Science and Arts of Chiapas, Chiapas, México, Tel: 9616170440; E-mail: dr.abumale@gmail.com

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possibly additional requirements (particular discharge conditions) that can be imposed by the environmental agency in Mexico SEMARNAT. Despite the lack of specificity, maximum permissible limits for BOD_5, total suspended solids, and other pollutants of the Mexican regulation are much lower than the corresponding parameter values in raw vinasse.

At present, anaerobic treatment is the most environmentally beneficial technology and energy-efficient production [10]. Besides that, it can decrease drastically contamination and it can generate bioenergy in form of biogas by utilization of locally available resources, so that a number of technologies have been explored for reducing the pollution load of distillery effluent.

The composition of biogas depends on the type of raw material and to a certain extent on the technique used in the digestion process; this biogas mainly in bioreactors is generated, also called anaerobic digesters. There are numerous designs and configurations of anaerobic digesters. Expanded granular sludge bed (EGSB) bioreactor is one type of designs of anaerobic digesters belongs to the third generation. It has been developed primarily to improve substrate-biomass contact within the treatment system by expanding the sludge bed with a high upward flow rate of liquid (>4 m/h), which intensifies the hydraulic mixture and results in better performance and stability to treatment wastewater [11].

After the organic molecules of vinasse are degraded in the bioreactor (Figure 3) by the action of microbial consortia, biogas is formed [12]. For all the above, the main aim of this research was to evaluate the treatability of the vinasses in an EGSB bioreactor. In addition, the production of biogas and BMP were determined.

Methodology

Vinasse characterization

The vinasse was obtained from sugar industry of the plant of ethyl alcohol from Chiapas, Mexico (latitude 16.277222 and longitude -92.452778). The samples were stored at -20°C until they were used. The vinasse characterization were analyzed according to the Standard Methods for Examination of Water and Wastewater [13], in order to determine pH, COD, BOD_5, total solids (TS), total volatile solids (TVS), sedimented solids, electrical conductivity (EC), color, turbidity, acidity, alkalinity, total nitrogen, total phosphorus and sulfates. The total organic carbon (TOC) was determined by the method of Walkley and Black [14], the biodegradability was calculated according by the method reported by Cruz-Salomón [15] and total phenols were determined by spectral analysis using the Folin-Ciocalteau reagent [16]. Additionally, density and viscosity were measured using a viscometer (Anton Paar SVM 300) in triplicate. The metal ions (Ca^+, K^+, Na^+, Cu^{2+}, Zn^+ and Mg^{2+}) were analyzed by flame atomic absorption spectroscopy (FAAS).

Inoculum acclimatization

Inoculum acclimatization was performed in batch bioreactor, using the fixed efficiency strategy [17] gradually increasing the feed concentration. This process consisted to feed an anaerobic inoculum with diluted vinasse with tridistilled water (30 to 50% V/V) the time required to achieve an elimination percentage, in this case, a COD elimination efficiency of 90% was established to terminate the reaction step. After which the treated effluent was drained from the bioreactor per batch to subsequently restart a new cycle of operation. The distribution of operating cycles of the acclimatization process were: 1) 30% v/v dissolution, 2) 40% v/v dissolution and 3) 50% v/v dissolution for 2 operating cycles, respectively.

EGSB bioreactor description and operation

This study was carried out using an EGSB fiberglass bioreactor, with a total volume of 3.3 L (operated at 90% of its capacity), with a reaction zone volume of 1.9 L, an interior diameter of 63.5 mm, and a height to diameter ratio of 15.8. The bioreactor was equipped with a gas–liquid–solid separator installed 10 cm below the exit. Vinasse was continuously fed entering at the bottom of the bioreactor using a peristaltic pump (Master Flex model 7534-04) connected to tank of 1.5 L that stored the vinasse, and the effluent was withdrawn from the top. $NaHCO_3$ (buffer and alkalinity source) was used to neutralize the influent. The EGSB bioreactor was inoculated with 1 L of granular sludge previously adapted (spherical in shape (Ø, c. 0.5-1 mm) and a grey-green color with a content of TS of 49.75 g/L and TVS of 29.5 g/L) from batch reactor. The bioreactor was operated at 25 ± 2°C for 140 days. The organic loading rate (OLR) and the hydraulic retention time (HRT) were maintained at 5.1 Kg COD/m^3 day and 7 days (optimum HRT in batch condition), respectively, with an average COD influent of 35762 mg/L.

Effluent analyses

Samples from bioreactor effluents were routinely taken for COD, pH and temperature measurement according to standard methods [13]. The COD removal efficiency (%) was determinate according to

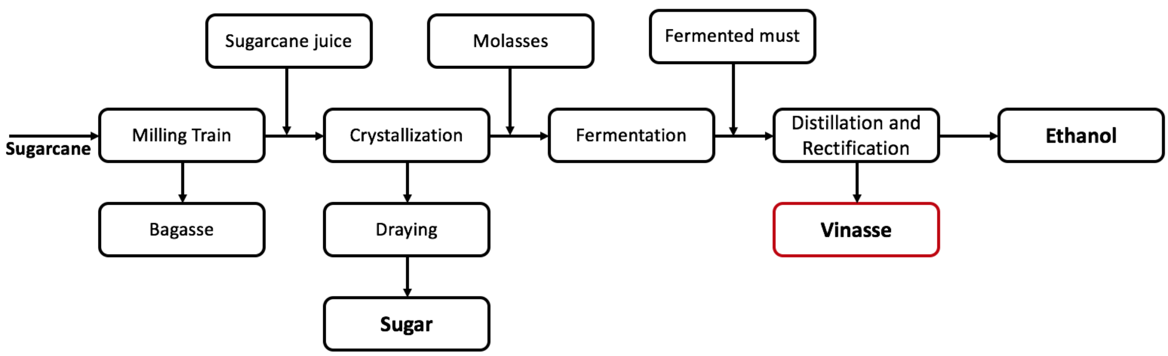

Figure 1: Flow diagram of the production of sugar and first-generation ethanol from sugarcane.

Figure 2: Environmental impact of vinasse without treatment in water bodies, soil and air.

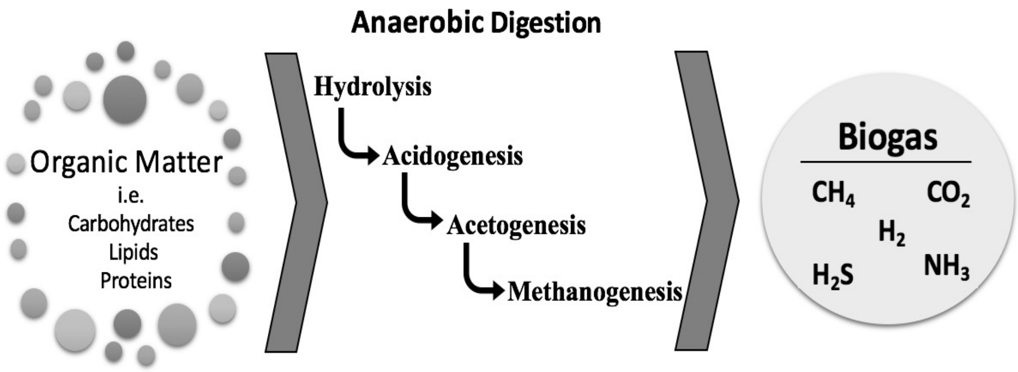

Figure 3: Anaerobic digestion process.

Cruz-Salomón et al. [18] and alkalinity factor according to following procedure: 10 mL of sample was taken and acidified with to a pH of 5.75 by recording mL of HCl (0.1 M) required (V_1) is corresponded to the part alkalinity (PA). Subsequently, the sample was brought to pH 4.3 (V_2) corresponding to intermediate alkalinity (IA) and the total alkalinity was determined as the sum of both (TA=PA+IA). The buffer index (BI) was calculated by the following equation (1) [8]:

$$BI = \frac{IA}{TA} \tag{1}$$

Methane production and biochemical methane potential

Methane production was measured by the Mariotte bottle technique adapted by Cruz-Salomón et al. [4], which involves the absorption of CO_2 present in biogas, in an alkaline liquid (3% NaOH solution) with phenolphthalein as indicator. To determine in situ composition of the biogas produced (methane and carbon dioxide), it was used Mariotte bottle technique but with a NaCl (3%) solution, coupled to the equipment Sewerin model Multitec® 540 [8].

The BMP and percentage of biodegradability were obtained using Eq. (2) and Eq. (3) respectively [4,15]. 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_4/g COD) took place.

$$BMP = \frac{V_{CH_4}}{OM} \tag{2}$$

Where BMP (mL CH_4/gCOD) is the biochemical methane potential, V_{CH4} is the methane-accumulated volume (mL CH_4) during the experiment in standard conditions of temperature and pressure, and OM is the organic matter (g COD).

$$Bio\deg radability(\%) = \frac{BMP}{350 mLCH_4 / gCOD} \times 100 \tag{3}$$

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

Results and Discussion

Physicochemical characteristics

Table 1 shows the physicochemical composition of vinasse. The majority of the values found were similar to those reported by other authors [3-5,8,15,19]. According to this characterization (Table 1), vinasse has a high pollution potential. Nevertheless, the consequences of the discharge of this effluent have been known for a long time. The high organic load of this vinasse (70,470 mg/L as COD, 54,800 mg/L as BOD_5 and 40,400 mg/L as TOC) can causes the proliferation of microorganisms that deplete the oxygen dissolved in the water, kill aquatic animals and plants, and make contaminated water bodies. This value is more difficult to be used as sources of potable water according to reported by Christofoletti et al. [20]. In addition, the discharge of vinasse in water bodies releases an unpleasant odor and contributes to disseminate endemic diseases such as malaria, amebiasis, and schistosomiasis [21], by absence of natural predators and/or vectors.

On the other hand, the turbidity (1745.7 NTU) and color (68700.3 Pt-Co) associated to vinasses suspended solids and melanoidins respectively, may impair light penetration and associated photosynthetic processes and severely impact aquatic life. The acidic pH (4.5) is due to the presence of the organic acids generated in the fermentation and to the acid salts with which the fermented must is conditioned so that the yeasts can produce alcohol, however this pH can cause acidification and heavy metal remobilization in water and soils. The relatively high concentrations of nutrients P (416 mg/L) and N (1600 mg/L) may cause eutrophication in water bodies, reservoirs, and channels, generating blooms of toxic or tainting phytoplankton forms; increasing plant/algal biomass production; occurrence of blooms of micro-algae which may be a nuisance and cause aesthetic pollution; decline or disappearance of certain perennial plants, often replaced by annual, fast growing opportunistic species such as foliose or filamentous green algae; reduced diversity of the flora (and associated fauna); changes to photic regime through shading, increase in microbial community and thus oxygen depletion, leading to hypoxic processes such as H_2S and CH_4 production; development of opportunistic macrobenthic populations; poor water quality, especially water column oxygen depletion, thus affecting fishes and zooplankton; causing mortalities of higher organisms through effects of neuro-toxins according to reported by Cruz-Salomón et al. [18] and Elliott and de Jonge [22].

Furthermore, the high concentration of metal ions presents in the vinasse like K^+ (5415.7 mg/L), Mg^{2+} (1399.2 mg/L), Ca^{2+} (1113.1 mg/L), Na^+ (182.5 mg/L), Cu^{2+} (<2.2 mg/L) and Zn^+ (<2.2 mg/L) can be poisonous. At the same time, high exposures to these metals can have serious health effects in the invertebrates and fish. Therefore, this type of wastewater has become a major concern, as it cannot be discharged into natural watercourses or a municipal sewage system without previous treatment due that they do not meet the permissible limits for discharging of effluent purpose by Official Mexican Environmental Regulations [9] and World Health Organization (WHO) [23].

Because of the implications of these effluents, it is necessary to carry out some type of treatment for this wastewater. Mohana et al. [19] reports the anaerobic treatment of distillery effluent (vinasse) is an accepted practice and it has been one of the most employed system for vinasses treatment because of low operational costs, aeration savings, low sludge production and the obtaining of by-products as methane gas [24].

So that we analyzed the results of the physicochemical characterization of the vinasse as raw material to examine some of the constituents and present factors that governed the anaerobic digestion process, as well as the biochemical characteristics these present to development of microbial activity for the greater efficiency of anaerobic treatment and methane generation.

Parameter	Values	Permissible limits	
		WHO	NOM-001-SEMARNAT-1996*
pH	4.53	6.5 - 8.5	5-10
Density (g/mL)	1.03	NA	NA
Color (Pt-Co)	68700.3	NA	NA
Viscosity (mPa.s)	1.13	NA	NA
Turbidity (NTU)	1745.7	5	NA
Conductivity (ms/cm)	15	NA	NA
Floating matter	Absent	NA	Absent
Sedimented solids (mL/L)	120	NA	2
TS (g/L)	64.89	650	NA
TSS	-	200	125
TVS (g/L)	49.82	NA	NA
Acidity (mg $CaCO_3$/L)	4975	NA	NA
Alkalinity (mg $CaCO_3$ L^{-1})	ND	NA	NA
BOD_5 (mgO_2/L)	54,800	100	150
COD (mgO_2/L)	70,470	300	NA
Biodegradability index	0.75	NA	NA
TOC (mg/L)	40,400	NA	NA
Total phosphorus (mg/L)	416	NA	30
Total Nitrogen (mg/L)	1600	NA	60
C/N	25-Jan	NA	NA
C/P	97/1	NA	NA
Total sulfates (mg/L)	360	250	NA
Total phenols	647	NA	NA
Competitiveness index	195	NA	NA
Zinc (mg/L)	< 2.2	5	20
Copper (mg/L)	< 2.2	1	6
Magnesium (mg/L)	1399.2	50	NA
Potassium (mg/L)	5415.7	NA	NA
Calcium (mg/L)	1113.1	75	NA
Sodium (mg/L)	182.5	NA	NA

Table 1: Physicochemical characteristics of sugarcane vinasse.
ND = not detected, NA = not applicable, *water discharged in rivers

The first parameter for good anaerobic treatment is the pH. The optimum pH for anaerobic digestion is neutral, and the vinasse has an acidic pH (4.53), so it was necessary to buffer with $NaHCO_3$ in order to neutralize and feed the bioreactor [25]. Other important parameter is biodegradability index [26], it indicates the percentage of organic matter able to be degradable. This vinasse presented a biodegradability index of 0.75 (indicates that 75% of the organic matter can be degraded). In the other hand, the quality of the anaerobic treatment depends on the C/N ratio (30/1 to 15/1) [8] and C/P ratio (130/1 to 60/1) (adapted ratio of the correlation COD vs TOC) [27]. Additionally, it presented a ratio of 25/1 of C/N and 97/1 of C/P, which makes to vinasse a good substrate for anaerobic digestion since it presents the right ratio for microbial growth or biomass production, biofilm formation and requirements necessary for its optimal treatment. The competitiveness index (COD/SO_4^{-2}) <10 facilitates methanogenesis, since there will be no competition between sulfate-reducing bacteria and methanogenic archaea [28]. The competitiveness index determined in vinasse was 195, having them within the appropriate parameters to carry out the anaerobic digestion.

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The metals ions are often present in industrial and agro-industry wastewater in significant concentrations, and the most frequent detected are copper (Cu), zinc (Zn), lead (Pb), mercury (Hg), chromium (Cr), Cadmium (Cd), iron (Fe), nickel (Ni), cobalt (Co) and molybdenum (Mo) [29]. Nevertheless, many metals are needed in low concentration for the activation or functioning of many enzymes and coenzymes in anaerobic digestion like Fe >> Zn ≥ Ni>Co=Mo>Cu [30]. However, an excessive amounts of metal ions can lead to inhibition or toxicity (Table 2) [31,32]. In this case, the vinasse presented a concentration of 182.5 mg/L of ion Na^+, 1113.1 mg/L of calcium (Ca^{2+}), <2.2 mg/L of copper (Cu^{2+}) and <2.2 mg/L of zinc (Zn^+). These metal concentrations do not cause any effect (do not inhibition) to the process of anaerobic digestion, whereas for the potassium (K^+) with 5415.7 mg/L and magnesium (Mg^{2+}) with 1399.2 mg/L present moderately inhibitory values without actually affecting the anaerobic digestion.

Inoculum acclimatization

Acclimatization of the inoculum was achieved in 75 days (Figure 4). Six feeds at different feed dilutions (30 to 50% V/V) were made. During acclimatization, the inoculum degraded organic matter with greater efficiency and in less hydraulic retention time (HRT). With the time, the degradation was gradually decreased and with each feed the removal efficiency improved relative to the previous feed dilution until to reach a 7-day HRT in a dilution of 50%, which (7-day HRT) was subsequently used in the EGSB bioreactor.

EGSB bioreactor operation

The behavior of the bioreactor is observed based on its pH and temperature control parameters during the 140 days of evaluation (Figure 5). It can be observed that the bioreactor operated under mesophilic condition (26°C) and the pH at the beginning was below for the optimum stable range (6.8-7.4) for the methanogenic archaea reported by Speece [33], so it was necessary to buffer with $NaHCO_3$, until the bioreactor was stabilized and operated in the appropriate range after 40 days.

The alkalinity factor is usually used for both to control the stability of the anaerobic process [33] and to measure the buffer capacity of the bioreactor (it is very important because it allows thus be able to quickly identify if there is an imbalance in the bioreactor). Rojas [34] reported that the anaerobic bioreactor operates properly with an alkalinity factor was within the optimal operating range of 0.2-0.4. This parameter of control is showed in the Figure 6, where it can be observed that during the first 40 days the bioreactor operated in an unstable condition since it was presented very high values of buffer index, which indicated acidification principles that as was corroborated in this study. Nevertheless, after 40 days the bioreactor began to operate stably generating values in a range of 0.2 to 0.3, concentrating most of them between 0.24, which indicates that the bioreactor works within the optimum range of operation all the time evaluation.

Figure 7 shows the COD analysis of EGSB bioreactor performance during at 140 days evaluation period. It can be observed that bioreactor operated in stable condition throughout the evaluation period, achieving a percentage of removal close to 75%, this value of COD removal efficiency is similar to reported by Qinglin et al. [35], Lopez and Borzacconi [36] and Cruz-Salomón [8]. However, even when the COD removal efficiency was moderate, effluents with a COD of 9648 mgO_2/L were generated (average in stable condition). The moderate COD removal efficiencies obtained in this investigation could be a consequence of the fermented material origin, the distillation process [37] as well as due to the presence of some organic compounds (phenolic compounds) that have been reported as being toxic and recalcitrant for the methanogenic systems [38]. Therefore, these effluents generated by the EGSB bioreactor still cannot be discharged to the sewerage system or to surface water bodies (e.g., rivers, lakes, etc.) since it does not yet comply with Environmental Regulations [9,23], so a second treatment is recommended like Anaerobic fluidized bed reactor (AFBR), Anaerobic filters (AF), Fungal treatment or Aerobic treatment combined with other methods.

Biogas quantification

The production of biogas through anaerobic digestion in addition to reducing the environmental pollution generated by the vinasse, offers significant advantages over other forms of bioenergy production

Substance	Moderately inhibitory	Strongly inhibitory
Zinc (mg/L)	-	30
Copper (mg/L)	200	200
Magnesium (mg/L)	1000-1500	3000
Potassium (mg/L)	2500-4500	12000
Calcium (mg/L)	2500-4500	8000
Sodium (mg/L)	3500-5500	8000

Table 2: Concentrations metal ions to inhibiting the anaerobic digestion.

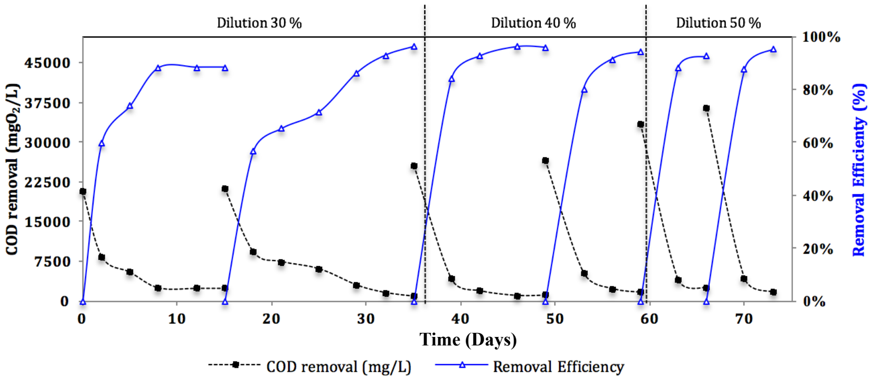

Figure 4: Kinetic of inoculum acclimatization.

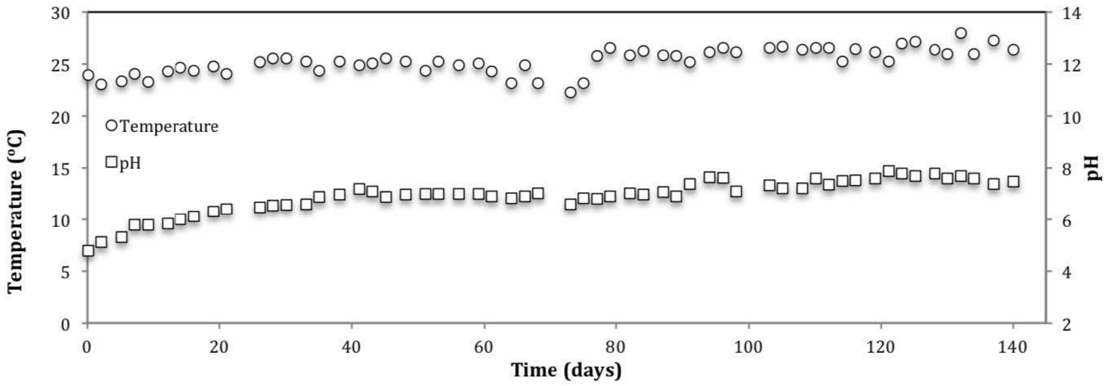

Figure 5: Monitoring of pH and temperature in the effluents of EGSB bioreactor.

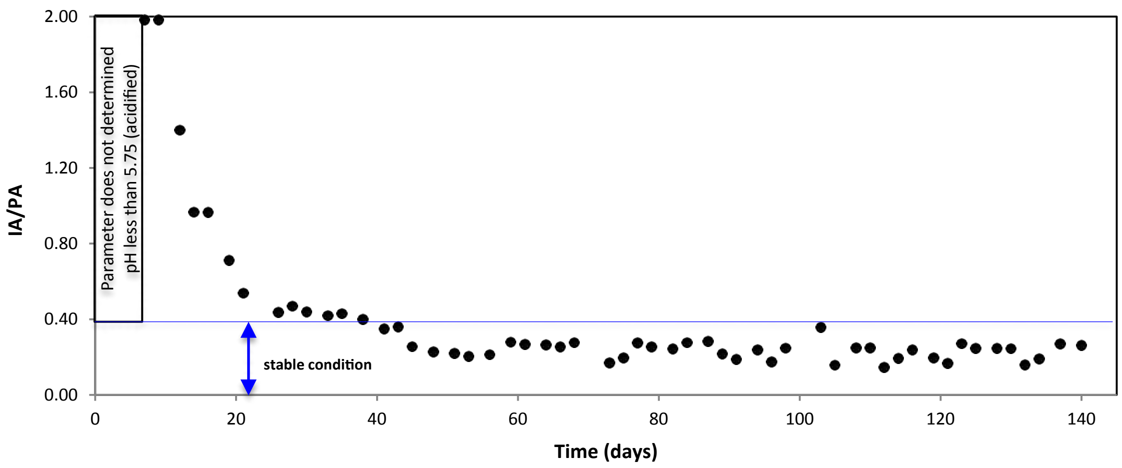

Figure 6: Monitoring of alkalinity factor (BI) in the effluent of EGSB bioreactor.

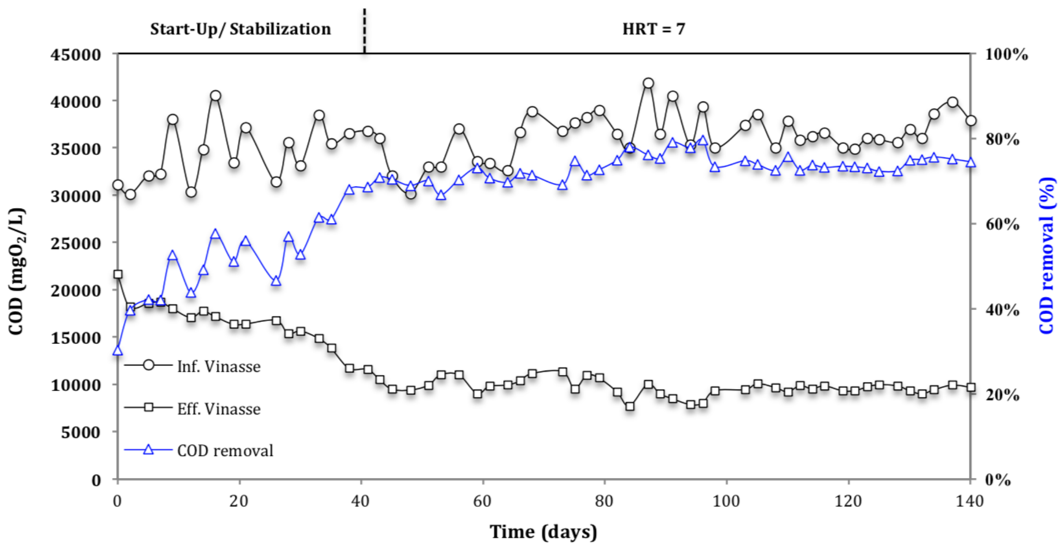

Figure 7: Process performance of the EGSB bioreactor.

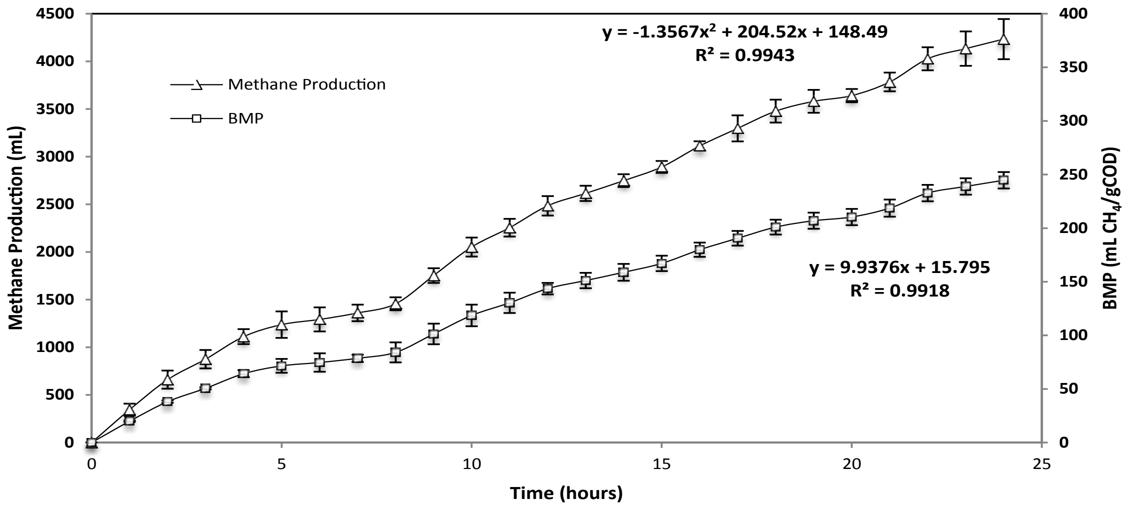

Figure 8: BMP and methane production.

Methane content (%)	Calorific power kcal/m³		Methane content (%)	Calorific power kcal/m³	
	At 0°C and 760 mm Hg	At 20°C and 760 mm Hg		At 0°A and 760 mm Hg	At 20°C and 760 mm Hg
50	4281	3955	66	5650	5261
52	4452	4145	68	5822	5420
54	4623	4304	70	5993	5579
56	4794	4463	72	6164	5739
58	4965	4623	74	6335	5898
60	5137	4782	76	6507	6058
62	5308	4942	78	6678	6217
64	5479	5101	80	6849	6376

Table 3: Biogas content and biogas calorific power.

[39], however, the methane quantity in the biogas mix decides the energetic value [40], which the biogas holds. Table 3 [41] portrays the energetic value associated with biogas, containing methane at different percentage concentration. Additionally, the table depicts the energetic value of biogas at a pressure of 760 mm Hg, at two different temperatures (0°C and 20°C).

The EGSB system working at 7 day HRT has a biogas production (4233.5 ± 110 mL CH_4/day) and a BMP of 244.7 mL CH_4/gCOD (Figure 8). This results indicate that a EGSB system is a possible sustainable alternative to solve environmental problems, compared to other conventional methods to treat vinasse. Biogas production by the EGSB bioreactor contained 76% methane which generates a calorific power of 6507 kcal/m³ at 0°C and 760 mm Hg, therefore these products can be considered as fuel (45% of methane is flammable). The numerical values of BMP of the sugarcane vinasse was of 244.7 mL CH_4/gCOD, this result was similar a value to the reported by Janke [42], Leite [43] and Cruz-Salomón [4] (220- 307.5 ml CH_4/g COD). Therefore, it has been determined as sugarcane vinasse in EGSB bioreactor is one of the most energy efficient and environmentally beneficial technologies for wastewater treatment and the production of bioenergy [10].

Conclusion

Sugarcane vinasse cannot be discharged into natural watercourses or a municipal sewage system without previous treatment due that they do not meet the permissible limits for discharging of effluent purpose by Official Mexican Environmental Regulations and World Health Organization, However it has a high potential as a nutrient alternative (substrate) for anaerobic treatment in EGSB bioreactor, because it contains a large amount of organic matter, capable of being degraded by the anaerobic microorganism and it does not has inhibition by metal ions.

The adaptation demonstrated a better efficiency of the inoculum in being operating in the EGSB bioreactor. The EGSB bioreactor with a 7 day HRT generated the COD removal efficiency close to 75% and biogas production above 4.2 L of CH_4/day, with 76% methane concentration (high quality) which generates a calorific power of 6507 kcal/m³ and BMP of 244.64 mL CH_4/gCOD. According to the obtained results, the anaerobic EGSB bioreactor can be a solution to the environmental challenges that this agro-industry confronts, since could be a sustainable alternative to simultaneously solve the environmental pollution caused by vinasse and produce bioenergy for the operation of the same agro-industry.

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