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Biogas production from vinasse derived from ethanol manufacturing using a continuous stirred tank reactor pilot plant

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ABSTRACT

Vinasse is considered the most important source of contamination in the ethanol beet production industry; it is also considered as a resource of high biogas production potential. The production of biogas from vinasse has economic and environmental benefits. In this research, the anaerobic digestion of vinasse derived from bioethanol manufacturing is studied on a laboratory-scale using a continuous stirred tank reactor pilot plant at mesophilic conditions (37°C). The quantity of produced biogas from the vinasse was carried out under different operating parameters: pH, agitation, electric conductivity and hydraulic retention time (HRT). Results of this study show that inoculum/vinasse ratio of 0.3 presents removal effectiveness of pollution abatement close to 91% for chemical oxygen demand (COD) and total suspended solids (TSS) which correspond to maximum biogas production up to 77% of methane for an optimal HRT of 21 d. However, inoculum/vinasse ratio of 0.5 leads to lower removal of COD (85%) and TSS (82%) which represent a total biogas production of only 52%. This study allows us to suggest that the inoculum/vinasse ratio of 0.3 is the appropriate value for the removal of pollution and the production of biogas from vinasse, which is approved by the t-student statistic test of the *p*-value significantly lower than the significance level $\alpha = 0.05$.

Keywords: Anaerobic digestion; Mesophilic conditions; Continuous stirred tank reactor; Vinasse; Biogas; Pollution load

1. Introduction

Renewable energy has become necessary to remove carbon from the global economy and mitigate global climate change by exploiting biomass. The consumption of renewable energy continues to grow strongly. In 2017, global renewable energy consumption has grown at an annual rate of 5.62%, which is 2.95 times higher than the annual growth rate of primary energy consumption [1]. According to statistics from the 21st Century Renewable Energy Policy Network (REN21), biomass energy accounted for around 12.8% of global energy consumption in 2016 [2]. Therefore,

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Morocco is turning to more interest in renewable energies; since 2009, the Kingdom of Morocco has adopted an energy strategy that has set for the objective of the rise of renewable energies, the strengthening of energy efficiency and regional integration [3]. Several factual elements attest to Morocco's commitment to building a carbon economy, with the objective of achieving at least 52% of renewable energy in the energy mix by 2030.

Vinasse is a waste from the distillery industries causing major environmental problems around the world. It is also called stillage or molasses wash in Asian countries [4]. It is the biggest source of pollution in the ethanol industry. The new increase is foreseen in the production of bioethanol dramatically increases the volume of distillation residue. This trend exacerbates worldwide the problem of its use since the quantity of vinasse obtained is more than ten times the quantity of bioethanol produced [5]. Over 95% of ethanol production comes from the sugar and starch industries and it contributes up to 42% and 58% of total production, respectively [6]. If these residues are released into the environment without a proper disposal method, it can lead to environmental pollution and harmful effects. In addition, this untreated waste creates different problems with climate change by increasing the number of greenhouse gases [7]. The high chemical oxygen demand (COD) of these untreated wastes are also suitable conditions for the growth of various pathogenic fungi and bacteria [8]. The restrictive laws prohibited the direct and indirect discharge of vinasse into water bodies [9], which encouraged the search for solutions to reduce the negative environmental effects of vinasse.

Anaerobic digestion (AD) or bio-digestion can be considered the primary alternative for the management of stillage in sugarcane and beet bio-refineries. AD has significant advantages, including a reduction in the polluting organic load of vinasse, the potential recovery of bioenergy from biogas and the potential for improving the profitability of bio-refineries through the production of surplus electricity, based on the combustion of biogas of the movers [10]. Bioethanol is by far the most widely used biofuel for transport around the world, as it is a non-toxic, biodegradable and oxygenated resource, offers the possibility of reducing particulate emissions in ignition engines by compression [11]. Around the world, fuel ethanol could reach 10%–20% of gasoline consumption by 2030, requiring a seven-fold increase in ethanol production capacity compared to estimate from 2005 [12], in previous work the advantages and the capacity of biogas production optimal from vinasse are provided [13,14].

The main objective of the present study is to examine the potential of biogas produced from vinasse derived from the manufacture of bioethanol using a laboratory-scale continuous stirred tank reactor (CSTR) pilot plant in a batch mode according to the inoculum/vinasse ratio. Particular attention has been paid to its efficiency in removing pollution load and to the amount of biogas produced according to operational parameters, such as pH, hydraulic retention time (HRT), agitation speed and temperature. Statistical analysis using software XLSTAT v.14 and Minitab v.18 is carried out to evaluate the performances of two ratios of inoculum/vinasse in the biogas and methane production of the AD of vinasse.

2. Materials and methods

2.1. Inoculum and substrate sampling

The start of the AD process requires the use of inoculum and the substrate to be treated.

The inoculum used in this study was recovered from inside an anaerobic digester at the wastewater treatment plant of Fez City.

The substrate used in this study is the vinasse that comes from the ethanol manufacturing process by the Sotrameg Company "Molasses Transformation Company" (Molasses Processing Company of Gharb, Souk EL HAD, Municipality Benmansour, Province of Kenitra, Morocco). The company is installed in the plain of Gharb (Region of Kenitra) of Morocco since 1975. The main activities of Sotrameg are the industrial production of alcohols and liquid gases. Sotrameg produces ethanol by mesophilic fermentation of molasses with *Saccharomyces cerevisiae*. Fig. 1 shows the scheme process of vinasse and ethanol production.

2.2. CSTR reactor configuration

The CSTR used in this study was provided by the company Cosimi/Deltalab, France. Fig. 2 shows an overview of the pilot plant that consists of four parts: a feed tank, a reactor tank and a liquid introduction lock installed at the top of the reactor manages the opening of the solenoid valve V13, it is filled to the high level using pumps P1, P3 or P4. The reactor tank is coupled to a heating group able to delivering regulated water up to 95°C. The temperature of the heat transfer fluid is programmed on the group. The resulting product temperature is displayed on the control cabinet temperature display. To monitor the produced biogas a gas flow meter was installed at the outlet of the reactor to the monitoring of the weight of the gas. Table 1 presents the characteristics of the equipment's CSTR reactor. The electrical box displays the temperature, gas flow, pH, redox potential, reactor stirrer timer and speed, pump timer.

2.3. Batch system experiments

Batch system experiments are carried out over a period of about 6 weeks in the mechanically stirred bioreactors with a digester volume of 50 L. Before feeding the reactor, the vinasse and the inoculum undergo a pre-filtration step through a filter to remove large particles, then the reactor is automatically inoculated with a predetermined quantity of the mixture composed under the following operating conditions:

- Mesophilic conditions (37°C);
- HRT of 3 weeks;
- Stirring speed of 20 tr/min.

Under these operating conditions, the experiment is subjected in batch mode for two ratios:

- Inoculum/vinasse of 0.3;
- Inoculum/vinasse of 0.5.

2.4. Analytical methods

The inoculum and vinasse are analyzed before and during the operation respectively. At the end of the digestion

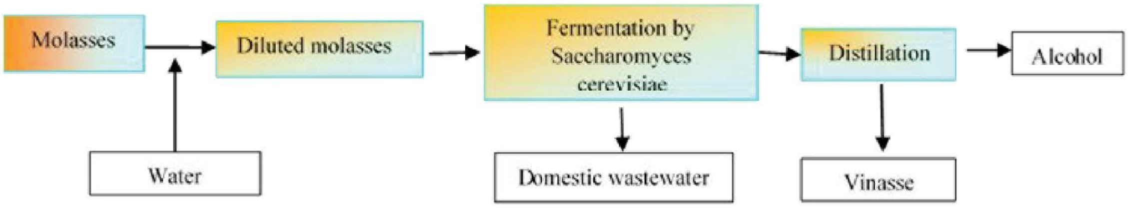

Fig. 1. Scheme of the Sotrameg plant for bioconversion process of molasses.

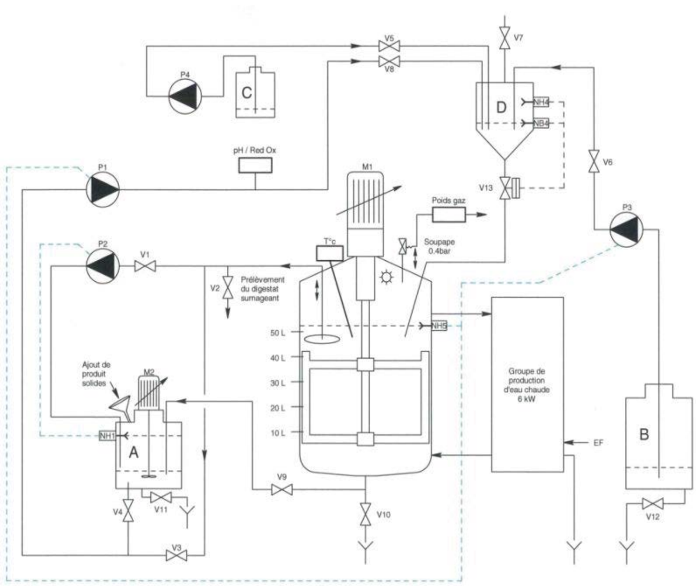

Fig. 2. Schematic diagram of the continuous stirred tank reactor system. A: Dilution tank; B: Feed tank; C: Antifoam tank; D: Introductory airlock; R: Stainless steel reactor; P1: Peristaltic recirculation pump; P2: Substrate recirculation pump; P3: Substrate feed pump; P4: Antifoam peristaltic pump.

Table 1
Characteristics of the equipment's continuous stirred tank reactor

Equipment	Characteristics
Reactor	Stainless steel reactor of 70 L total
Dilution tank	Substrate dilution tank of 25 L
Feed tank	Feed tank of 60 L
Gas flow meter	Bronkhorst brand
Heating group	Capacity: 6 kW, 95°C

process, that is, after 3 weeks, the collecting digestate (mixture composed) is analyzed. Various physicochemical parameters are determined such as COD, dry matter (DM), total suspended solids (TSS), volatile solids, mineral solid in accordance with standard methods [15,16]. All these parameters are measured daily, Table 2 reporting the type of analysis and the instrument used in this study. The main compositions of raw inoculum and vinasse used in the experiments are presented in Table 3.

The reactor stability operation has been controlled by monitoring the volatile fatty acids (VFA) and the pH. Together VFA and pH are good indicators for the AD process. The composition of the produced biogas (CH_4, CO_2, H_2S and O_2) is analyzed with a gas analyzer (Multitec 545).

The multivariate statistical method of samples is applied by multivariate statistical study through statistical software XLSTAT v.14 and Minitab v.18 to evaluate whether there is a statistical difference in the average biogas production of the samples tested in the reactor.

3. Results and discussion

3.1. Stability of the digester

To study the stability of the digester, the monitoring of pH is part of the daily monitoring, its value may vary depending on the phase of AD. The biogas production rate will decline at pH conditions higher or lower than the appropriate range [17]. The optimum for the pH of the mesophilic AD range is close to neutrality, varying for each type of bacteria and it is between 6.5 and 7.5 [18]. In our study, Fig. 3 shows that the pH in the reaction medium is controlled for the ratio 0.3 and is in the neutrality range during the 21 d of AD, this is explained by the consumption of VFA and the presence of bicarbonates produced by methanogenic bacteria, which promote better biogas production. For the 0.5 ratio, the pH is kept constant during

Table 2
Parameters analyzed and instrument used

Parameters	Instrument used
Chemical oxygen demand	Programmable DCO Reactor 12 Workstations: LABO-MODERNE France References: BOT1200
Biochemical oxygen demand	Oxitop IS 12: Labo and Co., French Company
pH	A JENWAY 3510 Type pH Meter: Fabrimat French Company
Electric conductivity	An inoLab Type Conductivity Meter: XYLEM ANALYTICS France
Volatile solid, mineral solid	Milian SA Dutscher Group

Table 3
Characteristics of raw inoculum and vinasse

Parameters	Inoculum	Vinasse
pH	8.1 ± 0.3	7.4 ± 0.2
Electric conductivity, mS/cm	11.87 ± 0.52	31.3 ± 0.78
Chemical oxygen demand, g/L	17.48 ± 0.25	75.00 ± 12.54
Total suspended solid, g/kg	151.74 ± 10.07	50.94 ± 3.22
Volatile solid, g/kg	52.63 ± 2.4	25.53 ± 1.3
Mineral solid, g/kg	99.12 ± 3.24	25.41 ± 1.32
Dry matter, g/L	26.23 ± 1.76	53.79 ± 3.64

the 21 d of AD around 8.4. This increase in pH promotes a decrease in the degradation of organic matter as a result of disturbance of the reaction medium caused by the presence of other compounds such as hydrogen sulfide ($H_2S/HS^-/S^{2-}$, pKa 7.1 and 13.3) which promoting pH imbalance and inhibition of bacterial flora.

3.2. Removal of COD, TSS and DM

The concentration of COD decomposition is called COD removal. In the literature, Lutosławski et al. [19] stated that 1 g of COD will be destroyed in to 0.395 L biogas at 35°C and 1 atm. In this research, the COD of the substrate to be treated will be decomposed by microorganisms in the digester during the AD process. Fig. 4 shows the variation of COD both ratio (0.3 and 0.5) as a function of time under the operational conditions shown below. COD is measured daily for three weeks. On the first day, a maximum increase in COD of around 60.000 mg/L is observed for 0.3 ratio compared to 0.5 ratio which is around 69,000 mg/L. Throughout the experiment, COD gradually decreases for the two ratios (0.3 and 0.5). In the last days, COD reaches minimum values of around 10,000 and 5,000 mg/L respectively for the ratio 0.3 and 0.5. In this research, the variable 0.3 exhibits a significant elimination of COD during 21 d of HRT compared to the variable 0.5 which represents the smallest elimination at the start of AD until the 18th day. However, these results show that the methanogenic bacteria of the reaction medium exhibits better acclimatization with a ratio of 0.3 than 0.5. This behavior could be explained by the fact that the fermentation process in the digester with the ratio of 0.5 is dominated by the acidogenesis step and the activity of methanogenic bacteria did not grow well in the system. In fact, these results have been clearly explained with the initial composition and the quantity of organic matter in the digester which represent the main factor in the advancement of AD and the adaptation of microorganisms in order to obtain a better degradation of organic matter which is resulted in significant biogas production. Many studies demonstrate that the COD removal in the digester was related to the nature of the substrate and the conditional operations [20]. In their study, Syaichurrozi and Sumardiono [21], stated that the COD removal was the biggest for vinasse/water ratio of 0.3 than the other variables under the operational conditions of room temperature in batch mode.

Figs. 5 and 6 illustrate the concentration of DM and TSS in the digester as a function of the time for both ratios (0.3 and 0.5) during AD. Fig. 5 shows that the DM concentration is reduced during AD of 21 d and it reaches the

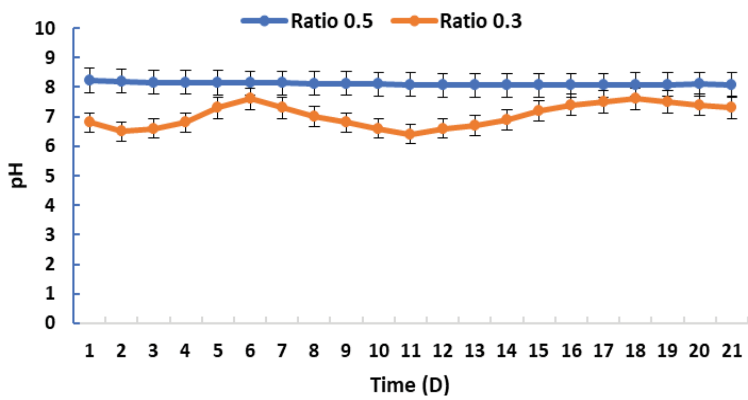

Fig. 3. pH vs. time during anaerobic digestion of vinasse for the two ratio inoculum/vinasse.

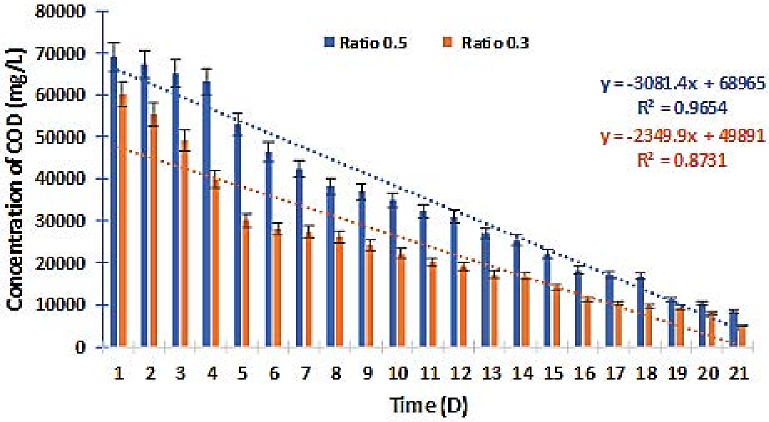

Fig. 4. Variation of chemical oxygen demand as a function of the time for both ratios (0.3 and 0.5).

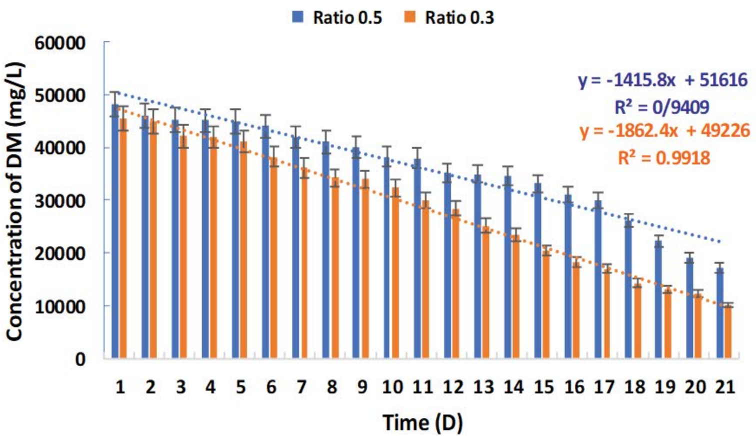

Fig. 5. Variation of dry matter as a function of the time for both ratios (0.3 and 0.5).

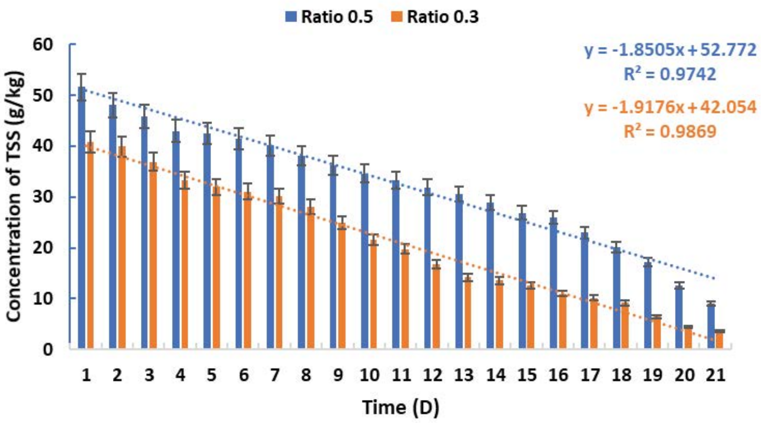

Fig. 6. Variation of total suspended solids as a function of the time for both ratios (0.3 and 0.5).

minimum values of 10,112 and 7,237 mg/L respectively for the ratio of 0.3 and 0.5. Following these results, a significant difference average between DM concentration between the ratios 0.3 and 0.5 is obtained. According to this result, the concentration of TSS (Fig. 6) is more significant for the ratio 0.3 than for 0.5 with minimal degradation of 8.9 and 3.5 for the ratios 0.3 and 0.5, respectively. This lowest concentration obtained with the ratio of 0.5 is explained by supplying the reactor with organic matter and the substrate and also by the accumulation of mineral matter in the acidogenesis phase which dominates and causes the deactivation of methanogenic bacteria and a disruption of the metabolism of the bacterial flora. This phenomenon is caused by the value of the pH in the reactor where the methanogenic bacteria did not grow in the high pH, greater than the optimum range of AD (pH = 6.8–7.5). This research is conducted to demonstrate the important quantity of TSS in the digester. Variation of inoculum/vinasse ratio causes a change in TSS concentration and COD content in the digester leading to the production of the highest biogas and methane. Another study stated that the vinasse/water ratio of 0.3 produces the biogas quantity of 35 mL for the content of TSS and COD of 7.015% and 76.42 g respectively [22]. So according to the results of this work, the degradation of the organic matter in the digester is related to the nature of the substrate and the operational conditions which activate the microorganism's and accelerate the acetogenesis stage; acetogenic bacteria converts ethanol, propionate acid and butyric acid into acetic acid [23].

3.3. Effectiveness of pollution abatement

Table 4 presents a comparison of the pollution reduction efficiency of the parameters COD, TSS and DM for both ratios of 0.3 and 0.5. It appears that 85% of the COD is removed for the ratio 0.5 ratio, in comparison to 91% for the ratio of 0.3. This result indicates that increasing COD concentration in the digester causes an increase in COD removal. Concerning the TSS and DM parameters, the results show a removal efficiency of 91.3% and 92% respectively for the ratio of 0.3 and 82 % and 86% for the ratio of 0.5. The highest effectiveness of pollution abatement is obtained with a ratio of 0.3. It shows that there has been a good decomposition of organic material by microorganism's activity.

Table 4
Effectiveness of pollution abatement of both ratios (0.5 and 0.3)

Parameter	Treated substrate	
	Ratio: 0.5	Ratio: 0.3
Chemical oxygen demand, mg/L	85%	91%
Total suspended solid, g/kg	82%	91.30%
Dry matter, mg/L	84%	92%

3.4. Biogas production and composition

The comparison of biogas production rate as a function of the inoculum/vinasse ratio is shown in Fig. 7. The vinasse is composed of mineral and organic matter such as magnesium, zinc, phosphorus, acetic acid [24]. In this study, the best output of biogas is recorded after the 15th day. The ratio of 0.5 exhibits lower biogas production than the ratio of 0.3. For the variable ratio of 0.3, the biogas production is highest and faster. It increases from the 16th until the 18th day, thereafter the biogas production decreases faster. The total biogas produced for the ratios of 0.3 and 0.5 is 5,500 mL/kg COD and 1,000 mL/kg COD respectively. These results explain the relationship between the initial concentration of organic matter and the activity of bacteria flora. The composition of the biogas produced in the digester for the two ratios is shown in Table 5. The ratio of 0.3 presents the highest gas methane production of 77% which corresponds to an interesting quality of biogas from the energy standpoint. According to Barrera et al. [25], a large quantity of organic matter presents in the vinasse under suitable operating conditions for the bacterial flora for hydrolysis and methanogenesis generate a large quantity of biogas and methane. Indeed, the smallest proportion of methane gas produced (40%) is obtained for the ratio of 0.5. These results can be explained by the presence of substrates rich in sulfur proteins and sulfates which can lead to an increase in the production of hydrogen sulfide (H_2S). H_2S is a toxic element for methanogenic bacteria because it causes inhibition in the methanogenic phase.

These results are confirmed by the proportion of H_2S produced at the level of the biogas for a value of 15% of the total quantity of the biogas produced. The level of hydrogen sulfide produced during the AD stage greatly impacts biogas production [26]. According to these results, several studies have demonstrated the feasibility of biogas produced from vinasse using AD. However, this approach has been suggested by the nature of the substrate and the operating conditions [27]. Marques dos Reis et al. [28] evaluated the methane and biogas production from sugarcane vinasse in an anaerobic fluidized bed reactor under the specific conditional experiment. In concluding this section, AD of the vinasse is a reliable solution for the production of biogas

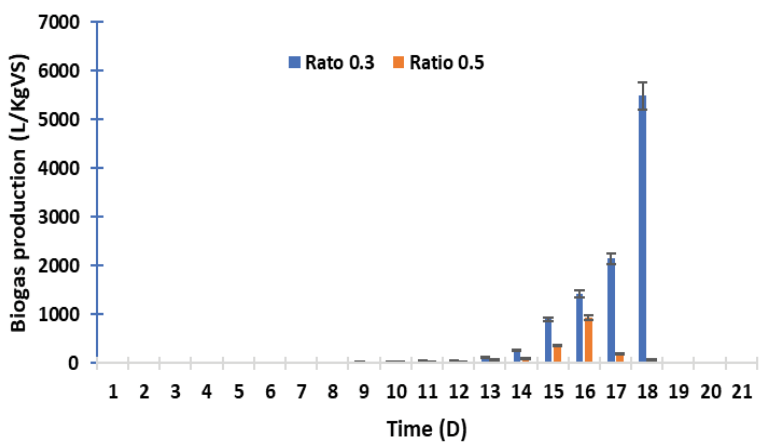

Fig. 7. Effect of inoculum/vinasse ratio on biogas production rate.

under specific operational conditions, which can be valued as a source of renewable energy, and that will allow the industry to save significant gains.

3.5. Statistical test

In this study, the statistical test is carried out to confirm the difference between the two ratios tested by the Boxplot comparison diagram (Fig. 8) which shows a clear difference in the studied parameters (COD, DM and TSS) with a better degradation of organic matter for the ratio 0.3 than 0.5.

The Student test is a parametric test which is consisted of comparing two independent samples under the satisfaction of the two conditions such as; the test of normality and that of equality of variance. The statistical test, including the Student and Wilcoxon test, shows the difference (the calculated p-value is lower than the level of significance $\alpha = 0.05$ and the degree of freedom ($DF = n_1 + n_2 - 2$) between the two ratios relative to the quantity of biogas produced during the AD (Table 6). However, the ratio of 0.3 is efficient in terms of organic matter degradation (COD and TSS of 91%; 91.3% respectively) and of the biogas production of methane (77%) compared to the ratio of 0.5 which exhibits the smaller biogas production corresponding to 40% of methane. These results are confirmed by the effectiveness of the pollution load abatement during the experiment of HRT 21 d. Indeed, concerning the biogas production of two ratios 0.3 and 0.5, the Wilcoxon test is used instead of the Student test since the normality test is not satisfactory by rejecting the null hypothesis (Table 6). In this case, the test is highly significant for the two ratios 0.3 and 0.5 with

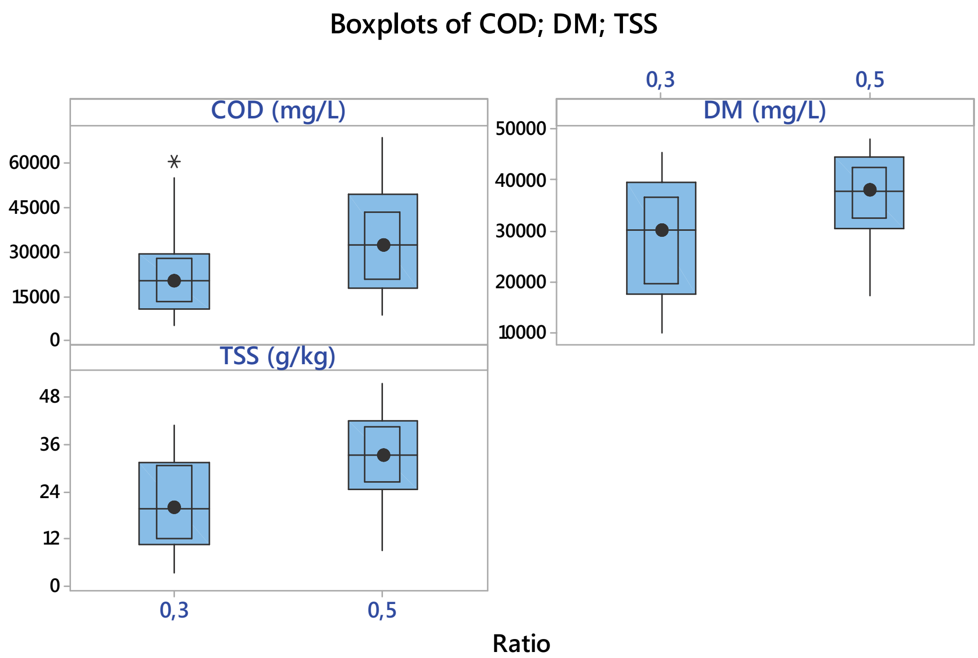

Fig. 8. Boxplot of the parameters chemical oxygen demand, total suspended solids and dry matter.

Table 5
Produced biogas composition by % volume

Biogas composition	Carbon dioxide (CO_2 %)	Hydrogen sulfide (H_2S %)	Dioxygen (O_2 %)	Methane (CH_4 %)
Inoculum/vinasse; ratio 0.3	17	2	2.2	77
Inoculum/vinasse; ratio 0.5	24	15	4	40

Table 6
Representation of test *t* student

Parameters	*T*-value	DF	*p*-value	Threshold meaning
Chemical oxygen demand	–2.03	40	0.047	0.05
Dry matter	–2.27	40	0.029	0.05
Total suspended solid	–4.22	40	0.000	0.05

Table 7
Results of the Wilcoxon statistics test

Sample	DF	Wilcoxon statistics	*p*-value	Meaning threshold
Biogas production (mL)	40	903	0.000	0.05

p-value = 0.000 which is lower than the threshold meaning $\alpha = 0.05$ (Table 7).

4. Conclusion

This study evaluates the biogas production of vinasse using AD by CSTR reactor. The amount of biogas produced is proportional to the degradation of organic matter. Inoculum/vinasse ratio of 0.3 presents removal effectiveness of pollution abatement up to 91%; 91.3% of COD and TSS respectively correspond to maximum biogas production up to 90% for an optimal time HRT of 21 d. However, the inoculum/vinasse ratio of 0.5 exhibits the smaller removal of COD and TSS which represents a total biogas production of 52%. This difference for both ratios is confirmed by the one-sided statistical test on the right of Wilcoxon with the *p*-value below the significance level $\alpha = 0.05$. This phenomenon is linked to the initial concentration of organic matter and the activity of the methanogenic bacteria in the digester. Finally, in this study, the inoculum/vinasse ratio of 0.3 is the best variable for removing pollution and producing the highest quantity of biogas from vinasse sewage.

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