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Influence of inoculum to substrate ratio on methane production in Biochemical Methane Potential (BMP) tests of sugarcane distillery waste water

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Abstract

The activated sludges and the sugarcane distillery wastewater (vinasse) are two different types of liquid wastes generated throughout the domestic wastewater treatment and sugarcane molasses fermentation and distillation respectively. In Reunion Island, sludge is currently discarded in landfills. Regarding the vinasse, a part of the production is treated by anaerobic digestion process. However, the remaining part is diluted then discharged into the sea 900 m of the coast. In this study, the mesophilic biochemical methane potential (BMP) test, with different inoculum to substrate (I/S) ratios, are presented in this paper as well as the methane production and kinetic results. The BMP is modelled by both the first order linear regression and the modified Gompertz model. In a future study, we will propose an alternative solution in order to treat both wastes simultaneously while producing energy with the co-digestion of the vinasse and the activated sludges in semi-industrial scale.

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Keywords: anaerobic digestion; biochemical potential test; I/S ratio; modified Gompertz model; sugarcane vinasse

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1. Introduction

Anaerobic digestion is a widely process for waste treatment. Moreover, it allows the production of renewable energy. In this context, many studies have been carried out in order to characterise the methanogenic potential of waste and to model the anaerobic process. This led to the publication of standardised protocol for the biochemical potential (BMP) tests to increase the probability of obtaining validated and reproducible results [1]. The I/S ratio is a point of interest as the obtained methane production of the BMP depends on this parameter. Moreover, the optimal I/S ratio depends on the tested substrate. For instance, the optimal I/S (based on VS) ratio of microalgae BMP is 2 in order to avoid volatile fatty acids (VFAs) accumulation [2]. In the case of the BMP of maize, the authors noticed only slight variations of methane yield with different I/S (based on VS) ratios (1, 1.5, 2 and 3) and the optimal ratio is 1 [3]. In a study on okara, S/I (based on VS) ratios in the range of 0.1 to 3 are tested [4]. The authors highlighted that the methane yield reaches its maximum at S/I values of 0.6 to 0.9, and inhibitions occur when the ratio exceeds 1, due to VFAs accumulations [4]. Others authors recommend to test different ratios, based on a study on piggery slaughterhouse waste [5]. Moreover, they recommend an S/I (based on VS) ratio inferior to 0.1 [5]. In the latest study, the recommended ratios based on VS contents are between 2 and 4 [1]. However, it is also mentioned that for easily degradable substrates where rapid accumulation of fermentation intermediates such as VFAs could lead to inhibition of anaerobic digestion, a ratio greater than or equal to 4 should be applied; and for less degradable substrates, such as lignocellulosic organic matter, a ratio less than or equal to 1 can be applied [1].

Regarding the BMP modelling, linear or non-linear regression can be used [6].

This paper deals with the study of the BMP tests of vinasse from sugarcane with vinasse sludge as inoculum. First, we present the BMP tests methods and results for four I/S ratios, indeed this ratio is a key parameter of BMP tests [1]. Second, the BMP is modelled with both linear and non-linear regressions and the results are compared to literature data.

Nomenclature

TS	total solid
VS	volatile solid
B	biodegradability
Y_{max}	methane production potential ($Nl_{CH4}.kg_{COD}^{-1}$)
t	time (days)
Y(t)	cumulative methane production ($Nl_{CH4}.kg_{COD}^{-1}$)
k	kinetic constant (day^{-1})
R	methane production rate ($Nl_{CH4}.kg_{COD}^{-1}.days^{-1}$)
λ	lag-phase time (days)
$Y_{m,i}$	measured cumulative methane production ($Nl_{CH4}.kg_{COD}^{-1}$)
$Y_{c,i}$	calculated cumulative methane production potential ($Nl_{CH4}.kg_{COD}^{-1}$)
n	number of measurements during the experiment

2. Materials and methods

2.1. The Inoculum and substrate

The vinasse and the inoculum come from the active mesophilic biogas plant of the sugar-cane distillery Rivière du Mât (Saint-Benoit, Reunion Island). They come from the same industrial plant, thereby the sludge is already acclimatized to the vinasse. The inoculum is stored at ambient temperature and incubated at 37°C before the BMP tests, which is the process temperature (mesophilic conditions). Regarding the vinasse, it is stored at -20°C and then at 4°C before the tests.

2.2. The physico-chemical characterisation

The substrate and inoculum are homogenised with the Ultra turrax IKA T25 digital at 12,000rpm for 10min before the characterisation tests. We carried out TS, VS, pH, chemical oxygen demand (COD), total organic carbon (TOC), VFA, ammonium (Am), alkalinity (Alk) and Kjeldahl nitrogen (Ni) measurements. The TS content is obtained after drying 20g of the samples for 24h at 105°C and the VS content after burning the dried samples for 4h at 550°C. The chemical tests were conducted on the Hach Lange DR5000 Spectrophotometer, using the Hach Lange tests LCK 914 (COD), LCK 381 (TOC), LCK 365 (VFA), LCK 303 (Am), LCK 362 (Alk) and LCK 338 (Ni). The physico-chemical characteristics of the vinasse and the sludge are given in the Table 1. The C/N ratio is 15.20, which is in the range of optimum C/N ratio from the study, i.e. 10-30, carried out by (Yadvika and al.) [7]. Nevertheless, other authors reported supper range, i.e. 16-25 and 25-35 [8].

Table 1. Physico-chemical characteristics of vinasse and inoculum.

Characteristics	TS %	VS %	pH	COD $g_{O2}.L^{-1}$	TOC $mg.L^{-1}$	VFA $mg.L^{-1}$	Am $mg.L^{-1}$	Alk $mg_{CaCO3}.L^{-1}$	Ni $mg.L^{-1}$	C/N ratio
Inoculum	2.25	0.97	4.84	9.93	-	-	517.67	-	-	-
Vinasse	6.64	4.04	7.57	79.67	27646.11	3045.00	239.00	3651.25	1815.00	15.20

2.3. The BMP tests

The BMP tests are carried out using the Automatic Methane Potential Test System II (AMPTS II - Bioprocess Control). We refer to the last recommendations for the BMP test organisation [1]. The digestion is carried out in mesophilic regime at 37°C. The total volume of the digesters is around 590mL. The operating volume is 400mL. The digesters volume are adjusted with distilled water in order to have the same test volumes. Then, the headspace is flushed with nitrogen gas. The experiment includes the substrate tests, the positive tests and the blank tests. All the tests are carried out in triplicates. The BMP value is expressed in the volume of methane produced per gram of organic matter which is expressed in COD as the vinasse is liquid.

The studied substrate to inoculum ratios (g_{COD}/g_{VS}) are 0.5 (ratio A), 0.75 (ratio B), 1 (ratio C) and 2 (ratio D). These ratios correspond to inoculum to substrate ratios (g_{VS}/g_{VS}) of 3.9, 2.6, 2.0 and 1.0. The digesters preparation is summarised in the following table.

Table 2. Digesters preparation for BMP tests.

Ratio	Inoculum volume ml	Inoculum VS g	Substrate	Substrate COD g	Substrate VS g	I/S ratio VS/VS	S/I ratio COD/VS
A	300	2.91	Vinasse	1.46	0.74	3.9	0.50
B	300	2.91	Vinasse	2.18	1.11	2.6	0.75
C	300	2.91	Vinasse	2.91	1.47	2.0	1.0
D	300	2.91	Vinasse	5.82	2.95	1.0	2.0

2.4. The biodegradability

The biodegradability is estimated by the following equation:

$$B = \frac{Y_{\max}}{350} \tag{1}$$

Where 350 represent the theoretical maximum biodegradability of methane expressed in liters of methane per kilogram of removal COD.

2.5. Regression analysis

In accordance with Kim and al. (2003) anaerobic degradation after initial lag time is limited by the terms associated with substrate and kinetics, which generally could be represented by a first order kinetic law with some assumptions [6]. According to the approach reported by Llabres-Luengo and Mata-Alvarez (1987), the first order linear regression is [9]:

$$Y(t) = -Y_{\max}\left(e^{-kt} - 1\right) \tag{2}$$

It has been shown that the BMP fits with the modified Gompertz equation in case of mono-digestion is [6], [10]:

$$Y(t) = Y_{\max} * \exp\left[-\exp\left(R * \frac{\exp(1)}{Y_{\max}} * (\lambda - t)\right)\right] \tag{3}$$

The root mean square error (RMSE) and determination coefficient R^2 are used to evaluate the model results. The RMSE is expressed as follows:

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(Y_{m,i} - Y_{c,i}\right)^2} \tag{4}$$

3. Results and discussion

3.1. BMP tests performances

The Table 3. shows that the highest methane production is obtained with the ratio D followed by the ratio C, then the ratio B and the ratio A with the lowest production. The methane production increase when the I/S ratio decreases. The methane potential of the vinasse (ratio D) is 109.58 $Nl_{CH4}.kg_{COD}^{-1}$ after 16 days with a biodegradability of 0.31. Consequently, in the case of BMP vinasse test, we should use an I(VS)/S(VS) of 1. As previously said, this ratio (inferior or equal to 1) is recommended for less degradable substrates. Moreover, the maximum methane production is reached between 12 and 19 days for the four ratios.

Table 3. Experimental performances of BMP tests at different I/S ratios.

Ratio	Methane yield $Nl_{CH4}.kg_{VS}^{-1}$	$Nl_{CH4}.kg_{COD}^{-1}$	$Nl_{CH4}.l_{sample}^{-1}$	Maximum production Days	Biodegradability
A	93.96	47.60	3.86	12	0.14
B	162.28	82.64	6.67	15	0.24
C	169.17	85.46	6.72	19	0.24
D	216.18	109.58	8.74	16	0.31

3.2. Results from regression analysis

The Fig. 2. shows the comparison between the measured data, the calculated data with the first order regression and calculated data with modified Gompertz model for each ratio, and the Table 4. shows the coefficients for the two models. The results from the first order regression don't fit exactly with the measured data, indeed, the determination coefficient for the four ratios are comprised between 0.82 and 0.91 and the RMSE between 4.547 and 15.210. However, the results from the modified Gompertz fit very well with the measured data, this time, the determination coefficient for the four ratios is upper than 0.99 and the RMSE lower than 2.6.

Regarding the kinetic constant, the first order regression gives values between 0.1137 and 0.1629 $days^{-1}$ for the ratios A, B and D. The kinetic constant for the ratio C is lower than the others with 0.0883 $days^{-1}$. This lower value is due to a higher lag-phase of 6.247 days. Indeed, the minimum lag-phase for the BMP with ratios A, B and D are

respectively 2.232, 3.728 and 4.110 days. This time is due to the fact that the inoculum is already acclimatised to the vinasse.

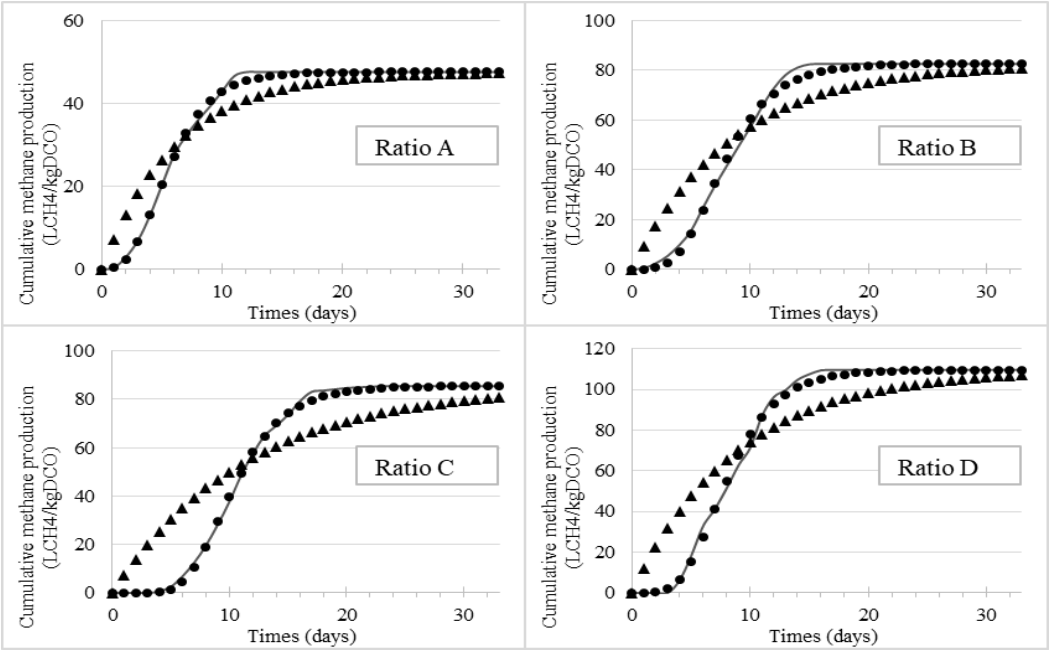

Fig. 2. Comparison between measured data (full line), calculated data with the first order regression (triangles) and calculated data with modified Gompertz model (points) for cumulative methane production at different I/S ratios.

Table 4. First-order regression and modified Gompertz model coefficients.

Model		Unit	Coefficients at different I/S ratios			
			A	B	C	D
First-order regression	Kinetic constant k	Day^{-1}	0.1629	0.1189	0.0883	0.1137
	Y_{max}	$Nl_{CH4}.kg_{COD}^{-1}$	47.60	82.64	85.46	109.58
	Correlation factor R^2		0.9196	0.8844	0.8246	0.8715
	RMSE	$Nl_{CH4}.kg_{COD}^{-1}$	4.547	10.560	14.790	15.210
Modified Gompertz model	R	$Nl_{CH4}.kg_{COD}^{-1}.days^{-1}$	7.35	10.50	10.65	14.23
	λ	Days	2.232	3.728	6.247	4.110
	Y_{max}	$Nl_{CH4}.kg_{COD}^{-1}$	47.60	82.64	85.46	109.58
	Correlation factor R^2		0.998	0.9954	0.9987	0.9963
	RMSE	$Nl_{CH4}.kg_{COD}^{-1}$	0.734	2.100	1.268	2.582

3.3. Comparison of kinetic constant and methane yield with literature data

From Table 5., we notice that the methane yield obtained in our study (ratio D) is low compared to the methanogenic potential of vinasse found in literature. This difference can be explained by a different of chemical compounds concentrations, resulting from different vinasse treatment. Indeed, as previously said, the C/N ratio may not be optimal.

The kinetic constant obtained in this study is 0.1137 $days^{-1}$ and the kinetic constants in literature are 0.17 day^{-1} [11] and 0.30 $days^{-1}$ [12]. Regarding the biodegradability, it is 0.31 in this study, and the maximum biodegradability is 0.73 in another study [11].

Table 5. Comparison of kinetic constant and methane yield of crude vinasse with literature data (mesophilic regime)

Reference	Kinetic constant $Days^{-1}$	Lag-phase Days	COD	Inoculum source	Methane yield $Nl_{CH4}.kg_{COD,Fed}^{-1}$	Biodegradability
Ratio D	0.1137	4.110	79.67 $g_{O2}.L^{-1}$	Sugarcane distillery waste water	109.58	0.31
[12]	0.30	1.99	62.63 $g_{O2}.kg^{-1}$	Pig and cow manure digestate	240	0.69
[11]	0.17	-	20.73 $mg_{O2}.L^{-1}$	reactors used for hydrogen production from sucrose based synthetic wastewater	255.4	0.73
[13]	-	-	68.56 $g_{O2}.L^{-1}$	Brewery wastewater reactor	250	0.71
[14]	-	-	120.221 $g_{O2}.L^{-1}$	Wastewater plant	264.83	0.77

4. Conclusion

This study focused on the variation of the substrate to inoculum ratio in BMP tests. We studied four ratios ranging from 1 to 4 (I_{VS}/S_{VS}). Based on the experimental results, the production of methane increases with the decrease of this ratio, thus the maximum production was obtained with the ratio 1. The methanogenic potential of vinasse is thus 109.58 $Nl_{CH4}.kg_{COD}^{-1}$. This production is obtained in 16 days. The regression analysis showed that the modified Gompertz model is suitable for the methane production of BMP tests with a coefficient of determination greater than 0.99 and an RMSE less than 2.6. Moreover, the first order regression allowed us to determine the hydrolysis constant of vinasse, which varies from 0.0883 to 0.1629 $days^{-1}$.

Compared to the literature, the methane production of vinasse obtained in this study is lower. Future studies will focus on pre-treatment of the vinasse for the optimisation of methane production.

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