ENHANCEMENT OF METHANE YIELD AND BIODEGRADATION RATE OF VINASSE BY USING A NON-CONVENTIONAL TWO-STAGE ANAEROBIC DIGESTION PROCESS

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ABSTRACT

A non-conventional two-stage batch anaerobic digestion process (TSAD) of vinasse was investigated. The novelty lies in the metabolic pathway involved in the hydrolysis and hydrogenesis. Initially, the first hydrolytic/hydrogenic stage was evaluated in a 3-L CSTR at 35 °C and pH-shift control of 6.5 to 5.8. Then, hydrolyzed and acidified vinasses were fed to the second methanogenic stage to evaluate CH_4 potential (YCH_4) and biodegradation rate at 37 °C and pH 7.0. One-single stage (OSAD) was also compared with TSAD. During hydrolysis, it was generated lactate (HLac) and acetate (HAc) of 12.6 and 6.6 g/L, respectively. At the hydrogenic phase, both HLac and HAc were metabolized into H_2 (115.6 NmL/g VS_{added}) and butyrate (5.1 g/L). The maximum YCH_4 (435.9 NmL/g VS_{added}), biodegradation rate (105.8 NmL/d) and COD removal (92.9%) were achieved from TSAD, which were enhanced by 46.2, 89.3 and 8.8%, respectively, compared with those attained from OSAD.

1. INTRODUCTION

Nowadays, the management of vinasse that is the effluent from distillation processes is going towards a bioeconomy approach rather than being considered as a waste. Vinasse can be classified according to the material from which it comes (*e.g.*, sugarcane juice, wine, cassava, beet, agave, etc.), so the characteristics of vinasse depend strongly on the type and quality of material, formulation, and processes [1]. However, regardless of vinasse type, vinasse is *per se* an acidic-brown color by-product with high organic content in terms of chemical oxygen demand (COD) and biochemical oxygen demand (BOD), and high inhibitory compounds such as phenolic compounds and melanoidins [2–4]. In Mexico, the production of Tequila, which is a traditional alcoholic beverage, is one of the most important activities for the economy of the country. In 2017, Mexican distillers produced around 271.4 million liters of tequila [5], taking into account that 1 L of produced tequila generates 10–12 L of vinasse [4], approximately 2,700 million liters of vinasse were generated in that year. Because of the large quantities of vinasse generated and its features of environmental pollution, it is mandatory to treat vinasse before its final disposal.

Anaerobic digestion (AD) is a well-established technology employed worldwide not only to treat several wastewaters before to discharge but also to recover gaseous energy carriers such as hydrogen (H_2) and methane (CH_4). Since vinasse is a feedstock with a high content of easily acidified compounds and zero alkalinity, perturbations during reactor performance are the main bottlenecks of the AD of vinasse in one-single stage (OSAD), resulting in low CH_4 yields (YCH_4) and ineffective organic removal. In an attempt to overcome this problem, it has been suggested the use of two-stage processes (TSAD) [6,7]. According to Ruggeri et al. (2015), TSAD consists in the separation of natural ecology and metabolism into H_2-producing bacteria (HPB) and methanogens. Thus, TSAD not only offers the possibility of producing CH_4 but also H_2 from organic-rich wastes. Compared with one-single stage, TSAD could result in more flexible operation, higher energy yield, stability and biodegradation rate,

and shorter hydraulic retention time in the methanogenic stage [8].

In TSAD processes, most of the literature reports the separation of the AD into a first stage comprised of the hydrolytic and acidogenic phases, and a second one comprised of the acetogenic and methanogenic phases. However, whether favoring the hydrolysis over acidogenesis (or vice versa) might lead to a better methanization, is still an unsolved question [8]. To answer this issue, it must be considered the intermediate metabolic products (gaseous and non-gaseous) generated during hydrolysis and acidogenesis for the further optimization in the second methanogenic stage. Generally, during the conventional hydrolysis and acidogenesis, the most common soluble metabolic products (SMPs) formed are acetate (HAc), propionate (HPr), butyrate (n-HBu), ethanol, butanol, and others at lower concentrations [8]. Lactate (HLac) has been reported to be important in the AD [9–11]. The biochemical reaction to produce CH_4 from HLac could lead to higher available energy in comparison with other SMPs [12]. However, high HLac may result in HPr accumulation, thereby decreasing the methanogenic performance [13,14]. Favoring hydrogenotrophic pathway is needed to avoid HPr accumulation [9].

Recently, it has been reported that HLac also plays a key role in the H_2 production from vinasse [1,3]. García-Depraect et al. (2017) reported a non-conventional pathway to produce H_2 from the co-digestion vinasse and *Nejayote*, where the complex carbohydrates were transformed into HLac and HAc as a first step (hydrolysis). These compounds were further metabolized to n-HBu and H_2 during the second step (hydrogenesis). However, there is a lack of information on testing the feasibility of producing CH_4 from this non-conventional hydrolysis/hydrogenesis. Thus, this study aimed to investigate the influence of hydrolysis/hydrogenesis via the HLac-HAc pathway on the performance of a TSAD treating vinasse, in terms of methane yield (YCH_4) and biodegradation rate.

2. MATERIALS AND METHODS

2.1. Hydrolytic/hydrogenic stage

2.1.1. Substrate

The first hydrolytic/hydrogenic stage was fed with vinasse from a tequila factory located in Tequila, Jalisco, Mexico. Fresh vinasse was collected in plastic containers and stored at 4 °C until use. The physicochemical characteristics are summarized in Table 1.

Table 1. Physicochemical characteristics of raw vinasse used in the experiments.

Parameter	Units	Value
Total COD	mg/L	58,450 ± 2,899.1
BOD	mg/L	28,167 ± 548.5
BOD/COD		0.48
Total nitrogen	mg/L	265 ± 21.2
Total phosphorous	mg/L	41.0 ± 2.8
TS	mg/L	41.2 ± 2.3
VS	mg/L	31.9 ± 5.1
TDS	mg/L	28.07 ± 2.2
TSS	mg/L	13.2 ± 0.2
pH		3.6 ± 0.03
Total alkalinity	mg $CaCO_3$/L	Negligible
Total acidity	mg $CaCO_3$/L	3,862.2 ± 47.3
TRS	mg/L	11,037.3 ± 25.4
Total carbohydrates	mg/L	18,698.2 ± 22.2
Sulfate	mg/L	820.6 ± 2.3
Total protein	mg/L	228.9 ± 14.35
Total phenols	mg GAE/L	1,551.2 ± 53.03

TS: total solids; VS: total volatile solids; TDS: total dissolved solids; TSS: total suspended solids; TRS: total reducing sugars; GAE: gallic acid equivalent.

2.1.2 Inoculum

A mixed culture (ATCC PTA-124566) mainly composed of lactic acid bacteria (LAB), acetic acid bacteria (AAB) and HPB was employed to inoculate the first hydrolytic/hydrogenic stage.

2.1.3 Experimental set-up

Triplicated batches were carried out to investigate how vinasse is metabolized at the first hydrolytic/hydrogenic stage. Liquor from the hydrolysis and hydrogenesis are hereinafter referred to as hydrolyzed and acidified vinasse, respectively. The fermentations were conducted in a 3-L CSTR with a working volume of 2 L. The operational pH value was fixed at 6.5 till the beginning of the acceleration phase referred to

H_2 production, then it was shifted and maintained constant to 5.8 till the end of the test. It has been observed that by applying this two-stage pH control strategy the extent of lag time is reduced and H_2 productivity and yield enhanced (García-Depraect et al., unpublished data). Sodium hydroxide (10 N) or sulfuric acid (3.5 N) solutions were used to control the pH culture. The temperature of the reactor was kept at 35 °C, while mixing was set at 500 rpm. The reactor was fed with 200 mL of activated inoculum and 1,800 mL of raw vinasse (in this work the term "raw" indicates the substrate without dilution and pretreatment for solids removal). Only nitrogen (NH_4Cl 2.4 g/L) and iron ($FeSO_4 \cdot 7H_2O$ 0.05 g/L) sources were supplemented. Free-oxygen conditions within the reactor were achieved by the benefits of the inoculum itself, thus it was not necessary to apply artificial techniques such as flushing gas or adding reducing agents. The accumulated volume of biogas was measured continuously using the μFlow digital biogas meter (Bioprocess control™, Lund, Sweden). Gas samples were taken periodically from the headspace of the reactor to analyze the composition of H_2, CH_4 and CO_2 by gas chromatography. Liquid samples were collected regularly to perform further analysis. Two different substrates were obtained from the first hydrolytic/hydrogenic stage: (1) hydrolyzed vinasse, which is the vinasse came from the beginning of the acceleration phase referred to the H_2 production, and (2) acidified vinasse, which was obtained once the production of H_2 took place (end of the stationary H_2 production phase).

2.2 Methanogenic stage

2.2.1 Substrate

Three different substrates were tested in the second methanogenic stage, namely hydrolyzed vinasse, acidified vinasse, and raw vinasse as the control (OSAD).

2.2.2 Inoculum

The methanogenic inoculum was anaerobic granular sludge, which was obtained from a stable up-flow anaerobic sludge blanket reactor treating vinasse at mesophilic conditions (35 ± 2 °C) from a tequila factory. Since the treated effluent had the following quality criteria: pH, 7.2 ± 0.2; SMPs, 0.12 ± 0.01 g HAc/L, ammonium 26.5 ± 0.7 mg/L; alkalinity, 3,032.4 ± 166.2 mg $CaCO_3$/L; VS/TS ratio, 0.8 ± 0.06, it can be inferred that the inoculum used had good quality characteristics, as recommended by [2].

2.2.3 Experimental set-up

The YCH_4 of the different substrates (*i.e.*, hydrolyzed vinasse, acidified vinasse and raw vinasse) was evaluated by using the Automatic Methane Potential Test System II (AMPTS, Bioprocess control™, Lund, Sweden). The biochemical methane potential (BMP) test was conducted following the recommendations described by [2,3]. Particularly, the operational conditions were as follows: working volume, 400 mL; temperature, 37 °C; mixing speed, 120 rpm (mixer on time, 60 s; mixer off time, 180 s); and inoculum to substrate ratio (on a VS basis), 4. No addition of nutrients was performed; however, since vinasse lacks alkalinity, sodium bicarbonate was added to provide buffering capacity to the system 5 g $CaCO_3$/L. The pH was controlled at 7.0 ± 0.2 using NaOH (5 N) throughout the operational time. Each substrate was evaluated in triplicate. Besides, blank reactors without substrate addition were run to measure the amount of endogenous CH_4 evolved from the inoculum. The endogenous CH_4 production from blanks was subtracted from the total CH_4 produced from substrates. Glucose was used as a model substrate to validate the quality of the inoculum used. The reactors were sealed with rubber stoppers, and then a leakage test was carried out to prevent biogas loss. The headspace was flushed with inert nitrogen gas (0.5 L/min) during 1 min to ensure free-oxygen conditions. Gas samples were taken daily from the reactor's headspace to analyze biogas composition. Liquid samples were taken to perform further analyses including COD, SMPs, ammonia, among others.

2.3 Analytical methods

Physicochemical parameters including COD, BOD, pH, total alkalinity, total acidity, total nitrogen, total phosphorus, sulfate, and solids were analyzed according to the Standard methods [4]. Total carbohydrates, total phenols, and biogas composition were analyzed as previously reported [1]. Ammonia was measured with the HACH kit 2606945 (HACH Company, Loveland, USA). Protein was measured by the method of Bradford [5]. The concentration of biomass was estimated based on the content of intracellular protein of a given weight of dry biomass (0.29 g protein/g dry cell biomass). For the determination of SMPs including HLac, HAc, n-HBu, iso-butyrate (i-HBu), valerate (HVal) and HPr, previously acidified samples (concentrate H_2SO_4) were filtrated through a 0.2 µm membrane and were then analyzed by high performance liquid chromatography (HPLC) with a Varian ProStar system model 230 (Varian Analytical Instruments, California, USA). The HPLC was equipped with a Varian 325 UV/VIS detector operated at 210 nm and a column Aminex HPX-87H (300 mm x 7.8 mm internal diameter, 9 µm; Bio-Rad, California, USA) operated at 55 °C. Sulfuric acid (5 mM) was used as the mobile phase at a flow rate of 0.5 mL/min.

2.4 Data analysis

The performance of the first hydrolytic/hydrogenic stage was evaluated in terms of YH_2, H_2 production rate, TRS consumption, biomass growth, hydrolysis degree, and metabolic profile. To characterize the kinetics of H_2 production, the cumulative H_2 production was fitted to the modified Gompertz model (Eq. 1) where, H is the cumulative H_2 production (NmL), λ is the lag phase time (h), t is the culture time (h), P is the maximum cumulated H_2 production (NmL), Rm is the maximum H_2 production rate (NmL/h), and $e \approx 2.718$. The performance of the second methanogenic stage was evaluated in terms of the YCH_4 and rate, VS reduction, COD removal, metabolic profile, and microbial structure. YCH_4 was calculated according to Eq. (2) where BMP is the volume of CH_4 produced per gram VS of substrate added (NmL CH_4/g VS_{added}), V_S is the mean value of the accumulated volume of CH_4 produced by the sample (NmL), V_B is the mean value of the accumulated volume of CH_4 produced by the blank (NmL), m_{IS} is the total amount of inoculum in the sample (g VS), m_{IB} is the total amount of inoculum in the blank (g VS), and m_{VS} is the total amount of organic material (g VS) [7]. Dixon's test was used to eliminate a single outlier from measurements [2]. YCH_4 was modeled by using the modified Gompertz model described in Eq. (1) (note that the term H_2 in this equation was replaced by CH_4). The coefficient of determination (R^2) was used to assess the goodness of fits. The kinetic parameters were estimated with the solver function in Microsoft Excel version 12 (Microsoft, Inc., USA). All data reported are the average and standard deviation of triplicate experiments. Analysis of variance (Tukey test with a significance level of 5%) was used to compare data. Biogas was normalized at standard temperature and pressure (1 atm, 0 °C).

$$H = P \exp\{-\exp[(Rm \cdot e)/P\,(\lambda - t) + 1]\} \quad (1)$$

$$BMP = [V_S - \left(V_B \cdot \frac{m_{IS}}{m_{IB}}\right)]/m_{VS} \quad (2)$$

3. RESULTS AND DISCUSSION

3.1. First hydrolytic/hydrogenic stage

3.1.1 Performance

Fig. 1 shows the performance of the first hydrolytic/hydrogenic stage in function of time, more particularly, the H_2 evolved, TRS consumption, biomass growth and hydrolysis degree. This first hydrolytic/hydrogenic stage lasted 94 h and was divided into two phases. The hydrolytic phase, which was associated with no H_2 production with an extent of 49 h, and the hydrogenic phase, which was associated with the H_2 production with an extent of 45 h. The maximum H_2 production was 7,542 ± 496.7 NmL, resulting in a H_2 yield of 115.6 ± 8.5 NmL/g VS_{added}, which was higher as compared with other studies evaluating the H_2 production from vinasse [1–

3]. The different characteristics of the substrate, type of inoculum, mode of operation (batch, continuous, *etc*.), working conditions (temperature, pH, *etc*.) may explain the differences in the H_2 yield obtained in this study and previous studies. The total consumption of TRS was 62.9%, of which 47.4 and 15.5% constituted the hydrolytic and hydrogenic phases, respectively. The total biomass growth was 0.58 g/L. However, lesser biomass growth was achieved at the hydrolytic phase (0.25 g/L), when compared with that formed at the hydrogenic phase, which was 0.33 g/L. The total hydrolysis degree was 45.3%, of which 85.3% was attained during the hydrolytic phase, and the remaining 14.7% was observed during the hydrolytic phase.

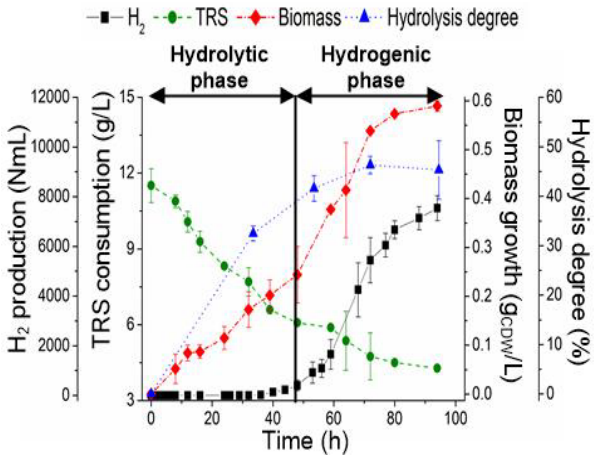

Fig. 1. Cumulative H_2 evolved, TRS consumption, biomass growth and hydrolysis degree during the first hydrolytic/hydrogenic stage. CDW: cell dry weight.

3.1.2 Soluble metabolic products

Fig. 2A illustrates the profile of the main SMPs during the first hydrolytic/hydrogenic stage. As shown in Fig. 2A, the major SMPs at the hydrolytic phase were HLac and HAc, contrarily, the presence of n-HBu was negligible during this phase. On the other hand, during the hydrogenic phase, both HLac and HAc were diminished with a concomitant generation of n-HBu. Other SMPs such as i-HBu, HPr, HVal, were found at very low concentrations (< 0.2 ± 0.1 g/L) throughout the whole experiment. Fig. 2B summarized the SMPs concentration at three key different times of the overall process: 0 h (beginning of the test), 49 h (end of the hydrolytic phase/start of hydrogenic phase) and 94 h (end of the hydrogenic phase). At 0 h, the concentrations of HLac, HAc and n-HBu were 5.9 ± 4.3, 3.9 ± 1.7 and 0.03 ± 0.02 g/L, respectively. At 49 h, 12.6 ± 2.03 and 6.6 ± 1.9 g/L of HLac and HAc were reached. n-HBu at 49 h was not detected. At 94 h, the concentrations of HLac, HAc and n-HBu were 2.7 ± 2.5, 3.2 ± 0.2 and 5.1 ± 1.1 g/L, respectively. Based on the patterns of SMPs, it can be concluded that in this study, the formation of H_2 implies the consumption of HLac and HAc, with simultaneous generation of n-HBu. The findings are in agreement with a previous report [1]. As expected, the microbial community determines the SMPs. The high amounts of HLac and H_2 were correlated with LAB (*i.e.*, *Sporolactobacillus* and *Lactobacillus*) and HPB (*i.e.*, *Clostridium*), respectively (data not shown).

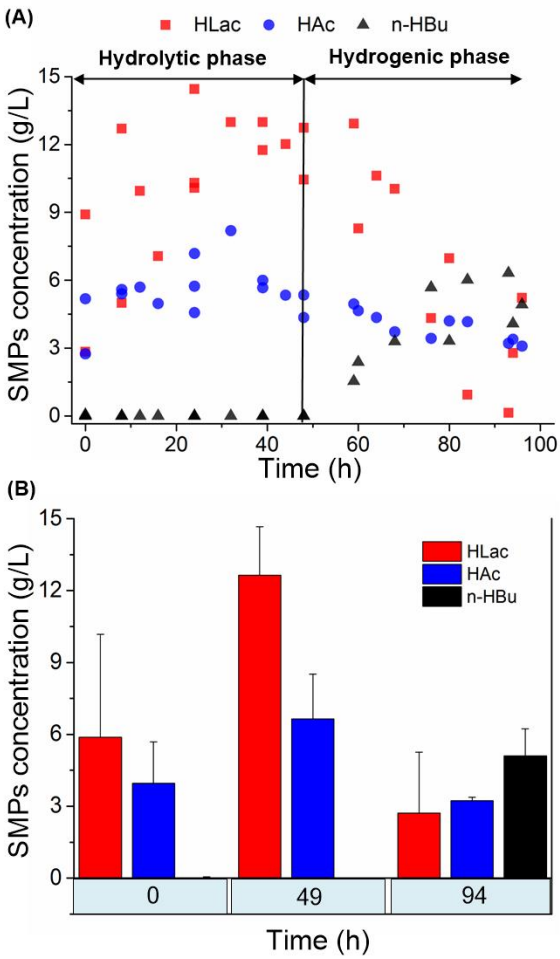

Fig. 2. (A) Profiles of the main SMPs during the first hydrolytic/hydrogenic stage over time. (B) SMPs concentration at the beginning of the test (0 h), end of

the hydrolytic phase/start of hydrogenic phase (49 h) and, end of the hydrogenic phase (94 h).

3.1.3 Kinetics

The cumulative H_2 production was fitted to the modified Gompertz model. The high R^2 obtained (> 0.99) indicates the good fitting between the experimental and modeled data. The extent of lag time was found to be 52.3 ± 6.8 h. While, *P* and *Rm* were 7,877.4 ± 1,758.1 NmL and 223.6 ± 21.8 NmL/h, respectively. Dividing the *Rm* by the working volume it was possible to obtain the maximum volumetric H_2 production rate ($VHPR_{max}$) of 111.8 NmL/L-h.

3.2. Second methanogenic stage

3.2.1 Performance

Fig. 3 shows the YCH_4 for the different types of vinasse tested. In general, the YCH_4 obtained from all assays was significantly different to each other. The highest YCH_4 of 435.9 NmL/g VS_{added} was achieved from the hydrolyzed vinasse, followed by the acidified vinasse (376.3 NmL/g VS_{added}). It must be stressed that OSAD of raw vinasse resulted in the lowest YCH_4 of 298.2 NmL/g VS_{added}. Other YCH_4 expressed in different units are shown in Table 2, which summarizes the performance of the process at the second methanogenic stage. The high YCH_4 obtained from the hydrolyzed vinasse could be explained by three different approaches. First, in this phase, there was no loss of reducing equivalents and carbon in the form of H_2 and CO_2, respectively. Second, the SMPs products formed (HLac and HAc) during this phase might be beneficial for the further CH_4 formation in the second methanogenic stage. In this study, high amounts of HLac did not result in HPr accumulation. In fact, there was no accumulation of SMPs at the end of the experiment in all conditions evaluated (data not shown), indicating that BMP tests were performed efficiently. And third, the microorganisms such as LAB and HPB might favor the syntrophic interactions towards the CH_4 formation through the hydrogenotrophic pathway rather than the acetoclastic one (data not shown). All BMP tests lasted 20 d, however, the t_{90} (time needed for achieving 90% of total CH_4 production) varied according to the type of vinasse. The hydrolyzed vinasse had a t_{90} of 5.3 d, which resulted significantly lower than the other conditions tested. The average concentration of CH_4 in the biogas during the entire BMP test was 46.3, 50.02, and 51.4% for the hydrolyzed, acidified and raw vinasse, respectively. However, during the exponential biogas production phase, the CH_4 concentration ranged from 59.2 to 65%. TSAD resulted in a significant increase in the removal of COD in comparison with that observed from OSAD.

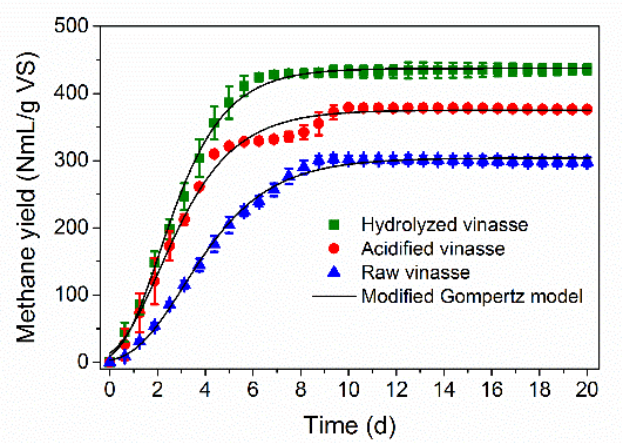

Fig. 3. Methane yields for the hydrolyzed and acidified vinasse in a function of time. Raw vinasse was tested as a control. Solid lines fitted the experimental values through the modified Gompertz model.

3.2.2 Kinetics

The YCH_4 was fitted with the modified Gompertz model. Table 3 summarizes the kinetic parameters obtained from Eq. 1. Based on the values of R^2 (> 0.99), the modified Gompertz model seems to be good for fitting the experimental data. Lower correlation coefficients were obtained from a first-order kinetics model (data not shown). Regarding the extent of lag phase, no significant differences were found between the different conditions tested, the average λ was 0.7 d. In contrast, *P* and *Rm* were strongly dependent on the condition evaluated. The highest *P* (437.1 NmL) was obtained when hydrolyzed vinasse was employed. While the lowest *P*

(303.7 NmL) was obtained from the raw vinasse (OSAD). The acidified vinasse yielded 374.9 NmL. The *Rm* among the hydrolyzed (105.8 NmL/d) and acidified (80.9 NmL/d) vinasse did not show significant differences. However, a significant decrease of the *Rm* was observed from OSAD (55.9 Nm/d).

In conclusion, higher ultimate YCH_4 and biodegradation rate can be achieved by using the TSAD proposed herein. To determine which approach (hydrolysis or hydrogenesis) is better as the first stage, it must be considered the target of the process. For instance, the first scenario "hydrolysis + methanogenesis" can result in value-added SMPs such as HLac, and higher YCH_4. In the second scenario "hydrogenesis + methanogenesis" high amounts of H_2, value-added SMPs such as n-HBu, and CH_4 can be achieved. Additionally, it is necessary to perform a mass and energy balance of the overall process at each scenario.

Table 2. Summary of process performance at the second methanogenic stage for the different types of vinasse tested

Parameter	Units	Hydrolyzed vinasse	Acidified vinasse	Raw vinasse
YCH_4	NmL/g VS_{added}	[a]435.9 ± 8.2	[b]376.3 ± 4.1	[c]298.2 ± 6.8
YCH_4	NmL/g COD_{added}	[a]215.4 ± 9.6	[b]196.4 ± 1.6	[c]166.09 ± 3.8
YCH_4	NmL/g $COD_{removed}$	[a]269.8 ± 17.2	[b]226.8 ± 0.8	[a]261.2 ± 19.0
YCH_4	$NmL/mL_{substrate}$	[a]9.9 ± 0.4	[b]8.2 ± 0.1	[a]9.57 ± 0.2
[1]CH_4 t_{90}	d	[a]5.3 ± 0.5	[b]8.0 ± 0.9	[b]7.3 ± 0.3
[2]CH_4 in biogas	%	46.3 ± 18.9	50.02 ± 14.3	51.4 ± 18.6
[3]Biogas purity		0.9 ± 0.2	1.0 ± 0.2	1.1 ± 0.2
COD removal	%	[a]91.4 ± 0.2	[a]92.9 ± 2.8	[b]85.4 ± 1.7

[1] time needed for achieving 90% of total CH_4 production; [2]Average CH_4 concentration during all BMP test. [3] CH_4/CO_2; Equal letters in the same row indicate no significant difference ($\alpha = 0.05$);

Table 3. Kinetics parameters of the second methanogenic stage for the different types of vinasse tested

Model	Hydrolyzed vinasse	Acidified vinasse	Raw vinasse
P (NmL)	[a]437.1 ± 8.1	[b]374.9 ± 0.7	[c]303.7 ± 6.5
Rm (NmL/d)	[a]105.8 ± 11.5	[a]80.9± 10.4	[b]55.9 ± 2.6
λ (d)	[a]0.5 ± 0.3	[a]0.4 ± 0.5	[a]1.1 ± 0.1
R^2	0.9978 ± 8.5 $x10^{-4}$	0.9952 ± 2.5 $x10^{-3}$	0.9975 ± 8.3 $x10^{-4}$

Equal letters in the same row indicate no significant difference ($\alpha = 0.05$).

3. CONCLUSIONS

This study evaluated the TSAD of vinasse, where the hydrolysis and acidogenesis phases (which were the influent of the second methanogenic stage) followed a non-conventional metabolic pathway. Based on the results, the hydrolysis of vinasse was associated with a high hydrolysis degree (45.3%), high accumulation of HLac (12.6 g/L) and HAc (6.6 g/L), and with no loss of reducing equivalents and carbon in the form of H_2 and CO_2, respectively. In contrast, the hydrogenesis of vinasse resulted in high amounts of H_2 (115.6 NmL/g VS_{added}) and n-HBu (5.1 g/L). At the second methanogenic stage, both hydrolyzed and acidified vinasse resulted in higher YCH_4, biodegradation rate and COD removal of 435.9 and 376.3 NmL/g VS_{added}, 105.8 and 80.9 NmL/d, and 91.4 and 92.9%, respectively, when compared to those obtained from OSAD (298.2 NmL, 55.9 NmL/d and 85.4%). In conclusion, this study proved the technical feasibility of using the non-conventional metabolic pathway to

perform the hydrolysis and/or hydrogenesis of vinasse as the first stage of a TSAD. This metabolic pathway may appear be the ideal because of the physicochemical features of vinasse such as high organic content and high amounts of HLac and HAc observed during collection, transport and storage. It should be highlighted that the conclusions drawn are limited to the conditions used in this study. Further experiments aimed at investigating the possible benefit of using this non-conventional pathway for long-term operation need to be addressed.

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Nomenclature

BMP Volume of methane produced per gram volatile solid of substrate added
H Cumulative hydrogen production
m_{IB} Total amount of inoculum in the blank
m_{IS} Total amount of inoculum in the sample
m_{VS} Total amount of organic material
P Maximum cumulated hydrogen production
Rm Maximum hydrogen production rate
t Culture time
V_B Value of the accumulated volume of methane produced by the blank
V_S Value of the accumulated volume of methane produced by the sample
AAB Acetic acid bacteria
AD Anaerobic digestion
AMPTS Automatic methane potential test system
BOD Biochemical oxygen demand
CH_4 Methane
CO_2 Carbon dioxide
COD Chemical oxygen demand
CRT Mexican Tequila Regulatory Council
CSTR Continuously stirred tank reactor
H_2 Hydrogen
HAc Acetate
HFor Formate
HLac Lactate
HPB Hydrogen-producing bacteria
HPLC High-performance liquid chromatography
HPr Propionate
HVal Valerate
i-HBu Iso-butyrate
LAB Lactic acid bacteria
n-HBu Butyrate
SMPs Soluble metabolic products
t_{90} Time needed to achieve 90% of the total hydrogen (or methane) production
TRS Total reducing sugars
TS Total solids
TSAD Two-stage anaerobic digestion
UV/Vis Ultraviolet-visible detector
VHPR Volumetric hydrogen production rate
VS Total volatile solids
YCH_4 Methane yield
YH_2 Hydrogen yield

Greek Letters

e Euler's constant
λ Lag phase time

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