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EFFECT OF PROCESS PARAMETERS FOR ENHANCED BIOHYDROGEN PRODUCTION FROM VINASSE

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ABSTRACT

Vinasse wastewater, which is a suitable feedstock for H_2 production, is commonly characterized by a high content of organic acids such as lactate (HLac) and acetate (HAc). This study evaluated the influence of the initial total solids content (TS), substrate concentration and inoculum addition on the H_2 production from vinasse by a mixed culture following the HLac-HAc pathway. Batch experiments were performed in a 3-L completely-stirred tank reactor with a two-stage pH-shift control (6.5 to 5.8) and at 35 °C. The results showed that the process performance was impacted by the inoculum addition > substrate concentration > TS. The highest H_2 yield was 124.3 NmL/g VS_{added}. The HLac-HAc pathway could be a feasible way to produce H_2 from vinasse.

1. INTRODUCTION

Biological hydrogen (H_2) production is considered one of the most promising clean energy resources of the future. H_2 production from vinasse wastewater is gaining increasing attention given its high organic matter content and abundant availability. However, despite great efforts have been made towards the H_2 production from vinasse even from different raw materials [1–3], there are still research gaps that need to be addressed to use vinasse in a technically feasible way.

The main bottleneck is the low H_2 yield caused by perturbations during reactor performance, which in turn are associated with the presence of large amounts of organic acids such as lactate (HLac) and acetate (HAc). These acids can be generated during the processes of collection, transport, and storage. Therefore, there is a need to seek practical solutions to deal with this scenario. Although, the production of HLac exhibits a zero H_2 balance, under certain conditions there is possible to produce H_2 from HLac and HAc as described the following reaction $HLac + 0.5HAc \rightarrow 0.75n\text{-}HBu + 0.5H_2 + CO_2 + 0.5H_2O$ (Eq. 1) [4,5], which differs from the direct production of H_2 from carbohydrates glucose + $2H_2O \rightarrow 2HAc + 2CO_2 + 4H_2$ (Eq. 2), and glucose $\rightarrow$ n-HBu + $2CO_2 + 2H_2$ (Eq. 3) [2,6,7]. In this context, this study proposed the production of H_2 via the HLac-HAc pathway as one possible alternative to cope with the high quantities of HLac and HAc contained in vinasse. However, there is a lack of knowledge regarding the production of H_2 through the HLac-HAc pathway, especially with complex substrates. Thus, this study aimed to investigate the effects of the initial total solids content (TS), substrate concentration and addition of inoculum, on the batch H_2 production from vinasse via the HLac-HAc pathway.

2. MATERIALS AND METHODS

2.1 Inoculum

A stock culture of the inoculum ATCC PTA-124566 was used for H_2 production.

2.2 Substrate

Vinasse from a tequila factory located in Tequila, Jalisco, Mexico was used as the substrate. Vinasse was collected in plastic containers and stored at 4 °C until used. The main physicochemical characteristics of vinasse are presented in Table 1.

Table1. Physicochemical characteristics of vinasse

Parameter	Value
COD (g/L)	63.1 ± 6.5
BOD (g/L)	29.2 ± 7.9
pH	3.6 ± 0.1
Total nitrogen (mg/L)	220 ± 63.6
Total phosphorous (mg/L)	526.8 ± 165.1
TS (g/L)	43.8 ± 3.6
VS (g/L)	37.4 ± 7.8
TRS (g/L)	10.8 ± 0.3

COD: chemical oxygen demand; BOD: biochemical oxygen demand; VS: total volatile solids; TRS: total reducing sugars.

2.3 H_2-producing reactor

A series of batch experiments were carried out to evaluate the influence of (i) the initial TS, (ii) the substrate concentration, and (iii) the addition of inoculum, on the H_2 production from vinasse via the HLac-HAc pathway. The experiments were performed as follows: (i) By comparing the use of raw vinasse with high TS of 39.9 ± 1.0 g/L and centrifuged vinasse (4,000 rpm for 10 min) with low TS of 34.1 ± 0.2 g/L. Both high and low TS were supplemented with minimal nutrients (NH_4Cl, 2.4 g/L; and $FeSO_4 \cdot 7H_2O$, 0.05 g/L); (ii) By ranging the content of VS from 5.4 ± 0.8 g/L to 39.1 ± 0.04 g/L. Raw and diluted vinasse were supplemented with complete nutrients, including NH_4Cl, 2.4; K_2HPO_4, 2.4; $MgSO_4 \cdot 7H_2O$, 1.5; KH_2PO_4, 0.6; $CaCl_2 \cdot 2H_2O$, 0.15; $FeSO_4 \cdot 7H_2O$, 0.05; (iii) By comparing the use of raw vinasse with and without addition of inoculum (self-fermentation). Both with and without inoculum processes were supplemented only with the minimal nutrients. Triplicate batches for each condition evaluated (i-iii) were conducted in a sterile 3-L complete-mix reactor with a working volume of 2 L (Applikon Biosciences, Schiedam, The Netherlands). The reactor was operated at 35 ± 0.1 °C by using a thermal jacket. Mixing was provided at 500 rpm. Operational pH was kept constant at 6.5 during the hydrolytic stage, then it was shifted to 5.8 until 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). For all conditions except the self-fermentation, it was fed to the reactor 200 mL (10% *v/v*) of inoculum. Cumulative biogas volume was continuously measured with the digital µFlow biogas meter (Bioprocess control, Lund, Sweden). Gas samples were collected and analyzed via gas chromatography for H_2, methane (CH_4) and carbon dioxide (CO_2) composition. Liquid samples were collected in different phases of the reactor operation for further analysis.

2.4 Analytical methods

Physicochemical features of the substrate were analyzed according to Standard Methods [8]. TRS, protein, and biogas composition were analyzed as previously described [1]. Biomass concentration as dry cell weight was estimated based on the relationship between the dry biomass and intracellular protein content, which was 0.29 g protein/g dry cell biomass. Soluble metabolic products (SMPs), including HLac, HAc, butyrate (n-HBu) and propionate (HPr) were measured by high performance liquid chromatography (HPLC) using a Varian ProStar system model 230 (Varian Analytical Instruments, CA, USA), which was equipped with a Varian 325 UV/VIS detector and a column Aminex HPX-87H (300 mm x 7.8 mm id, 9 µm; Bio-Rad, CA, USA). The column temperature was kept at 55 °C. The mobile phase was a solution of sulfuric acid 5 mM at a flow rate of 0.5 mL/min. Analytes were detected by determining absorbance at 210 nm.

2.5 Kinetic analysis

The modified Gompertz model was used to fit the H_2 production (Eq. 4). On the other hand, Monod type model (Eq. 5) was used to describe the variation of H_2 yields as a function of substrate concentration. In Eq. (4), 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 ≈ 2.718. In Eq. (5), $YH_2(S)$ is the H_2 yield (NmL/g VS_{added}) at initial substrate concentration S (g VS/L), YH_2m is

the maximal H_2 yield (NmL/g VS_{added}), and *Ks* is the half-saturation constant (g VS/L).

$$H = P \exp\{-\exp[(Rm \cdot e)/P(\lambda - t) + 1]\} \quad (4)$$

$$YH_2(S) = YH_2m \cdot S / (S + Ks) \quad (5)$$

Biogas volume was corrected to dry gas at standard temperature and pressure (0 °C, 1 atm). Data presented are the mean values of triplicate experiments. ANOVA (Tukey test with a significance level of 5%) was used to compare differences between results.

3. RESULTS AND DISCUSSION

3.1 Effect of initial total solids content

Fig. 1 shows the cumulative H_2 production at low and high TS. Compared with high TS, the low TS resulted in higher H_2 production of 5,732.6 ± 200.7 NmL. As a result, the H_2 yield was enhanced by 47% (114.9 ± 3.7 NmL/g VS_{added} compared to 77.7 ± 3.3 NmL/g VS_{added}). The evolved biogas was comprised of H_2 and CO_2.

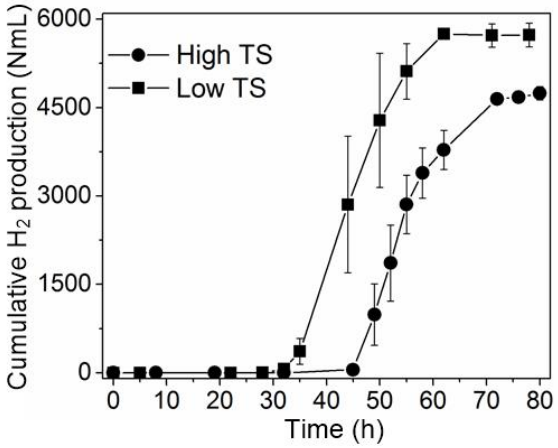

Fig. 1. Effect of TS on H_2 production

For low and high TS, HLac and HAc were the predominant SMPs throughout the hydrolytic phase, which was associated with no H_2 production. However, during the hydrogenic phase, both HLac and HAc were metabolized to H_2 and n-HBu. The maximum amounts of HLac and HAc of 8.2 ± 0.2 g/L and 9.7 ± 0.1 g/L, respectively, were found at high TS, which were 22.4 and 42.3% higher than those attained at low TS. The consumption of TRS did not show a significant difference between low and high TS, indicating that the range of TS tested did not alter the ability of microorganisms to consume sugars. On the other hand, the biomass growth was slightly higher (1.0 ± 0.1 g/L) at low TS as compared with that observed at high TS (0.7 ± 0.2 g/L). It must be stressed that in this study instead of carbohydrates, H_2-producing bacteria (HPB) metabolized HLac and HAc to produce H_2. This could justify the independence of TRS consumption.

The kinetic parameters obtained from the modified Gompertz model are summarized in Table 2. The correlation coefficients higher than 0.99 indicate the quality of the fit. Interestingly, the initial TS also influenced the extent of lag phase and H_2 production rate. It can be drawn that higher solids led to higher substrate loads as VS. However, as no H_2 inhibition was observed because of the substrate concentration (discussed in section 3.2), it seems that the lower amount of H_2 observed at high TS could be a consequence of less favorable conditions in the reactor caused by the particulate material rather than the inhibition by organic overload.

Table 2. Parameters values obtained from the modified Gompertz model for low and high TS

TS	P (NmL)	Rm (NmL/h)	λ (h)	R^2
Low	5,810.5[a] ± 275.4	449.9[a] ± 133.9	38.2[a] ± 3.9	0.9994
High	4,858.5[b] ± 164.3	286.6[a] ± 74.6	44.7[a] ± 4.5	0.9997

Equal letters in the same column indicate no significant difference (α = 0.05).

Based on the previous results, it can be concluded that the partial removal of solids enhanced not only the H_2 yield and volumetric H_2 production rate (VHPR) but also the adaptation period of bacteria.

3.2 Effect of substrate concentration

Fig. 2 presents the H_2 yields as a function of the concentration of substrate. As indicated in Fig. 2, the highest (124.3 ± 9.01 NmL/g VS_{added}) and lowest (39.7 ± 10.9 NmL/g

VS_{added}) H_2 yield was observed at both limits of tested substrate concentration range. The evolved biogas was composed of only H_2 and CO_2 in all experiments. On the other hand, no significant differences were found in the TRS consumption for all conditions evaluated, which was 52.7 ± 4.2% on average. The no dependence of TRS consumption shows that HPB mainly consume HLac and HAc rather than carbohydrates to produce H_2, as previously discussed. The major SMPs were HLac, HAc and n-HBu. Thus, the metabolic pathway for the H_2 production observed herein entails as a first step the degradation of carbohydrates to generate HLac and HAc, and their further consumption to form H_2 and n-HBu. The low amount of H_2 obtained at low substrate concentration could be explained by the fact that the concentrations of SMPs increased with increasing the substrate concentration (data not shown).

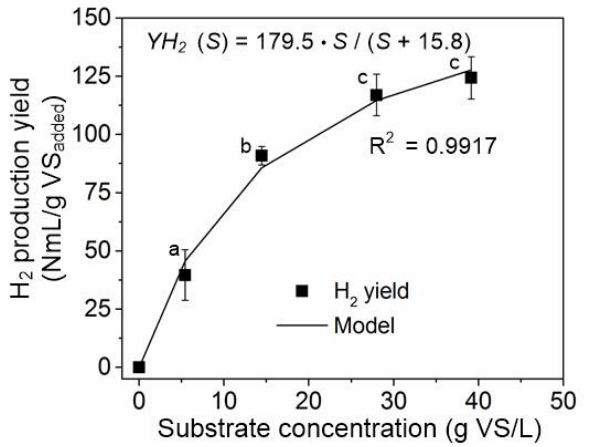

Fig. 2. Effect of substrate concentration on H_2 production. Equal letters indicate no significant difference (α = 0.05).

There are no general rules to fix an appropriate range of the substrate concentration since inhibition threshold depends on numerous factors. Commonly, HPB may exhibit low metabolic activity at very low substrate concentration, thereby decreasing the H_2 production. Contrarily, too high concentrations may lead to inhibition due to organic overload [9,10]. Here, it is worth noting that the higher substrate concentration of 39.1 ± 0.04 g VS/L (57.7 g COD/L) did not result in inhibition. Similar results were reported by Buitrón et al. (2014), who obtained the highest VHPR of 57.4 ± 3.9 NmL/L-h by increasing the substrate concentration from 2 to 16 g COD/L.

The variation of H_2 yields as a function of substrate concentration was described by the Monod model. The Monod type model fitted the experimental yields with a value of R^2 of 0.9917. The estimated parameters were 179.5 NmL/g VS_{added} for YH_2m and 15.8 g VS/L for *Ks*. From results, it is evident that substrate concentration fairly impacts on the H_2 production from vinasse via the HLac-HAc pathway. It is recommended to fix an appropriate concentration to avoid underestimations of H_2 production.

3.3 Effect of inoculum addition

H_2 production was not observed in the self-fermentations throughout the operation time (7-12 d). In contrast, the fermentations with external inoculum addition yielded 2,371.4 ± 56.9 NmL/L with a t_{90} (time needed to achieve 90% of the total H_2 production) of 67.6 h. The absence of H_2 could be explained by the fact that raw vinasse was not pre-treated to select only desirable bacteria. Kim et al. (2009) evaluated three different pre-treatments (heat, acid, and alkali) on the H_2 production from food waste by self-fermentation. Because carbohydrate removal efficiencies in all cases did not show significant differences, but untreated food waste only produced a minimal amount of H_2, the authors inferred that the role of pre-treatment was the selection of microbial population rather than the enhancement of hydrolysis.

Besides the evolved biogas from the reactor, the profiles of dissolved oxygen intake, bacterial growth, and SMPs formed were found to be significantly different for the self-fermentations in comparison with the fermentations aided by external inoculum (data not shown). However, the TRS consumption did not show a significant difference (57.8 ± 3.2%), which agrees with the previously reported [11]. Thus, it can be suggested that the bacteria present in vinasse

lead more energy for cellular maintenance and metabolism than to form new cells. Remarkable differences exist when compared the microbial ecology found in the raw vinasse with that of the inoculum used (data not shown). Because microorganisms are the key actors in the process, it was suggested that the autochthonous microflora present within the vinasse was less specific for H_2 production under the conditions tested. Contrarily, the more specialized microbial structure of the inoculum used in this study was responsible for the proper functioning of the H_2 production through the HLac-HAc pathway.

4. CONCLUSIONS

The present work showed that the HLac-HAc pathway could be feasible and robust via to produce H_2 from vinasse. The results showed that the process performance was impacted by the inoculum addition > substrate concentration > TS content. The microbial community contained in the inoculum used was found to be responsible for the supporting of the fermentative H_2 production from vinasse, attaining the highest H_2 yield of 124.3 NmL/g VS_{added}.

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Nomenclature

ANOVA Analysis of variance
BOD Biochemical oxygen demand
CH_4 Methane
CO_2 Carbon dioxide
COD Chemical oxygen demand
H_2 Hydrogen
H Cumulative hydrogen production
HAc Acetate
HLac Lactate
HPB H_2-producing bacteria
HPLC High-performance liquid chromatography
HPr Propionate
Ks Half-saturation constant
n-HBu Butyrate
P Biogas production potential
Rm Maximum hydrogen production rate
S Initial substrate concentration
SMPs Soluble metabolic products
t_{90} Time needed to achieve 90% of the total hydrogen or methane production
t Culture time
TRS Total reducing sugars
TS Total solids
UV/Vis Ultraviolet/visible detector
VHPR Volumetric hydrogen production rate
VS Total volatile solids
YH_2 Hydrogen yield
YH_2m Maximal hydrogen yield

Greek letters

e Euler's constant
λ Lag phase time

References

[1] García O, Gómez J, León E, López A, A novel biohydrogen production process: Co-digestion of vinasse and *Nejayote* as complex raw substrates using a robust inoculum, *International Journal of Hydrogen Energy*, Vol. 42, No. 9, 2017, pp.5820-5831.

[2] Fuess L, Júnior A, Botelho C, Zaiat M, Temporal dynamics and metabolic correlation between lactate-producing and hydrogen-producing bacteria in sugarcane vinasse dark fermentation: the key role of lactate, *Bioresource Technology*, Vol. 247, 2017, pp. 426-433.

[3] Buitrón G, Kumar G, Martinez A, Moreno G, Hydrogen and methane production via a two-stage processes (H_2-SBR + CH_4-UASB) using tequila vinasses, *International Journal of Hydrogen Energy*, Vol. 39, No. 33, 2014, pp. 19249-19255.

[4] Matsumoto M and Nishimura Y, Hydrogen production by fermentation using acetic acid and lactic acid, *Journal of Bioscience and Bioengineering*, Vol. 103, No. 3, 2007, pp. 236-241.

[5] Tao Y, Hu X, Zhu X, Jin H, Xu Z, Tang Q, Li X, Production of Butyrate from Lactate by a Newly Isolated *Clostridium* sp. BPY5, *Applied Biochemistry and Biotechnology*, Vol. 179, No. 3, 2016. pp. 361-374.
[6] Jo J, Lee D, Park J, The effects of pH on carbon material and energy balances in hydrogen-producing *Clostridium tyrobutyricum* JM1, *Bioresource Technology*, Vol. 99, No. 17, 2008, pp. 8485-8491.
[7] Stavropoulos K, Kopsahelis A, Zafiri C, Kornaros M, Effect of pH on Continuous Biohydrogen Production from End-of-Life Dairy Products (EoL-DPs) via Dark Fermentation, *Waste and Biomass Valorization*, Vol. 7, No. 4, 2016, pp. 753-764.
[8] APHA. Standard Methods for the Examination of Water and Wastewater. Stand Methods 2005:541.
[9] Wang B, Strömberg S, Li C, Nges I, Nistor M, Deng L, Effects of substrate concentration on methane potential and degradation kinetics in batch anaerobic digestion, *Bioresource Technology*, Vol. 194, 2015, pp. 240-246.
[10] Elbeshbishy E, Dhar B, Nakhla G, Lee H, A critical review on inhibition of dark biohydrogen fermentation, *Renewable and Sustainable Energy Reviews*, Vol. 79, 2017, pp. 656-668.
[11] Kim D, Kim S, Shin H, Hydrogen fermentation of food waste without inoculum addition, *Enzyme and Microbial Technology*, Vol. 45, No. 3, 2009, pp. 181-187.