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THE INFLUENCE OF PARTIAL PRESSURE ON HYDROGEN PRODUCTION FROM SUGARCANE VINASSE

Sofia Lück T. [1]; Caroline V. S. Campos [1]; Felipe F. de Almeida[1]; Fabrício E.S. de Lima [1]; Jose L. S. Victor [2]; Bruna Fernandes [1]; Osmar Menezes [1] & Sávia Gavazza [1*].

[1] Federal University from Pernambuco, Recife, Brazil
[2] University from Pernambuco, Recife, Brazil
** Corresponding author's email address: savia@ufpe.br*

ABSTRACT

The current study aimed to observe the effects of partial pressure on hydrogen production in an anaerobic microcosm from 50% diluted sugarcane vinasse from the ethanol industry, using Manipueira (Cassava wastewater) as inoculum. The inoculum was pre-treated (90ºC, 10 minutes) to select microorganisms favorable for hydrogen production. The experiment lasted 166 hours and obtained results that condition B (without purge) showed a higher efficiency (49%) compared to condition A (with purge), which had a hydrogen yield of 20%, indicating the need for a minimum hydrogen partial pressure to improve the production. No methane was found in the headspace during the experiment, suggesting that the pH control and pre-treated inoculum successfully selected the microorganisms.

Keywords: Dark Fermentation. Sugarcane Vinasse. Biohydrogen. Waste Valorization. Microcosms.

1 INTRODUCTION

Brazil stands as the world's leading producer of sugarcane, a fundamental resource for ethanol production[1], a biofuel derived from the fermentation of starch or other plant-based sugars. It emits approximately 80% fewer greenhouse gasses into the atmosphere when sourced specifically from sugarcane than gasoline. [2] Despite its renewable and more sustainable, ethanol production generates considerable waste, with one of the primary byproducts being sugarcane vinasse. Vinasse is a residue produced after the fractional distillation of fermented sugarcane juice to obtain ethanol. Given that, on average, 12 liters of vinasse are generated as waste for every liter of ethanol, the imperative for effective reutilization becomes apparent.

This waste is used by ethanol plants for fertigation in sugarcane cultivation, as it contains high concentrations of nitrate, potassium, and organic matter. This practice enhances agricultural productivity and diminishes the reliance on chemical fertilizers. Nevertheless, over time, this disposal method alters the physical and chemical properties of the soil, eventually leading to groundwater or surface water contamination.[3] Therefore, developing a higher value-added product is essential, providing a more sustainable option for vinasse utilization and generating fewer pollutants. a more sustainable option for vinasse utilization and generating fewer pollutants.

The microcosms were inoculated using cassava wastewater, the primary liquid residue produced during cassava processing. Improper disposal of this wastewater can pose a high toxicity risk due to its elevated organic content and cyanogenic glycosides in its composition.[4] The choice of cassava wastewater as an inoculum is due to the common occurrence of hydrogen-producing microorganisms in this residue.

One of the challenges in hydrogen generation through anaerobic fermentation using mixed cultures is the presence of methane-producing microorganisms and hydrogen consumers or inhibitors.[5] Therefore, pre-treatment methods and pH control are employed to ensure that the metabolic pathway favors hydrogen production. There is no consensus on the best method, but thermal and chemical pre-treatments have shown the most promising results[6] and combinations of methods are also possible.

The anaerobic digestion of vinasse inoculated with cassava wastewater is a promising alternative for producing hydrogen, a compound that can be used as fuel, does not cause pollution during combustion, and holds higher value in the market. It is considered a promising alternative in the energy sector for the coming decades.

2 MATERIAL & METHODS

In this study, sugarcane vinasse obtained from the Petribu Plant in Lagoa do Itaenga-PE served as substrate and was stored in a freezer at -4°C until the beginning of the experiment. As inoculum was utilized, Manipueria, collected from a cassava factory in Taquarana – AL, was stored in a refrigerator at 4°C until the experiment. Six flasks of 120 mL each, with a reaction volume of 87.5 mL, served as the reactor, with sugarcane vinasse as the substrate. The Manipueira was pre-treated thermally (90°C for 10 minutes) to avoid the methanogenic pathway and used as the inoculum. The pH was adjusted to 5.5 using a 5M NaOH solution, and a 0.05 Mol/L phosphate buffer solution was utilized to minimize pH variations and the methanogen. After assembly, the reactors were sealed, locked, and purged with nitrogen for two minutes. The microcosms were then depressurized using a glass syringe to equalize the internal pressure with atmospheric pressure. Following this, they were placed in a shaker incubator at a constant temperature of 37.5°C and rotated at 150 rpm for the entire experiment.

The study aimed to analyze the feasibility of hydrogen production from sugarcane vinasse, assessing the influence of removing the produced biogas. In this way, triplicates were performed under two conditions:

- **Condition A:** headspace is purged every 24 hours with nitrogen.

- **Condition B:** no headspace purges.

The experiment lasted 166 hours, with gas chromatography analysis conducted twice daily at an 8-hour interval. Biogas analysis was performed using a GC-TCD gas chromatograph, injecting 0.2 mL of the system's atmosphere. Before each biogas analysis, the volumetric biogas production in the reactor was measured using a 100 mL glass syringe from the Arti Glass brand. Analyses of fatty acids, sugars, and alcohols were done using GC-FID and HPLC twice weekly. For this purpose, 1 mL of the sample was withdrawn from the reactor, centrifuged (10 min; 14,000 rpm), filtered using disposable RC0.22 µm filters, and diluted by 50% for use in the analyses. Additionally, initial analyses of Chemical Oxygen Demand (COD), Solid Series, sulfate, and nitrate in the collected vinasse were performed.

3 RESULTS & DISCUSSION

It has been considered the primary hydrogen production route based on the main carbon sources present in sugarcane vinasse to evaluate and compare the suggested conditions.

Acetic Acid Oxidation: acetic acid + $2H_2O \rightarrow 4H_2 + 2CO_2$ (1)
Lactic Acid Oxidation: lactic acid + $3H_2O \rightarrow 3CO_2 + 6H_2$ (2)
Fructose Oxidation: fructose + $2\ H_2O \rightarrow 2\ CH_3COOH + 2\ CO_2 + 2\ H_2$ (3)

Table 1 Theoretical Hydrogen Production.

Stage	Fructose (mmol/L)	Acetic Acid (mmol/L)	Lactate (mmol/L)	Acetic from Fructose (mmol/L)
Start	10,54	27,64	32,21	21,07
Final	0,56	6,85	0,07	1,12
Consumed	9,98	20,79	32,14	19,95
Theoretical H2 produced	19,95	83,16	192,84	79,80

As indicated in Table 1, the routes consider the products generated in the microcosm, which have the potential to produce H_2, with the reactors achieving an average of 32,9 mMol of theoretical hydrogen considering headspace volume.

Figure 1 illustrates the H_2 production in mMol/L over 166 hours of the experiment, demonstrating how condition B had a superior hydrogen production compared to condition A. It is also noticeable that there was CO_2 production, as expected from the predicted routes, and it is further noted that only condition A (Figure 1a) had nitrogen in its atmosphere due to the purges carried out during the assay. Additionally, it was verified that there was no methane production during the experiment, demonstrating the effectiveness of the chosen process as a pre-treatment for inhibiting methanogenic bacteria.

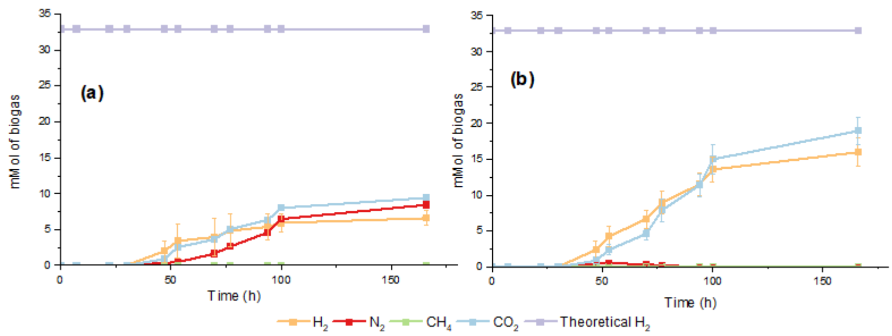

Figure 1 Hydrogen production. (a) stands for the microcosms without purge, while (b) the microcosms with purge

Condition A (Figure 1a) had a production of 6.6 mMols of H2, equivalent to about 20% of the theoretical efficiency expected with the routes. In a similar analysis, condition B (Figure 2b) had a production of 15.97 mMols of H_2 and 49% of the theoretical efficiency expected. This result suggests the need for a minimum partial pressure of hydrogen to optimize the process of producing this fuel through anaerobic digestion.

Compared with the literature, it was observed that Lazaro et al. (2014) evaluated the effect of different temperatures (37°C and 55°C) and concentrations of sugarcane vinasse (2, 5, 7, and 12 g COD L-1) on H2 production using 2L glass bottles (1.2 L reactional volume and 0.8 L of headspace). They also used a thermal pre-treatment (90°C for 10 min), with wastewater treatment as the inoculum. The results showed H_2 production in all conditions, but the increase in vinasse concentration had a positive impact on production at 37°C, while at 55°C there was a decrease. In Lazaro et al. (2014), the most similar condition was essayed at 37°C and 12 g COD/L, achieving a production of 23.7 mMol/L of reactional volume (28.4 mMol) over 150 hours. This work, essayed at 37.5°C and 13g COD/L, achieved a higher production of 182,5 mMol/L of reactional volume (15.97mMol) of H_2 over 166 hours, highlighting the importance of this study.

The influence of tequila vinasse concentration (40 to 120 g COD/L) on hydrogen production for continuous reactors was also compared to Allerano-García et al. (2021), using inoculum wastewater sludge from tequila factory pre-treated thermally (120°C for 24h) at a pH of 5.5. The condition closest to that evaluated in this study (13 g COD/L) achieved a production of 0.58 mol H2/mol sugar consumed, compared to 0.8 mol H2/mol sugar consumed in the 40 g COD/L condition, thus highlighting the influence of substrate concentration on hydrogen production. Furthermore, it can be observed that sugarcane vinasse, despite its lower concentration, reached a satisfactory production level, demonstrating the potential of this substrate.

4 CONCLUSION

The high efficiency of using low partial hydrogen pressure plus vinasse as a substrate has been demonstrated, in addition to the effects of pre-treatment methods and microbial diversity of manipueira as an inoculum. This work shows its importance for those who want to practice sustainability and optimization while producing hydrogen by anaerobic digestion as result of the low acquisition costs of both bioproducts. The findings in this research on hydrogen production levels point out higher values than the ones found in many literature sources- showing hope for converting vinasse into biohydrogen, which underscores the need for sustainable production through proper selection of biological materials and process parameters. In the future, research needs to focus on the optimization of the process, increasing the yield of products and production volume. These measures are crucial in moving closer to the realization of a biorefinery concept that involves a synergy of different processes toward optimal utilization of bio-based materials. This will help introduce biohydrogen favourably as one promising innovation in the energy industry-expected to be widely adopted and lead to environmentally friendly and cost-effective energy production systems in upcoming years.

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