I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM ** XV SHEB ** XV ENZITEC FLORIANÓPOLIS ** 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
ENVIRONMENTAL BIOTECHNOLOGY

HYDROGEN PRODUCTION THROUGH CO-FERMENTATION OF GLYCEROL AND SUGARCANE VINASSE IN A BATCH REACTOR

Beatriz P. Moreira[1] & Edson L. Silva[2]*

[1] *Department of Chemical Engineering, ANP Human Resources Program, Federal University of São Carlos, São Carlos, Brazil.*
[2] *Department of Chemical Engineering, Federal University of São Carlos, São Carlos, Brazil.*
** Corresponding author's email address: edsilva@ufscar.br*

ABSTRACT

Amidst the challenges faced by fossil fuels this study aims to investigate hydrogen (H_2) production as a sustainable alternative. The experiment involved co-fermentation of vinasse and glycerol subproducts in equal proportions (1:1 $v.v^{-1}$) of organic matter concentration per liter. The pH was adjusted between 4.5 - 5.5 and the temperature was maintained under thermophilic conditions (55 ± 1 °C). In order to identify the optimal approach for H_2 production, different substrate concentrations were considered, namely 5, 10, and 15 g COD L^{-1}. Detailed analysis of gas production over 1000 hours revealed that the batch with 10 g COD L^{-1} stood out, demonstrating significantly higher production, with a volume of 365.9 mL. Using the modified Gompertz equation, a Maximum Production Potential (P) of 327.8 mL H_2 L^{-1} and a Maximum Production Rate (R_m) of 7.3 mL H_2 L^{-1} h^{-1} were observed for the batch with the highest production. This result was attributed to the ideal nutritional balance for anaerobic bacteria at this concentration. Additionally, variations in COD removal efficiency (16.3-36.3%) were evaluated, with the highest removal observed in the batch with 15 g COD L^{-1}, and carbohydrate removal (56.3-74.4%) falling within the expected range.

Keywords: Dark fermentation. Gas. By-products. Biofuels.

1 INTRODUCTION

The increasing utilization of petroleum is driven by its myriad consumer benefits. However, its consumption, along with that of its derivatives, can engender implications, particularly within the environmental sphere, stemming from the combustion and subsequent release of pollutant gases[1]. Consequently, there arises a pressing demand for sustainable alternatives that mitigate or entirely obviate the emission of these gases. Among the array of biofuel investigations, gas stands out as a flexible renewable energy source capable of replacing fossil fuels in electricity and heat generation. Gas production is a process derived from dark fermentation, where biomaterials are decomposed by bacteria in an oxygen-free environment. This method, which is flexible and can be applied to a variety of biodegradable substrates, results in the production of a gaseous mixture[2]. Co-fermentation, a fundamental process in optimizing gas production, has emerged as a promising alternative to overcome the limitations of mono-digestion, as it can address the system's nutritional deficit and diversify microorganisms[3].

Vinasse, a by-product of ethanol production, encompasses a high content of potassium and nitrogen, as well as a high organic matter concentration. Glycerol, a by-product obtained from biodiesel production, contains various other substances in its composition, such as long-chain fatty acids, methanol, salts, among others. Both by-products can be highly polluting to the environment, causing various environmental problems if improperly disposed of. Thus, the utilization of these two substrates in dark fermentation for gas production can favor the attainment of excellent production values, given that glycerol is a by-product rich in biodegradable carbon, serving as an alternative carbon source, contributing to the C/N balance (carbon-to-nitrogen ratio), while vinasse contains various other nutrients that together can provide more stability to the medium. Therefore, this study aims to enhance the efficiency of hydrogen (H_2) production, a gas with high energy potential, through anaerobic co-fermentation of vinasse and glycerol in a batch reactor.

2 MATERIAL & METHODS

Vinasse and glycerol were obtained from Usina da Pedra in Serrana/SP and Biobrotas Oleoquímica in Brotas/SP, respectively. The substrates were added to Duran flasks (2 L) with a headspace volume of 1 L. The co-substrates were added in proportions of 50% COD L^{-1} each, followed by the addition of 10% of the total liquid volume with the inoculum (sludge) from an Upflow anaerobic sludge blanket (UASB) at Usina São Martinho. This inoculum underwent a thermal treatment method[4] (10 minutes at 90°C) to deactivate the methanogenic archaea and stimulate anaerobic spore-forming bacteria capable of withstanding adverse conditions such as high temperatures, nutrient deficiency, or exposure to chemicals. Additionally, to stimulate cell growth during the process, a nutrient medium was incorporated into the substrates and inoculum as proposed by Del Nery[5], mainly composed of urea, monobasic potassium phosphate, and calcium chloride.

The organic matter concentration in the batches was 5, 10, and 15 g COD L^{-1}, which, distributed equally between the co-substrates, resulted in 2.33, 4.65, and 6.97 g L^{-1} of glycerol, as well as 44.25, 88.5, and 133 mL L^{-1} of vinasse. The pH was adjusted in the range of 4.5 to 5.5 using hydrochloric acid and sodium bicarbonate as a buffer solution. The batch was kept in an oven at 55 ± 1 °C, ensuring the ideal conditions for H_2 production during a residence time of 1000 hours.

The volume of gas produced was evaluated using a method involving a saline solution and an inverted graduated cylinder, as described by literature[6]. The collected samples were subjected to pH and Chemical Oxygen Demand (COD) analyses using the

methods established in Standard Methods for the Examination of Water and Wastewater[7], utilizing a pH meter and spectrophotometer, respectively. Gas chromatography was used to analyze the gas to determine the composition generated in each batch. This analysis was performed using a GC 2010 gas chromatograph (Shimadzu). Gas samples were collected from the reactors using a gas-tight syringe and then injected into the chromatograph. Argon was used as the carrier gas to transport the gas or vaporized samples through the chromatographic column. A thermal conductivity detector (TCD) was used to identify the gases H_2, nitrogen (N_2), methane (CH_4), and carbon dioxide (CO_2)[8]. Finally, the Statistica software was used to apply and determine the parameters of the modified Gompertz equation, such as Maximum Production Potential (P) and Maximum Production Rate (R_m)[9].

3 RESULTS & DISCUSSION

Analysis of gas production over a 1000-hour interval allowed for the assessment of performance, as depicted in Figure 1. A higher H_2 production was observed in the batch with 10 g COD L^{-1}, yielding 365.9 mL (Table 1). Thus, it's evident that this concentration provides an ideal nutritional balance for anaerobic bacteria. At this concentration, the behavior can be justified, compared with the other concentrations analyzed, for a lower presence of inhibitors or toxic substances that could impair bacterial activity, and adequate amounts of vinasse and glycerol at this concentration result in effective decomposition of organic matter, leading to robust gas production over time. Extremely high or low concentrations may lead to detrimental pH fluctuations in the anaerobic process, negatively impacting gas production. Furthermore, higher concentrations, such as 15 g COD L^{-1}, may introduce compounds that could inhibit dark fermentation, thereby reducing gas production, while lower concentrations may have deficits in essential nutrients for system maintenance and consequent gas production. The Gompertz equation (Equation 1), that associate the accumulated production of H_2 (H) with the Maximum Pr-oduction Potential (P) and Maximum Production Rate (Rm) of H_2, as well as the onset time of production (λ), total production time (t) and the Euler number (e), provided a more detailed insight into the potential of the 10 g COD L^{-1} batch, with a P of 327.8 mL H_2 L^{-1} and a R_m of 7.3 mL H_2 L^{-1} h^{-1}. These values address the notable capacity of the batch with the highest production to continuously generate H_2.

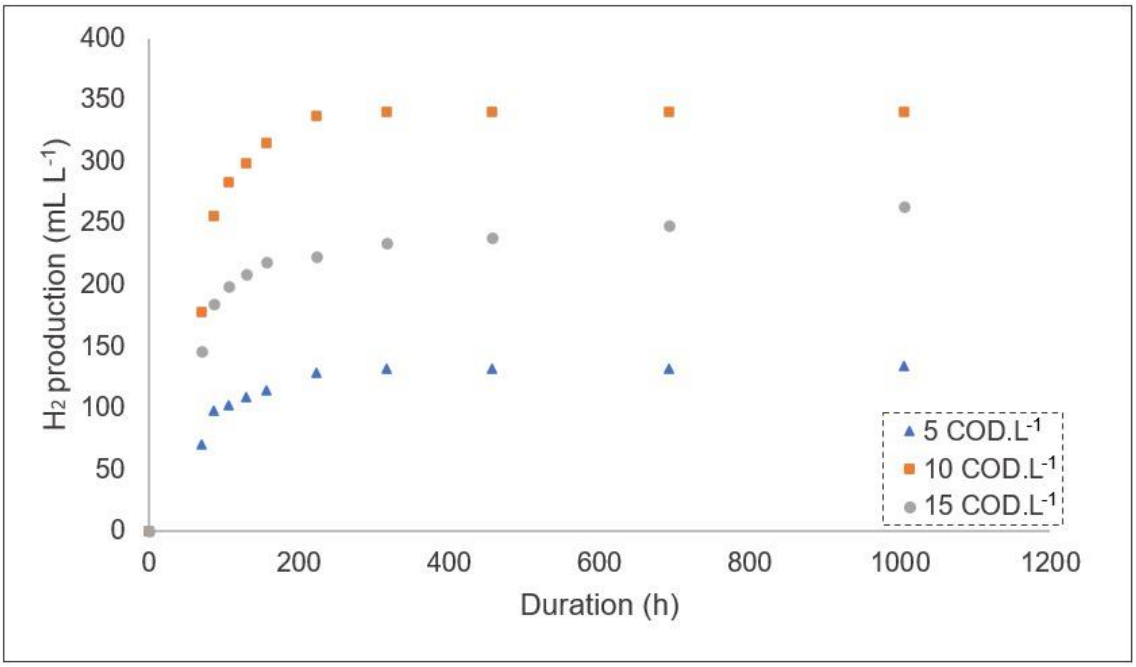

Figure 1 Hydrogen production (mL h^{-1}) for each COD L^{-1} condition throughout the experiment duration.

Table 1 Hydrogen production for each batch.

Concentration (g COD L^{-1})	H_2 (mL)
5	136.3
10	365.9
15	263.0

$$H = P \cdot exp\left\{-exp\left[\frac{R_m \cdot e}{P}(\lambda - t) + 1\right]\right\} \quad (1)$$

Analyzing the removal of organic matter (Table 2), a range of 16.3% to 36.3% was observed, with the highest value related to the 15 g COD L^{-1} batch and the lowest found in the 10 g COD L^{-1} batch. These values also represent the lowest and highest H_2 volume values, respectively. With a higher concentration of organic matter (15 g COD L^{-1}) there is a significantly larger amount of substrate available for microorganisms comsumption in the reactor. Under proper conditions, a higher organic matter concentration can stimulate microbial activity, resulting in more efficient degradation of organic matter. Increasing substrate concentration can favor the growth of a wider range of anaerobic microorganisms in the reactor.

Table 2 COD and carbohydrate removal for each batch.

Concentration (g COD L^{-1})	COD removal (%)	Carbohydrate removal (%)
5	29.0	74.0
10	17.3	68.5
15	36.0	58.9

The high removal of carbohydrates (ranges of 56.3% to 74.4%) is mainly due to the anaerobic fermentation process involving bacteria that consume carbohydrates, capable of converting them into H_2. In a dark fermentation system, bacteria involved in carbohydrate degradation are crucial. If the system maintains a stable and diversified microbial population over time, the metabolic activity of bacteria can remain stable, leading to consistent removal of carbohydrates. The proper C/N ratio in the substrates is vital for bacterial growth. If the C/N ratio is balanced, bacteria will have the necessary nutrients to thrive, resulting in effective removal of carbohydrates.

4 CONCLUSION

In summary, this study conducted detailed analyses of gas production over a 1000-hour interval, providing valuable insights into its process performance. The results revealed that the batch with 10 g COD L^{-1} stood out, demonstrating significantly higher H_2 production. This concentration offered an adequate nutritional balance for the anaerobic bacteria involved in the process, resulting in optimized microbial activity.

Regarding organic matter removal, variations in the removal efficiency among the batches (16.3% to 36.3%) were observed. The highest removal occurred in the batch with 15 g COD L^{-1}, indicating that the presence of a higher substrate concentration stimulates microbial activity, leading to more efficient organic matter degradation. Additionally, consistency in the range of carbohydrate removal (56.3% to 74.4%) in a dark fermentation system for H_2 production can be attributed to various factors influencing the process, such as maintaining a stable and diversified microbial population over time, along with appropriate operational parameters ensuring adequate removal.

Thus, the results of this study underscore the importance of carefully controlling substrate concentrations in the dark fermentation process to maximize gas and H_2 production. Through understanding the complex interactions among microorganisms, substrates, and environmental conditions, it is possible to optimize the process for achieving sustainable and effective gas and H_2 production. These findings have significant implications for the practical application of these technologies and, consequently, for the sustainable and efficient replacement of fossil fuels.

REFERENCES

[1] SANTOS, M. A., LUNA, S., QUINTELLA, C. M. 2014. Prospecção tecnológica sobre o cenário brasileiro no que tange o petróleo e seus derivados com a substituição pelos biocombustíveis. Cad. Prosp., 6(3), 337. https://doi.org/10.9771/cp.v6i3.11404

[2] KUNATSA, T., XIA, X. 2022. A review on anaerobic digestion with focus on the role of biomass co-digestion, modelling and optimization on biogas production and enhancement. Bioresour. Technol. 344. 126311.

[3] HAGOS, K., et al. 2017. Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives. Renew. Sustain. Energy Rev. 76. 1485–1496.

[4] KIM, S. H., HAN, S. K., SHIN, H. S. 2006. Effect of substrate concentration on hydrogen production and 16S rDNA-based analysis of the microbial community in a continuous fermenter. Process Biochem. 41(1). 199–207.

[5] DEL NERY, V. 1987. Utilização de lodo anaeróbio imobilizado em gel no estudo de partida de reatores de fluxo ascendente com manta de lodo. Dissertação de Mestrado, Escola de Engenharia de São Carlos, Universidade de São Paulo, São Carlos. doi: 10.11606/D.18.2016.tde-05122016-090836.

[6] WALKER, M., ZHANG, Y., HEAVEN, S., BANKS, C. 2009. Potential errors in the quantitative evaluation of biogas production in anaerobic digestion processes. Bioresour. Technol. 100(24). 6339–6346.

[7] APHA - Standard Methods for Examination of Water and Wastewater. 21st ed. Washington DC, American Public Health Assoc., 2005.

[8] RIBEIRO, A. R., DEVENS, K. U., CAMARGO, F. P., SAKAMOTO, I. K., VARESCHE, M. B. A., SILVA, E. L. 2024. Evaluation of the effect of increasing the organic load in the thermophilic co-fermentation of sugarcane industry by-products on hydrogen, ethanol and lactic acid generation. Int. J. Hydrogen Energy. 57(1). 549–561.

[9] RIBEIRO, A. R., SILVA, E. L. 2022. Potato waste as feedstock to produce biohydrogen and organic acids: A comparison of acid and alkaline pretreatments using response surface methodology. J. Environ. Manage. 323. 116308.

ACKNOWLEDGEMENTS

I would like to thank the Human Resources Program of the National Agency of Petroleum, Natural Gas, and Biofuels (PRH 39.1/ANP), as well as FINEP for the financial support. Additionally, my heartfelt gratitude goes to my advisor, Dr. Edson Luiz Silva, and the members of the Environmental Control Laboratory II (LCA II) at the Department of Chemical Engineering of the Federal University of São Carlos (DEQ/UFSCar), for their consistent support and assistance in my academic and research endeavors.