

## OBTAINING ETHANOL FROM THE ACID HYDROLYSIS OF CELLULOSE FROM LIGNOCELLULOSIC BIOMASS USING A BIOFERMENTER

João P. C. Bonfim<sup>1</sup> & Leila M. A. Campos<sup>1,2,3</sup>

<sup>1</sup>Postgraduate Program in Chemical Engineering / Federal University of Bahia (UFBA), Salvador, Brazil.

<sup>2</sup>Salvador University (UNIFACS), Anima Institute – IA, Salvador, Brazil.

<sup>3</sup>Brazilian Institute of Technology and Regulation, Salvador, Brazil.

\* Corresponding author's email address: [jpdr.calheira@gmail.com](mailto:jpdr.calheira@gmail.com)

### ABSTRACT

The world is experiencing huge population growth and this could lead to shortages of both food and energy, in addition to the heavy use of fossil fuels that contribute to the increase in global warming. Therefore, it is essential to work with biomass in order to generate new forms of energy and thus minimize economic and environmental crises. Sugarcane bagasse is one of the main lignocellulosic materials to be used as an alternative source of energy, in addition to having great potential for generating ethanol with a view to meeting global demand. Therefore, this work aimed to carry out the acid hydrolysis of biomass using maleic acid with the purpose of determining the rate of hydrolyzed material and, using a specific biofermenter, optimizing the process of obtaining ethanol with a consequent reduction in costs. Acid hydrolysis and fermentation were carried out in a bioreactor, the result of which achieved a rate of 25% of hydrolyzed material, producing around 135g/L of ethanol. Based on the results obtained, considered promising, the prospect of replacing fossil fuels with ethanol with consequent minimization of environmental impacts appears.

**Keywords:** Ethanol. Biofermenter. Acid hydrolysis. Sugar Cane.

## 1 INTRODUCTION

Through studies carried out by Salgado et al. (2024), the world population could grow by 2 billion people in the next 30 years, currently exceeding 7 billion people to 9.7 billion in 2050, and by 2100 reaching the mark of 11 billion individuals. This growth is expected to generate enormous impacts on the environment, including the increased use of fossil fuels. According to Silva et al. (2021) constant CO<sub>2</sub> emissions into the atmosphere is an aggravating factor that has occurred over time, but unfortunately it became a concern for countries only recently, making the search for solutions immediate, making a change in the energy matrix necessary, in order to increase the share of renewable sources. Therefore, in order to create a better future, it is essential to include biofuels in order to contribute positively to environmental sustainability. Currently, the most promising example of biofuels is ethanol, whose production sector in the area is growing and which, according to the ANP (2022), there is the objective of expanding production capacity in 51 existing units. In this scenario, Brazil plays a prominent role in both the production and consumption of biofuels, presenting an institutional base with a legal framework, installed capacity, a scientific body of excellence in areas such as biofuels and biochemistry and technical knowledge that are important instruments to overcome the challenges of expanding biofuels in the country's energy matrix (Vidal, 2022).

According to the Sugarcane Industry Union (Unica, 2021), the amount of sugarcane generated had a satisfactory growth of 120% (326.1 million tons to 717.4 million tons), while the Sugar production rose by 136% (from 16.1 million to 38 million tons) and ethanol production by 160% (from 10.5 billion to 27.3 billion liters). This fact is due to issues such as increased taxation on liquid fossil fuels, which contributed to greater domestic consumption of biofuel and the country's tropical advantages such as climatic factors in rainy environments, which make production efficient, contributing significantly to the development of sugarcane (Santos et al, 2023). In addition to bagasse, sugarcane straw has great energy potential, being important for the generation of heat, electricity and ethanol production. According to Cargnin et al. (2021), sugar cane is the most effective input for generating ethanol, a fuel with great potential to deal with the global search for renewable sources. Even with the combustion of ethanol, pollution rates are even lower compared to gasoline.

Being made up of renewable raw materials, ethanol becomes viable for developing and improving technological processes in order to reach the ideal level of sustainability. Sugarcane waste contains significant amounts of cellulose, lignin and hemicellulose, making it viable to use it in the production of ethanol, using the biofermenter, which is of great importance due to the fact that it optimizes the processes for obtaining the product, increasing yield. In the conversion of sugars into ethanol and, thus, providing greater production, with consequent cost reduction. In this way, the study of obtaining ethanol from glucose extracted from biomass opens up paths for the search for new technologies, both for obtaining different types of alcohols, as well as expanding research into the compounds present in different biomasses and molecules with high added value.

## 2 MATERIAL & METHODS

Acid hydrolysis: To carry out this step, approximately 0.6 g of sugarcane bagasse was used, on a dry basis, previously treated with maleic acid, and added in a rotary evaporator together with 12 mL of maleic acid, at concentrations 0, 07% and 0.14% separately. Then, the system was heated in a thermostated glycerin bath at 200 °C for 30 minutes. After this time, the system was cooled in an ice bath in order to interrupt the reaction kinetics. The rate of material hydrolyzed in maleic acid at low temperatures was calculated according to Equation 1.

$$\%AH = \frac{m2-m1}{m2} \quad (1)$$

%AH – percentage of hydrolyzed material.

m1 – mass (g) of residual material (dry basis)

m2 – mass (g) of the starting material (dry basis).

Ethanol Production: In a beaker, 35 g of glucose was added to 300 mL of ultrapure water and transferred to a 500 mL volumetric flask and topped up with water. Then, 680 mL of the glucose solution were removed and transferred to the biofermenter vessel. After this process, the bioreactor vessel with the vacuum tube containing 120 mL of ultrapure water were taken to the autoclave and, after complete sterilization, were removed and placed to cool. After cooling, the vessel was inserted into the biofermenter and quickly closed to prevent contamination. To carry out the fermentation process, the biofermenter was turned on, initially without the heating blanket until the fermentation reached a temperature of 27 °C. Soon after, 5 g of biological yeast (*Saccharomyces cerevisiae*) were added to the bioreactor, whose process of obtaining ethanol lasted 5 days.

### 3 RESULTS & DISCUSSION

The acid hydrolysis step was carried out in duplicate, obtaining on average with maleic acid, at a temperature of 200 °C, a rate of hydrolyzed material. The result achieved was considered coherent, when compared to the results found by Agustini et al., (2007) who carried out the natural enzymatic hydrolysis of cassava to produce alcohol and obtained a rate of 26%, and concluded that the yield was considered positive, taking into account the process costs that were reduced.

To carry out the fermentation, we worked with the TEC-BIO-FLEX-II compact biofermenter, using the biological yeast *Saccharomyces cerevisiae*, as it is the most suitable yeast for ethanol production. Six fermentation tests were carried out, with different concentrations for glucose and yeast. Table 1 presents the concentrations of glucose and yeast worked.

**Table 1. Fermentation conditions**

Fermentation	Glucose (g/L)	Yeast (g/L)
1°	10	5
2°	15	5
3°	20	10
4°	25	35
5°	30	40
6°	35	45

For each fermentation, the biofermenter operated for 96 consecutive hours, initially without the heating blanket due to the high temperature that the bioreactor vessel presented, around 45 °C. After reaching the temperature recommended in the literature of 27 °C, the bioreactor was covered with the heating blanket so that the temperature remained constant, obtaining the following concentration of ethanol solution for the respective fermentations, as shown in Table 2.

**Table 2. Fermentation results**

Fermentation	Ethanol (g/L)
1°	5
2°	10
3°	15
4°	30
5°	35
6°	40

In these fermentations, an average of 135g/L of ethanol solution was obtained. The results obtained from the 4th, 5th and 6th fermentation were satisfactory, when compared with the results of Cinelli (2012), who produced ethanol from simultaneous fermentation, during 20h of fermentation they obtained 32.3g/L and, in 48h obtained 53.6g/L, concluding that yeast acts quickly and efficiently to produce ethanol. Regarding the result of the 3rd fermentation, which was 15g/L, it was observed that it is in accordance with Ferreira (2016), who carried out a study of the fed-batch process in the production of ethanol in a stirred bioreactor, obtaining maximum production of ethanol at approximately 15g/L in 60h of fermentation, concluding that aeration

and the geometry of the fermenters influence the final result of ethanol concentration. The ethanol concentration of 5g/L obtained from the 1st fermentation corroborated what was found by Sousa et al. (2013), who produced ethanol using different compositions of rice bran and sugarcane bagasse, reaching a content of 5.3g/L of ethanol in 72 hours, concluding that biomass is an important variable in the alcoholic fermentation process. As for the 2nd fermentation, they obtained 10 g/L of ethanol, a satisfactory result compared to those presented by Souza et al. (2019), who produced ethanol using cellulosic hydrolyzate in a bioreactor, obtaining a concentration of 9.25 g/L in 120 hours of fermentation.

## 4 CONCLUSION

The percentage of hydrolyzed material achieved and the yeast used in these fermentations demonstrated effectiveness in different conditions, corroborating previous studies that highlight the importance of variables such as biomass, aeration and geometry of the fermenters in the final yield of ethanol production. Therefore, this work denotes the importance of investments in biofuels from lignocellulosic biomass and the effectiveness in obtaining ethanol via biofermenter.

## REFERENCES

- <sup>1</sup> SALGADO, J.R.F., NAHIME, B.O., OLIVEIRA, G.A. Urban sustainable agriculture in homes with artificial intelligence and the internet of things. *Concilium*, vol. 24, no. 6, p. 184-195, 2024.
- <sup>2</sup> SILVA, M.L.S., CAMPOS, A.M.A., AVILA, M., BATISTA, L.D., FRAGA, V., CRUZ, L.V., NASCIMENTO, A.L.B., ASSIS, P.S. Analysis of the impact of biomass addition on the cri and csr parameters of coke. Technical contribution to the 50th Seminar on Iron Ore Reduction and Raw Materials and 8th Brazilian Symposium on Iron Ore Agglomeration, an integral part of ABM Week 6th edition, São Paulo, SP, 2021.
- <sup>3</sup> ANP. NATIONAL AGENCY FOR PETROLEUM, NATURAL GAS AND BIOFUELS. Statistical yearbook 2022. Available at: <Statistical Yearbook — National Petroleum, Natural Gas and Biofuels Agency (www.gov.br)> Accessed on Jun 13. 2024.
- <sup>4</sup> VIDAL, M. DE F. Biofuels: Biodiesel and Ethanol. Technical Office for Economic Studies of the Northeast – ETENE. 2022.
- <sup>5</sup> UNICA. Union of the sugar cane industry. Sugarcane Observatory, 2021. Available at: <unicadata.com.br> Accessed on: Jun 13 2024.
- <sup>6</sup> SANTOS, H.F., SAMPAIO, M.A.P. Factors determining the recent crisis in the sugar-energy sector in Brazil. *Geo UERJ*, n. 44, p. e72588-e72588, 2024.
- <sup>7</sup> CARGNIN, J. M. R., CUBAS, A. L. V., DUTRA, A. R. D. A., JOÃO, J. J., WHEELER, R. M. Potential applications of sugarcane vinasse aiming for cleaner production: an integrative review. *Periodical*. 2021.
- <sup>8</sup> AGUSTINI, D., JUNIOR, H. E. Production of cassava alcohol from natural enzymatic hydrolysis. *Synergismusscientifica*, Pato Branco, v. 2, p. 1-4, 2007.
- <sup>9</sup> CINELLI, B. A. Ethanol production from simultaneous fermentation and hydrolysis of granular starch from agro-industrial residue. 2012. 200 f. Thesis (Master's) – Federal University of Rio de Janeiro.
- <sup>10</sup> FERREIRA, A. D. Efficient production of 2G ethanol from hemicellulosic hydrolyzate of sugarcane bagasse: optimizing cultivation and operational conditions. 2016, 153 f. Thesis (Doctorate) - University of São Paulo, Lorena School of Engineering.
- <sup>11</sup> SOUSA, V. C., GUEDES, K., PLÁCIDO, M., LOPES, V. S., FISCHER, J., CARDOSO, V. L., FILHO, U. C. Production of cellulosic ethanol by enzymatic complex from cerrado fungus: effect of the solid medium on enzyme production and bagasse load in ethyl fermentation. *Blucher Chemical Engineering Proceedings*, Vassouras – RJ, v. 1, no. 1, p. 1-5, 2014.
- <sup>12</sup> SOUZA, T., BONATTO, C., BAZOTI, S. F., SÉRGIO, L., ALVES, J. R., TREICHEL, H. Production of second generation ethanol using cellulosic hydrolyzate in the presence of inhibitors in a bioreactor. *JOURNEY OF SCIENTIFIC AND TECHNOLOGICAL INITIATION IX*. 2019, Chapecó.

## ACKNOWLEDGEMENTS

The authors would like to thank the financial support from the Human Resources Program of the National Petroleum, Natural Gas and Biofuels Agency (PRH/ANP – PRH36/UFBA), supported by resources from the investment of oil companies qualified in the RD&I Clause of ANP Resolution No. 50 /2015, to PPEQ (Postgraduate Program in Chemical Engineering), Núcleo de Química Verde (NQV), the University of Salvador (Campus Tancredo Neves) and the Brazilian Institute of Technology and Regulation, for the use of laboratory spaces.