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# 2G ethanol fermentation process integrated with biochar derived from post-hydrolysis sisal mucilage

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## ABSTRACT

This study aimed to produce ethanol from sisal mucilage via biochemical methods (acid and enzymatic hydrolysis) and fermented with biochar from post-hydrolysis. The solid residue fraction of the hydrolysis (acid and enzymatic) was converted into biochar. The biochar was an adsorbent of fermentation inhibitors of ethanol production. The biomass was characterized and showed 35.7% cellulose, 14.1% hemicellulose, and 6.2% lignin content. Fermentation occurred with the yeast *Saccharomyces cerevisiae* applied to the hydrolysates with and without biochar. The biochar from the enzymatic hydrolysis produced at 550°C showed a better response to the adsorption of the fermentation inhibitors 89.3% of the initial furfural solution, 99.7% of the HMF solution, and 29.71% of the acetic acid solution, as for the ethanol production, this biochar increased the ethanol production from 37.47 g/L to 51.82 g/L.

**Keywords:** Biomass. Biochar. Bioprocess. Fermentation. 2G Ethanol.

## 1 INTRODUCTION

Biochar is a carbon-rich material produced by carbonization of low-cost biomass waste in a limited-oxygen atmosphere.[1,2]. Biochar's unique properties, such as its large surface area, high porosity, diverse functional groups, high cation exchange capacity, and stability, make it suitable for various applications.[3]. Determining the characteristics of biochar is fundamental for its use as an adsorbent and for other applications. Biochar is a promising alternative to traditional adsorbents such as activated carbon. [4]. In the adsorption of fermentation inhibitors, studies have evaluated the application of biochar in hydrolysates and fermentation, increasing the glucose conversion to ethanol and adsorbing inhibitors such as furfural, 5-hydroxymethylfurfural and acetic acid. [5]. The addition of biochar promotes the removal of more than 94% 5-HMF and 99% furfural after 24 h of contact. Various methods for the removal of furfural and HMF are also of wide interest because it is detected as an environmental contaminant in oil refineries and activated carbon has been suggested as an adsorption treatment method.[6]. The present work aimed to valorize the sisal mucilage in the production of second-generation ethanol (2G) with biochar, in the Brazilian semiarid region.

## 2 MATERIAL & METHODS

**Biochar production:** The pyrolysis experiments were at the Ceramic Materials Laboratory (LACER) at the Federal University of Rio Grande do Sul (UFRGS). To define the pyrolysis conditions, the samples were subjected to thermal analysis tests (TG/DTG), in Shimadzu equipment, with a nitrogen flow of 20 mL/min, a heating rate of 10 °C/min, and a temperature range from ambient to 900 °C. The temperatures used in the pyrolysis resulted from the bending points of the TG curve that occurred at 400, 500, and 650°C, which represented the decomposition of the cellulose, hemicellulose, and lignin fractions, respectively.

**Characterization of biochar:** The surface structure was identified by scanning electron microscopy (SEM) on a Zeiss DSM 960 microscope operating at 20 kV in SEI (secondary electron) mode. After preparing the samples, carbon was deposited by the sputtering method using a Balzers SCD 50 sputter coater. Analysis of the elemental composition (C, N, H, O) provides the dry mass percentage (wt% dm) of carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) present in the sample. Sulfur was disregarded in this study. The total elements present in a sample are expressed according to Equation 1, where the difference estimates oxygen (O) according to DIN 51733:

$$[wt.\ \%\ dm] = C + H + O + N + ash\ content = 100\% \quad (1)$$

Elemental analysis was in triplicate using a Vario Macro elemental analyzer at the Fuels and Energy Laboratory (POLICOM) of the Polytechnic School of the University of Pernambuco (POLI-UPE).

The analyses were on a micro-Raman model RENISHAW via Spectrometer with a power of 50mW. A 532 nm wavelength laser was used, focusing on the sample through a 50x objective lens at room temperature. The measurement range was 200–900 $cm^{-1}$. To determine the crystalline phases and crystallite sizes of the samples obtained in powder form, a SmartLab SE Rigaku diffractometer was used with CuKα radiation, a wavelength of 1.5406 Å, a voltage of 40 kV, a current of 30 mA, and a graphite monochromator. The diffractograms were obtained by varying the 2θ angle between 10 ° and 50° in increments of 0.02° at a speed of 1° $min^{-1}$. To determine the specific surface area ($S_{BET}$) of the biochar samples using the Brunauer-Emmett-Teller (BET)

method, a Quantachrome model NOVA 1000e. During pretreatment, the samples were kept at 300 °C for approximately 2 h under vacuum.

**Adsorption of inhibitors:** Using furfural, HMF, and synthetic acetic acid. In the first study, have been added 0.1 grams of biochar at different temperatures (400, 500, and 650 °C) of the hydrolyzed residual biomass (acid and enzymatic) and 10 mL of the fermentation inhibitor solution, at 3 g/L acetic acid and 1 g/L HMF and furfural. After 24 h of contact and stirring (90 rpm), the supernatant was obtained, filtered through a 0.22 µm membrane, and analyzed by HPLC (High-performance liquid chromatography).

**Fermentation with biochar:** Using the data obtained from the inhibitor adsorption stage, was defined the application of biochar, hydrolysate, and yeast for the fermentation process. 26.5 mL of the enzymatic hydrolysate was added to a 50 mL Falcon tube, 3 grams of JP1 yeast (*Saccharomyces cerevisiae*), and 0.5 g of the biochar from its respective hydrolysate. The entire experiment was performed in triplicate at room temperature in a static manner. Fermentation took place over 8 h, with samples of 1 mL taken each hour. The samples were filtered through 0.22 µm membrane filters and analyzed by HPLC. Carbohydrates and inhibitors (furfural and HMF) were analyzed on the Aminex HPX-87H and C-18 columns.

## 3 RESULTS & DISCUSSION

After acid and enzymatic hydrolysis, approximately 30% of the initial mucilage was converted into a solid residue. Table 1 shows the characterization results of these biomasses. The removal of the cellulose and hemicellulose fractions resulted in the concentration of the lignin fraction, which is important for the production and quality of biochar. Concentrations of Ca, K, and Mg showed a decrease compared to the initial biomass.

**Table 1**: Characterization of post-hydrolysis biomass

| Characterization | Components | Initial biomass (mucilage of sisal) | Biomass of Acid hydrolysate | Biomass of Enzymatic hydrolysate |
|---|---|---|---|---|
| Chemical characterization | Lignin (%) | 6.41 | 42.51 | 48.45 |
| | α-Cellulose (%) | 39.68 | 21.14 | 6.89 |
| | Hemicellulose (%) | 13.12 | 9.94 | 6.30 |
| | Ash (%) | 4.11 | 5.21 | 3.41 |
| | Fiber (%) | 19.45 | 24.13 | 20.12 |
| | $C_{organic}$ (%) | 44.20 | 32.92 | 41.12 |
| | N (%) | 1.20 | 1.12 | 1.01 |
| | Ca (g /Kg) | 7.4 | 2.9 | 4.8 |
| | Mg (g /Kg) | 3.1 | 0 | 0.5 |
| | K (g /Kg) | 5.2 | 0.1 | 1.8 |

The conversion of post-hydrolysis residual biomass showed the same proportion as the conversion of sisal mucilage into biochar, bio-oil, and pyrolysis gases. The solid phase was produced at the lowest temperature of 400 °C (47.3% biochar, 35.9% bio-oil, and 16.7% pyrolysis gas). The lowest temperatures tended to result in the highest biochar yields, and higher temperatures increased the yields of pyrolysis gases and bio-oil yields.[7].

In terms of pore type, biochar mostly has mesopores and macropores. In acid hydrolysis biochar, there is a greater surface area ranging from 1.3 to 3.0 $m^2/g$, when compared to enzymatic hydrolysis biochar with 0.01 to 0.3 $m^2/g$, which is related to the start of the activation that occurs during the hydrolysis process, acid activation. However, as the area is low (less than 300 $m^2/g$), it cannot be considered an activation of the biochar [8].

Subsequently, the adsorption of the inhibitor was tested in 24 h of contact between the biochars and inhibitors separately (HMF, furfural, and acetic acid). The biochar obtained from enzymatic hydrolysis solid fraction at 550°C adsorbed 89.3% of the furfural solution, 99.7% of the HMF solution, and 29.7% of the acetic acid solution. In another study, the application of biochar produced from a dry digestate was evaluated and obtained 94% removal of 5-HMF and 99% furfural after 24 h of contact with corn hydrolysate and wood hydrolysate. [9]. These results are comparable to this work.

The concentration profiles of ethanol and glucose during fermentation of the enzymatic and acid hydrolysates without (WB) and with biochar (BE) produced at temperatures of 400, 550, and 650 °C are shown in Figure 1. The highest glucose conversion was achieved in the presence of biochar produced at 550 °C, achieving an efficiency of 97.8 % and production of approximately 52 g/L ethanol for the enzymatic hydrolysate. Fermentation of the acid hydrolysate resulted in higher yields using biochar produced at 550 °C, resulting in an efficiency of 91% and an ethanol production of approximately 17 g/L. However, there was no significant adsorption of the fermentation inhibitors HMF and furfural. In other words, biochar is suitable for adsorbing these compounds, but further studies should be conducted to investigate its best application.

No statistically significant differences in ethanol production between fermentation without and with biochar at 400 and 650 °C from the enzymatic hydrolysate. However, the biochar adsorbed HMF, furfural, and partially adsorbed acetic acid. Figure 1 shows a reduction in fermentation time and an increase in ethanol production in the presence of biochars. Between the two fermentations, there was greater production of the enzymatic hydrolysate with the biochar from the biomass produced after enzymatic hydrolysis at 550 °C. This demonstrates the potential of using biochar during ethanolic fermentation, reducing the duration of the industrial

process and increasing the rate of conversion of carbohydrates into ethanol. In another study using biochar in fermentation with *Zymomonas mobilis* under stress from HMF and furfural was analyzed, the presence of biochar favored fermentation and decreased the reaction time. [10]. The present study corroborates previous studies on the application of biochar for the adsorption of inhibitors and in fermentation.

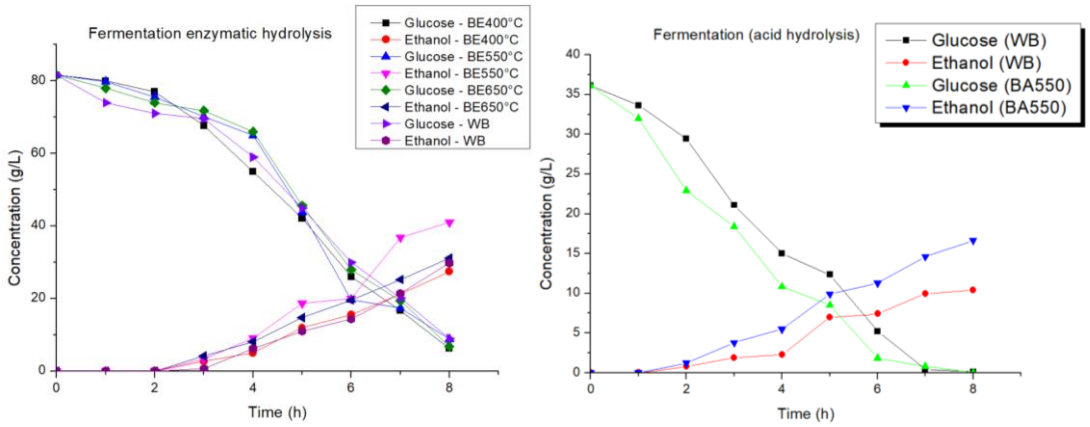


**Figure 1** Concentration profiles of ethanol and glucose during fermentation of the enzymatic and acid hydrolysates without (WB) and with biochar (BE or BA) produced at temperatures of 400, 550, and 650°C

## 4 CONCLUSION

This work demonstrated the possibility of producing second-generation ethanol from the sisal mucilage and the use of the biomass waste after hydrolysis to produce biochar, which has proven to be of excellent quality for application as an adsorbent of inhibitors and favored the fermentation ethanolic.

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