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Characterization of acid hydrolysis of sisal

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HIGHLIGHTS

- Pre hydrolysis at 105 °C was effective.
- Significant portion of xylose and arabinose was removed.
- The main product formed by acid hydrolysis at 160 °C was glucose.
- Ethanol was produced with 92% conversion of initial total reducing sugars.
- Sisal is a material that demonstrates feasibility for ethanol production.

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ABSTRACT

The present study aimed to characterize and evaluate the hydrolysis of sisal fibers for ethanol production. Acid hydrolysis of the fiber was performed and main products determined were xylose and glucose. However, xylose and arabinose (pentoses) are not fermented by the conventional yeast *Saccharomyces cerevisiae*. Prehydrolysis at 105 °C was efficient, removing most of the pentoses. The main product formed in acid hydrolysis at 160 °C was glucose, which can be metabolized by the yeast *S. cerevisiae* through alcoholic fermentation. In these conditions ethanol was produced with 92% conversion of initial total reducing sugars (0.47 g/g of ethanol yield). Sisal has potential as a raw material for ethanol production since it is not a food source, has high cellulose content and low biomass cost.

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1. Introduction

The population growth, higher vehicle numbers and atmospheric pollution caused by fossil fuels indicate the need for clean and renewable energy sources. Among the most widely studied new alternatives are biodiesel and bioethanol [1].

New technologies for producing second-generation bioethanol have enabled the use of agroindustrial wastes in bioethanol production; that is, converting the cellulose in this waste into fermentable sugars through acid or enzymatic hydrolysis [2].

Sugar as a renewable resource, can be derived from a variety of biological feedstocks such as lignocellulosic biomass. The sugar derived from lignocellulosic biomass can be further converted to a number of high-value bio-based fuels. The selection of the appropriate feedstock for biochemical production strongly depends on its local availability and cost. Currently, the most widely used feedstocks in tropical and sub-tropical regions, are from sugar cane; while in temperate regions, starchy products from crops such as corn, sorghum, wheat, rye, barley and potatoes are being used.

However, there is a drawback in producing biofuel and biochemical from sugar containing material or starch since these feedstocks are at the same time used as human food source and tends to be expensive and may cause the shortage of food supply. Therefore, recent interest is focused on the application of the low cost and abundantly available lignocellulosic biomass as potential feedstock for biofuel and biochemical production [3,4].

Lignocellulosic wastes, such as sisal and sugar cane bagasse, are being increasingly studied owing to their chemical composition, consisting mainly of cellulose, hemicellulose and lignin [2].

Originally from Mexico, the sisal plant (*Agave sisalana*) is cultivated in developing countries. In Brazil, plantations are concentrated in the states of Paraiba and Bahia. Sisal cultivation in the country has a planted area of around 154,000 ha, producing almost 800 kg/ha [5].

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Sisal is a potential raw material for ethanol production given its relatively low biomass cost, the fact that it is not a food source and its high cellulose content, about 10% higher than sugar cane bagasse. In regard to hemicellulose, 90% of sisal fiber is material that generates fermentable sugars, which form ethanol through fermentation. These sugars are produced by hydrolysis, a reaction where bonds linking several glucose units in cellulose and other sugars in hemicellulose are broken [6,7].

In order to be employed as raw material, cellulose must be exposed to a catalyzer. To that end, lignocellulosic material must undergo pretreatment to completely or partially remove lignin and hemicellulose. Its compact and complex structure requires physical and/or chemical pre-treatment of the biomass before hydrolysis for ethanol production. Pre-treatment aims to remove lignin and hemicellulose, reduce cellulose crystallinity and increase porosity in order to make cellulose susceptible to hydrolysis. Regardless of the type of hydrolysis used, pretreatment is essential and several methods can be applied, including physical, chemical and biological [8].

Among all the pretreatment methods, dilute sulfuric acid pretreatment has been extensively studied because it is generally inexpensive, convenient and effective for a broad spectrum of lignocellulosic biomass (herbaceous, softwood, and hardwood) [9]. The dilute sulfuric acid pretreatment can effectively hydrolyze hemicellulose into monomeric sugars (arabinose, galactose, glucose, mannose, xylose, etc.) and soluble oligomers, thus improving cellulose conversion [10].

Pentosans (C5), namely xylose and arabinose, are more susceptible to thermal degradation than hexosans (C6), namely glucose, galactose, and mannose. Therefore, especially in those substrates containing significant amounts of C5, the pretreatment process should produce an effective biomass destructuration without compromising the recovery of C5 [11].

Fermentation is the phase where sugar-rich liquid from hydrolysis (hydrolyzed liquor) is fermented by the yeast *Saccharomyces cerevisiae*, the same fungus used to bake bread. In the final stage liquids from different fermentations are distilled. The product of this distillation is ethanol, which exhibits the same characteristics as those produced from sugar cane in industrial processes [12].

Brazil is the world's largest producer of sisal, which has a rapid growth cycle and high cellulose content. As such, it displays potential as raw material for the production of ethanol.

This work aims to realize preliminary characterization and evaluate the hydrolysis process of sisal fiber for ethanol production.

2. Materials and methods

Sisal samples (*A. sisalana*) were provided by a farmer from the municipality of Nova Floresta, in the state of Paraiba (Fig. 1).

Sisal fiber was dried to constant weight at 75 °C in a circulating air oven and ground in a Tecnal Marconi TE340 knife mill.

2.1. Chemical composition of sisal

2.1.1. Moisture content

Fresh sisal samples in triplicate were placed in an oven at 105 °C. Five grams of sample was added to each crucible and kept in the oven for 24 h. Samples were then removed, cooled in a desiccator and weighed [13]. Moisture content was determined using the following equation:

$$Moisture\% = \frac{(initial\ weight\ \ - final\ sample\ weight)}{final\ sample\ weight} \times 100 \qquad (1)$$

Fig. 1. Sisal.

2.1.2. Ash content

Total ash content was measured by calcination. Two grams of dry material was placed in a muffle furnace at 700 °C for 12 h and weighed after cooling in a desiccator [14].

2.1.3. Extractive content

Thirty grams of sample was submitted to extraction in a Soxhlet extractor for 6 h. Solvents used were n-hexane and ethanol at a ratio of 1:1 [15].

2.1.4. Lignin content

Lignin content was determined using the Klason method with insoluble lignin in 75% sulfuric acid. Samples were treated with 75% sulfuric acid and submitted to agitation for 2 h. The system was then heated to boiling point, followed by the addition of 560 mL of distilled water, diluting the acid solution to 3%. Lignin content was measured after filtration and drying [15].

2.1.5. Holocellulose

For the determination of holocellulose content was added 5 g sample in erlenmeyer, 0.75 g of sodium chlorite, 0.5 mL of concentrated acetic acid and 100 mL of distilled water, and the mixture kept under stirring until the chlorite was dissolved. The mixture was stirred at 75 °C for 1 h. The same amount of reagent was added every hour for a period of 3 h. The system was cooled with ice to prevent oxidation, and then filtered by vacuum filtration. The residue was washed with distilled water and ethanol, dried at 60 °C to constant mass and weighed. The holocellulose content was determined by the difference between initial and final mass [15].

2.1.6. Cellulose and hemicellulose

Chemical methods of separation of cellulose and hemicellulose were based on the methodology for sugarcane bagasse proposed by Xu et al. 2006 [16]. Adapted for sisal, 2 g sample was added 4.1 mL of distilled water, 14.5 mL of acetic acid and 1.37 mL of nitric acid. The samples were digested for 20 min at 60 °C under stirring.

After this time was added 200 mL of distilled water and the solution was digested for 40 min to separate the particles and facilitate filtration. After cooling, the samples were vacuum filtered and the precipitate was determined gravimetrically as cellulose total.

The alpha cellulose was determined by transferring the residue remaining from the previous procedure to a 250 mL erlenmeyer then added 100 mL of sodium hydroxide at 17.5% m/v and the mixture kept under stirring at room temperature, for 30 min.

After this time was added 100 mL of distilled water and remained 30 min of settling. The material was vacuum filtered with a quantitative filter paper, the filtrate was set aside for determination of beta cellulose, and the residue was washed with distilled

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