EFFECT OF LIQUID FRACTION RECYCLING IN THE ACID PRETREATMENT OF SUGARCANE BAGASSE ON THE FORMATION OF THE INHIBITORS FURFURAL, HMF AND ACETIC ACID

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ABSTRACT

The study on the impact of liquid fraction recycling in the acid pretreatment of sugarcane bagasse investigated how this practice affects the formation of inhibitors such as furfural, HMF and acetic acid. During the acid pretreatment, which aims to hydrolyze hemicellulose and to maximize subsequent steps, recycling the liquid fraction can result in the accumulation of these inhibitory compounds. Furfural and HMF are products of the degradation of hemicellulose and cellulose sugars respectively while acetic acid is released by the hydrolysis of acetyl groups in hemicellulose. High concentrations of these inhibitors can compromise the efficiency of subsequent biotechnological processes. High-performance liquid chromatography (HPLC) analysis showed that the increase in xylose concentration in the acid hydrolysate, over the course of the recycles, was accompanied by an increase in acetic acid and, to a lesser extent, furfural. However, except for acetic acid, the severity degree used in these experiments did not significantly increase other inhibitors to the point of necessitating their detoxification for subsequent use in fermentative processes.

Keywords: Acid hydrolysis. Liquid fraction recycling. Inhibitors. Sugarcane bagasse. Furfural.

1 INTRODUCTION

Biofuels have emerged as a promising technological solution to reduce dependence on fossil fuels and their derivatives, offering an environmentally sustainable and promising alternative for bioenergy generation. Among the sources with lower environmental impact, biomass has shown high potential due to its low cost, ease of handling, and large-scale production[1]. Among agro-industrial residues, sugarcane bagasse stands out, being mainly composed of three macro-components: cellulose, hemicellulose, and lignin. For the production of second-generation bioethanol, pretreatment steps are necessary to break down the organic complex and facilitate the removal of lignocellulosic components, providing greater accessibility to enzymes during hydrolysis and a higher biotechnological yield[2].

In the literature, various types of pretreatment are described, among which dilute acid treatments have proven effective in solubilizing hemicelluloses, hydrolyzing polysaccharides into xylose and arabinose monomers, and increasing the enzymatic digestibility of biomass[3]. This pretreatment step is one of the most expensive stages in the production of 2G ethanol, which has driven research towards cost reduction strategies for this step. Several strategies have been adopted, with the recycling of the acid solution being one of the most promising for reducing the consumption of chemical agents and water in the process[3]. However, depending on the severity used in the treatments (temperature, % of acid, and reaction time), the sugars in the hemicellulosic fraction degrade, forming a pentose liquor with high concentrations of inhibitors such as furfural and hydroxymethylfurfural, formic, levulinic and acetic acids, and phenolic compounds such as vanillin and syringaldehyde[4,5,6]. In subsequent steps, these inhibitors can divert the metabolism of cell growth and ethanol production, damage the cell walls and membranes of yeasts, or bind to enzymes, reducing enzymatic activities and consequently ethanol production[4,7].

In this context, the purpose of this study was to analyze the generation of inhibitory compounds in the acid hydrolysate produced during the pretreatment of sugarcane bagasse with sulfuric acid, through successive recycling of the liquid fraction. This approach aims to promote water and chemical input savings, concentrate the pentoses in the solution, and reduce the costs of this stage of the process.

2 MATERIAL & METHODS

The biomass, originating from the Alcoolquímica Plant of the JB Group in Pernambuco, was processed according to the flowchart below (Figure 1). Initially, the sample underwent a drying and grinding process at the Experimental Biorefinery of Organic Solid Waste, located at UFPE, to standardize its chemical composition.

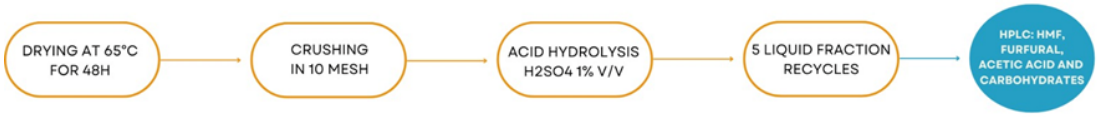

Figure 1: Experimental processes applied to sugarcane bagasse biomass.

The acid pretreatment with recycling of the acidic solution (H_2SO_4) was conducted in triplicate, and the solution underwent 5 cycles. The experiments were carried out in 500 mL Erlenmeyer flasks, where half of the volume consisted of 10% sugarcane bagasse (25g) and 90% 1% v/v H_2SO_4 solution (225 mL). The samples were autoclaved at 121°C for 1 hour, and then the biomass was separated by filtration using TNT fabric. The solid was washed until neutral pH and subsequently dried in an oven at 65°C. The liquid fraction was reused, maintaining 90%, and only the mass of bagasse was added after filtration of each cycle, maintaining the proportion of 10% (C1: 25g for 225 mL; C2: 17.62g for 158.58 mL; C3: 12.29g for 110.61 mL; C4: 8.33g for 74.97 mL; C5: 5.5g for 50 mL). All liquid fractions were analyzed by High-Performance Liquid Chromatography (HPLC) to determine the levels of carbohydrates, acetic acid, furfural, and HMF, using an Aminex HPX 87H column and a refractive index detector 2414 on a Waters high-performance liquid chromatography.

3 RESULTS & DISCUSSION

The analysis of the acid hydrolysate by high-performance liquid chromatography (Figure 2) showed that the recycling of the acidic solution proved effective up to the fifth cycle[8], the acidic solution provides protons that promote the breakdown of bonds between polymeric chains of hemicelluloses and celluloses. The xylose concentration in the fifth cycle was 70.8 g/L, a value higher than that obtained[3], which was 68 g/L under conditions of 1.5% H_2SO_4 and 30 minutes of reaction. In the first cycle, the xylose concentration was 19.2 g/L, in the studies by Vaz et al.[3] it was 12.3 g/L, and 25.6 g/L in other works[6] (2% sulfuric acid, 60 min, 121°C). In this same study, a factorial design revealed that the factors time (10-60 min), temperature (120-180°C), and % acid (2-20%) alone did not have a direct influence on xylose levels, but rather the combination of time × temperature and acid × temperature.

Regarding the inhibitors of microbial metabolism generated during the acid hydrolysis process, high concentrations of acetic acid were observed in the liquid fraction of the pretreatment. These concentrations are directly related to the hydrolysis of hemicellulose into xylose and the release of acetyl groups from hemicellulose[9]. Acetic acid reached 10.4 g/L in the fifth cycle of acid hydrolysis, contrasting studies that obtained 8.1 g/L in just one cycle under conditions of 2% sulfuric acid, 60 min, 121°C[6]; however, in other studies[10], the values in the first cycle were similar, 2.21 and 2.89 g/L (1% sulfuric acid, 10 min, 190°C, solid loads of 15% and 10% respectively) to the present study (2.2 g/L).

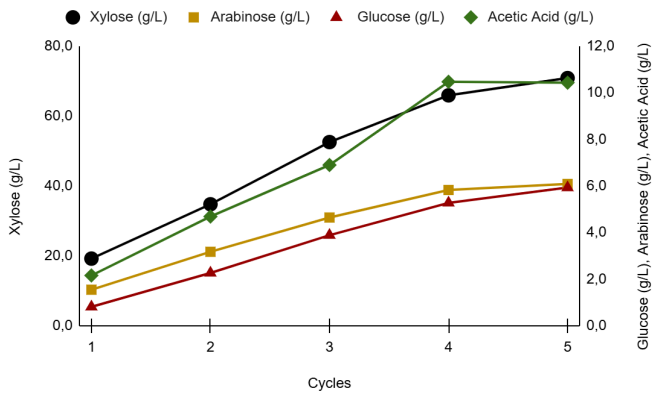

Figure 2: Concentration of carbohydrates and acetic acid in the acid hydrolysate at each pretreatment cycle of sugarcane bagasse biomass.

As for the inhibitors generated as byproducts of sugar degradation in the samples, only furfural and 5-hydroxymethylfurfural (HMF) were identified and quantified by HPLC (Figure 3), where it is possible to observe a gradual increase, especially in furfural, in relation to the cycles of acid hydrolysis, as this is derived from the dehydration of xylose, which is the most abundant monomer present in the liquid fraction. However, the values found in the fifth cycle in this study are well below those found in the acid hydrolysates[10], with 0.01 g/L of furfural and 0.02 g/L of HMF.

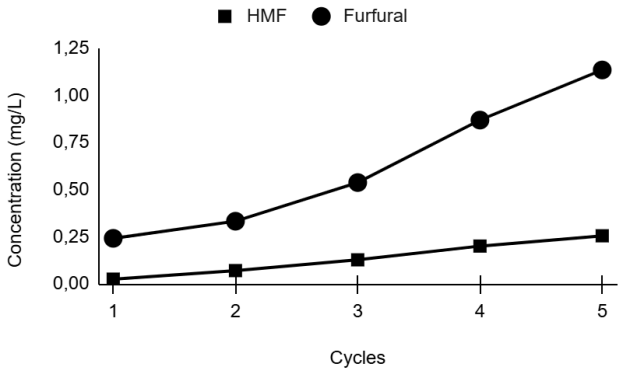

Figure 3: Concentration of HMF and furfural in the acid hydrolysate in each pretreatment cycle of sugarcane bagasse.

Other inhibitors, such as formic acid and levulinic acid, as well as phenolic compounds vanillin and syringaldehyde derived from lignin degradation, were not found in the chromatograms of the acid hydrolysates in the present study. Thus, pretreatment with recycles of sulfuric acid (1%) proved efficient in concentrating xylose throughout the cycles of recycling of the acid solution. However, concerning acetic acid concentrations, they appear high, since studies on fermentation for ethanol production from acid hydrolysates indicate that 2.5 g/L of acetic acid are the maximum inhibition limits with *Scheffersomyces stipitis*[4]. Regarding furfural and HMF, the concentrations in the fifth cycle in the present study are well below the inhibition limits (2.5 g/L)[4].

4 CONCLUSION

Given the results obtained, it is possible to conclude that for the chosen conditions (1% sulfuric acid, 121°C, 60 minutes), it is possible to recycle the liquid fraction of the pretreatment process without a severity level high enough to generate high levels of inhibitors such as furfural, HMF, formic acid, levulinic acid, and phenolic compounds. However, for the utilization of the hydrolysate for fermentative purposes, additional detoxification processes for the removal of acetic acid are indicated.

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