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Alkaline hydrogen peroxide pretreatment, enzymatic hydrolysis and fermentation of sugarcane bagasse to ethanol

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HIGHLIGHTS

- Evaluation of sugarcane bagasse pretreated with hydrogen peroxide.
- Evaluation of necessity of particle size reduction.
- Influence of increasing solids loadings in the pretreatment and hydrolysis stages.
- Performance evaluation of batch fermentation of pure hydrolysate.
- Evaluation of continuous fermentation of hydrolysate with sugarcane molasses.

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ABSTRACT

The pretreatment of sugarcane bagasse with alkaline hydrogen peroxide was evaluated for second generation ethanol production via enzymatic hydrolysis and fermentation using *Saccharomyces cerevisiae*. Factorial designs were used to determine the need for particle size reduction as well as to optimize pretreatment conditions and enzymes loadings in the hydrolysis. The influence of increasing solids loadings in the pretreatment and hydrolysis stages was determined; batch fermentation of pure hydrolysate, as well as continuous fermentation of hydrolysate concentrated with sugarcane molasses were performed. Furthermore, mass balances were used to determine the mass of ethanol obtained by mass of raw bagasse in different operational conditions. The pretreatment increased bagasse enzymatic digestibility without the need for prior size reduction. In the optimal pretreatment (1 h, 25 °C, 1.84 mL hydrogen peroxide/g bagasse) and hydrolysis conditions (3.5 FPU/g bagasse of cellulase and 25 CBU/g bagasse of β-glucosidase), 416.7 kg glucose/ton of raw bagasse were obtained. Fermentation of pure hydrolysate led to an ethanol yield of 187.85 kg/ton of raw bagasse.

1. Introduction

Pretreatment is an important stage in the production of ethanol from biomass. Different pretreatments lead to materials with different characteristics, which influence the enzymatic hydrolysis and fermentation steps. The appropriate choice of a pretreatment can be determinant for the process economical and technical viability and should be made considering aspects of the whole process [1], including the energy input and output and the environmental impact [2]. Thus, in the last years many researchers have been trying to develop a cost-effective technology that leads to maximum fermentable sugars recovery, with minimum inhibitors production and energy input, low demand of post-pretreatment processes and low costs for reactors, water and chemicals [3].

Alkaline hydrogen peroxide has been shown to be a good choice for the pretreatment of lignocellulosic biomass [4–8], as it leads to high glucose yields and can be carried out in conditions of moderate temperature and pressure without acids, which leads to minor inhibitors formation [9]. It is a typical environment-friendly agent used for delignification in wood pulping processes [10] and leaves no residues in the biomass, as it degrades in oxygen and water, thus minimizing the need for waste treatment.

In this work the process of second generation ethanol production from sugarcane bagasse was studied considering alkaline hydrogen peroxide as the pretreatment agent. All the steps in the study were performed using bagasse from a same harvesting as the used in the work of Rabelo et al. [11], which evaluated lime

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as the pretreatment agent. The objective was to compare the pretreatments considering their influence in all the stages of the production process. First, the need for particle size reduction before pretreatment was assessed using a complete factorial design. Then, a central composite design was performed to determine the optimal values for time, temperature and peroxide concentration in the pretreatment. The influence of increasing solids loading in the pretreatment was determined and the composition of bagasse pretreated at different solids loadings were compared. The lignin in the pretreatment liquor was recovered and characterized by differential scanning calorimetry and gel permeation chromatography. The optimization of enzymes loading in hydrolysis was performed using a central composite design and the influence of increasing solids loading in the hydrolysis was assessed. Finally, the hydrolysate was fermented using *Saccharomyces cerevisiae* in batch mode and the hydrolysate concentrated with sugar cane molasses was fermented in continuous mode for 21 days using the same microorganism. Mass balances for the process considering different process alternatives were performed and compared to the obtained in the work of [11].

2. Materials and methods

2.1. Raw material

Fresh sugarcane bagasse, from a single harvest (2005/06), was provided by the sugar plant Usina São Luiz – Dedini S/A (Pirassununga/SP, Brazil). The material was obtained by manual harvesting of burnt sugarcane and from the last milling resulting after juice extraction without washing. It was dried at 45 °C for 48 h, left for 48 h at room temperature, and stored into plastic bags. The average dry matter content (DM) was 95.0%.

2.2. Particle size

In order to study the influence of particle size on the release of fermentable sugars, the bagasse was divided into two parts. One part was used as it comes from the mill, without prior screening, and presented highly heterogeneous particle sizes, with 85.59 ± 2.89% of the mass presenting average particle size larger than 1.397 mm (non-screened bagasse). The other part of the material was screened (without grinding), and the portion which passed a 12 mesh and was retained by a 60 mesh screen (average particle size between 0.248 and 1.397 mm) was selected. Material of finer mesh corresponded to sand mainly and was not used.

2.3. Pretreatment

Pretreatment was carried out in 250 ml flasks in an orbital shaker MA-832 (Marconi, Piracicaba, SP, Brazil), agitated at 150 rpm. 4.00 ± 0.01 g of bagasse was used in each pretreatment run, at a concentration of 4% (w/w) DM, except for the runs performed to evaluate the influence of solids concentration in the pretreatment. Hydrogen peroxide concentration, temperature and pretreatment time were varied according to factorial designs as shown in the results and discussion section. After the pretreatment step, the liquors were separated from the solid fraction by filtration. They were then reserved for the precipitation and recovery of lignin and determination of sugars (monomeric and oligomeric fraction), furanic aldehydes, and organic acids concentrations. The solids fraction was washed several times for removal of the water-soluble solids (WS) and used to determine the water insoluble solids (WIS) recovery (pretreatment yield) and chemical composition [12,13].

2.4. Batch enzymatic hydrolysis

The enzymatic hydrolysis of the washed material was performed using 3.00 ± 0.01 g substrate at solid concentration of 3.0% (w/w) DM (except for the tests to determine the influence of solids loading in hydrolysis conversion) in 250 ml flasks incubated in an orbital shaker MA-832 (Marconi, Piracicaba, SP, Brazil) agitated at 100 rpm at 50 °C. The pH was adjusted to 4.8 with 0.05 mol/L sodium citrate buffer. Initially, cellulase from *Trichoderma reesei* ATCC 26921 (Sigma–Aldrich Corporation, St. Louis, MO, USA) was added at a loading corresponding to 3.5 FPU/g pretreated bagasse and β-glucosidase from *Aspergillus niger* (Sigma–Aldrich Corporation, St. Louis, MO, USA) was added at a loading corresponding to 1.0 CBU/g pretreated bagasse. After the evaluation of enzymes loading, experiments were performed at the enzymes concentrations determined.

Cellulase activity was determined as filter paper units per milliliter, as recommended by the International Union of Pure and Applied Chemistry [14]. β-glucosidase activity was determined through a solution of cellobiose 15 mmol/L and expressed in units per milliliter (CBU/mL) [15]. Enzyme activity was 47.44 FPU/mL for cellulases and 343.63 CBU/mL for β-glucosidase.

2.5. Fed-batch enzymatic hydrolysis

The hydrolysis started with 5% (w/w) DM (5.00 ± 0.01 g) in 250 ml flasks incubated in an orbital shaker MA-832 (Marconi, Piracicaba, SP, Brazil) agitated at 100 rpm at 50 °C. The pH was adjusted to 4.8 with 0.05 mol/L sodium citrate buffer. Cellulase and β-glucosidase were added at a concentration corresponding to 3.5 FPU/g pretreated bagasse and 25 CBU/g pretreated bagasse, respectively. After 6 and 12 h of reaction, 2.5 g of pretreated bagasse and the amount of enzymes to maintain enzymatic loads of 3.5 FPU/g and 25 CBU/g were added. The total solids concentration reached 10% (w/w) DM.

2.6. Fermentation

Hydrolysate obtained by fed-batch enzymatic hydrolysis of pretreated sugarcane bagasse (approximately 70 g/L glucose) was used for ethanol batch fermentation. In addition, fermentation was conducted using standard glucose (70 g/L) for comparison. The microorganism was an industrial strain of *S. cerevisiae* provided by Santa Adélia ethanol plant, SP, Brazil. Fermentation was performed in shaker at 34 °C and 150 rpm.

Continuous fermentation was performed by using sugarcane molasses to increase the hydrolysate sugars concentration (sucrose + glucose) to around 70 g/L. The hydrolysate was from hydrolysis performed at 3% (w/w) solids. Fermentation was conducted in bioreactors Bioflo III (New Brunswick Scientific Co., Inc., Edison, NJ) with 700 mL of working volume, stirred by two flat blade turbines, with six blades each, at 300 rpm. The flow rate was 0.6 mL/min and the temperature 34 °C. In order to evaluate the influence of adding hydrolysates in different proportions on the kinetics of molasses fermentation, the fermentation was initiated with pure molasses and in predetermined time intervals different percentages (mass) of hydrolysate was added to the feeding medium.

2.7. Inoculum and culture media

The media composition for inoculum growth was 50 kg/m^3 of glucose, 5 kg/m^3 of KH_2PO_4, 1.5 kg/m^3 of NH_4Cl, 0.7 kg/m^3 of $MgSO_4{\cdot}7H_2O$, 1.2 kg/m^3 of KCl, and 5 kg/m^3 of yeast extract. The inoculum growth was performed in erlenmeyer's flasks maintained in a shaker at 30 °C and 150 rpm for 24 h. Prior to fermentation, the molasses sterilization was performed in an autoclave Phoenix