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Enzymatic saccharification of lignocellulosic materials after treatment with supercritical carbon dioxide

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A B S T R A C T

Lignocellulosic materials, such as agricultural residues, are abundant renewable resources for bioconversion to sugars. The sugar cane bagasse was studied here to obtain simple sugars for the production of alcohols and other chemicals. The crystalline structure of cellulose and the lignin that physically seals the surrounding cellulose fibers makes enzymatic hydrolysis difficult by preventing the contact between the cellulose and the enzyme. Two different samples of sugar cane (bagasse pulp and skin) were used and compared with microcrystalline cellulose (Avicel). The investigated samples were pretreated with SC-CO_2 explosion before hydrolysis. The experiments were conducted at 12, 14 and 16 MPa at a temperature of 60 °C. In this process, particles of celluloses within the size range from 0.25 to 0.42 mm were placed in defined amounts inside the experimental vessel, CO_2 was injected and let stand for 5 and 60 min. The explosion pretreatment of cellulosic materials by SC-CO_2 was performed in an apparatus of a static type with 300 ml of volume. The hydrolysis reaction using cellulose enzyme was carried at 55 °C for 8 h. After the pretreatment, the glucose yield increased in 72% to the bagasse sample. The SC-CO_2 pretreatment together with alkali increased the glucose yield in 20% as compared with alkali only. X-ray, microscopy and thermal analysis were used to investigate the effect of the pretreatment.

1. Introduction

A significant amount of research is conducted worldwide in trying to find renewable sources of liquid fuels to replace fossil fuels. Burning fossil fuels such as coal and oil releases carbon dioxide in the environment, which is a major cause of global warming [1]. Lignocellulosic materials are a potential resource that is not used for the production of biogas and ethanol. For a long time studies have been performed to pursue alternatives to increase the digestibility of these materials in order to obtain efficient conversion of cellulose to ethanol, methane and, more recently, also to hydrogen [2]. The conversion of abundant lignocellulosic biomass to biofuels represents a viable option for improving energy security and reducing greenhouse emission. Pretreatments represent a potential process to improve the digestibility of the lignocellulosic biomass. Sugar cane bagasse is among these materials that can be used as raw material for conversion into products of commercial interest.

The composition of these materials is almost identical with some variation in the content of the components. According to Pandey et al., bagasse consists of approximately 50% cellulose with the remainder content distributed between hemicellulose, lignin and other compounds in small amounts [3]. The advantage of using sugarcane bagasse compared to other lignocellulosic materials is due to its levels of ash at below 5%, which is lower than usually identified in other materials.

The role of pretreatment is to increase the permeability of the material for subsequent hydrolysis and conversion to fuels. The pretreatment techniques change the structure of the lignocellulosic biomass and are classified into: physical and chemical.

Physical treatments can be divided into mechanical and thermal. The mechanical pretreatment consists in cutting the bagasse in small pieces, reducing the particle size and crystallinity, increasing the contact surface and improving the hydrolysis of the material. The power requirements for mechanically comminuting agricultural materials depend on the targeted final particle size and the waste biomass characteristics [4]. Thus energy costs for size reduction can make the treatment expensive.

Heat treatment (steam treatment) consists basically in heating the lignocellulosic material at temperatures around 150–180 °C or higher. Depending on the methodology used, hemicellulose and lignin can be degraded by thermal treatment. These components solubilize at temperatures close to the ones generally used. Steam explosion and LHW (*Liquid Hot Water*) can be included in this class of treatment. The disadvantage of the heat treatments is the risk of the formation of inhibitory compounds for the production of ethanol.

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Chemical treatments are widely utilized such as the use of acids and bases, as well as the use of peroxides to increase the digestibility of lignocellulosic materials.

The search for a method to adequately increases the digestibility of these materials and pretreatment techniques including physical, thermal, chemical and biological procedures have long been studied. In these studies the need for identifying methodologies that combine high yield and low waste is outstanding.

Treatment with supercritical carbon dioxide represents an interesting alternative because it can be operated at lower temperatures compared to thermal treatments, thus eliminating the problem of inhibitors formation with the degradation of hemicellulose and lignin.

The easy recovery of the solvent used enables the reutilization of the CO_2 after the process. It is nonflammable, essentially nontoxic, and very volatile. The properties of supercritical CO_2 are widely known and its applications in the pharmaceutical industry are also broad; its high power diffusion and low viscosity represent advantages over conventional solvents. The particular behavior of supercritical CO_2 in the structure of biomass is not well known; however it is known that it works more easily in rigid and wet biomass than in more flexible lignocellulosic materials. Kim and Hong investigated the effect of SC-CO_2 on raw lignocellulose with different moisture contents at various pretreatment conditions [5]. Similar studies performed with Avicel indicated that the explosion with supercritical CO_2 causes a decrease in the crystallinity of the material and promotes the hydrolysis [6]. A similar work reported that the treatment of explosion using supercritical CO_2 in lignocellulosic materials increased (50–70%) digestibility of the material and that the process was improved with increased pressure [7] and a best result of delignification (94.5%) was obtained with the combination of lower pressure and lower amount of 1-butanol [8].

Therefore the objective of this work is to investigate and compare the effect of pretreatment with supercritical CO_2 explosion in sugarcane bagasse and Avicel. A secondary objective is to evaluate the improvement of treatment combining supercritical explosion and alkaline treatment. In order to assess the effects of each form of treatment and the importance of the pretreatment tool over the material structure changes, methods of structural analysis (X-ray, microscopy, and thermal analysis) were used. The pretreatment process was used to enhance the digestibility of the lignocellulosic biomass.

2. Materials and methods

2.1. Material

The bagasse was chosen as a source of lignocellulosic material and Avicel which has also been used as a sample of micro crystalline cellulose was donated by FMC in Brazil. The bagasse was washed and oven dried for 24 h at a constant temperature of 60 °C. This material was submitted to a separation process to obtain two types of samples, bagasse pulp and skin. Both materials were then grinded and sieved to attain particle sizes in the range of 0.25–0.42 mm. Samples were stored in a dry environment at room temperature in order to avoid contamination.

2.2. Pretreatment using supercritical CO_2

The pretreatment was performed in a high pressure vessel as the experimental unit with an effective volume of 300 ml. The equipment was designed to operate with pressure levels up to 20 MPa and temperatures up to 300 °C. A thermostatic bath was used to maintain the constant temperature at 60 °C. The scheme used is shown in Fig. 1. The experiments were performed by changing the pressure and exposure time of CO_2. The pretreatments with SC-CO_2 were executed at 12, 14 and 16 MPa for time exposures of 5 and 60 min. The procedure consisted in placing 5 g of sample inside the vessel and feed with CO_2. After the sample was exposed to supercritical CO_2 for a designated length of time and maintained at a certain pressure and temperature, a quick pressure release was performed by opening a valve attached to the vessel thus bring the pressure levels to atmospheric pressure.

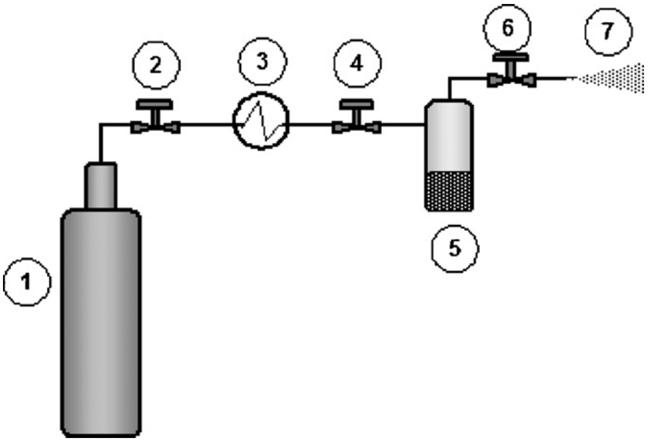

Fig. 1. Schematic diagram of the apparatus used in SC-CO_2 explosion. 1, liquid CO_2 tank; 2, outlet valve; 3, heating (water bath); 4, inlet valve; 5, reactor with sample; 6, pressure relief valve; 7, gas expansion.

2.3. Pretreatment with alkali + SC-CO_2 explosion

The alkali pretreatment used followed the method described in Fox et al. [9]. The pretreatment involved cooking the bagasse in a caustic solution for a given time at a specified NaOH concentration and solid contents. A proportion of 0.20 g NaOH/g of bagasse was used in a volume of 100 ml of distilled water. The process consisted in thermal and alkali treatment of the material at 100 °C for 60 min in atmospheric pressure. After the material was washed with distilled water to neutrality, squeezed and most of the moisture removed it was air dried at 37 °C and finally submitted to the SC-CO_2 explosion at 16 MPa during 60 min.

2.4. Enzymatic saccharification

Saccharification reactions were carried out by shaking the samples at 130 rpm at a temperature of 50 °C for 8 h. The enzyme complex used was donated by Novozymes of Brazil and consisted of two enzymes: cellulase (NS50013) and β-glucosidase (NS50010). The initial activity of the first one was 700 EGU/g (EGU, endoglucanase units) ~70 FPU/g (FPU, filter paper unit); the initial activity of the second one was 250 CBU/g (CBU, cellobiase units). β-Glucosidase was used to hydrolyze the cellobiose and other disaccharides thus enhancing the action of cellulose [10]. The enzymatic reactions were conducted in 0.5 M sodium acetate and acetic acid buffer solutions at pH 4.7, in a volume of 100 ml, using 1% (v/v) of the enzyme solution and 1.5 g of cellulosic material. After the incubation time for the hydrolysis reaction, the material was filtered on filter paper and 45 ml of the hydrolyzed sample separated for the later determination of reducing sugars. The enzyme was inactivated with 5 ml of 0.5 N NaOH and the sample stored under refrigeration. This methodology has been presented and discussed in a previous study [11]. The experiment was performed in triplicates, the temperature and pH were used according to Novozymes instructions and the time of saccharification was the same recommended by Santa'anna et al. [12].