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Effects of the pretreatment method on enzymatic hydrolysis and ethanol fermentability of the cellulosic fraction from elephant grass

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HIGHLIGHTS

- Pretreatment with NaOH was the best method.
- Optimization of the alkaline pretreatment was carried out.
- 95% Of theoretical ethanol yield was obtained.
- 88% Of lignin was removed.
- 99% Of the cellulosic fraction in the solid was recovered.

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ABSTRACT

Elephant grass (*Pennisetum purpureum*) is a lignocellulosic material that has high potential for ethanol production in tropical countries due to their high availability and adaptability. Chemical and physico-chemical pretreatments like alkaline delignification, diluted acid hydrolysis, steam explosion, alkaline peroxide and aqueous ammonia soaking were performed in order to determine their effect on the hydrolysis and the fermentability of the cellulosic fraction of this material. In an initial screening of the methods, the alkaline pretreatment with NaOH yielded the highest concentrations of reducing sugars (34.4 g/L) and ethanol (15.1 g/L). A more detailed study of the effect of the alkaline pretreatment conditions (temperature, solid to liquid ratio, NaOH concentration and residence time) on the fermentability of elephant grass was carried out. Results showed that under pretreatment conditions of 120 °C for 1 h with 2 wt.%. NaOH and a solid to liquid ratio of 1: 20 (wt.) the highest yield of ethanol was obtained, i.e., 26.1 g/L (141.5 mg ethanol/g dry biomass, 95% of theoretical yield). Furthermore, this pretreatment allowed the removal of most of the lignin present in this material, i.e., 88% lignin removal. Besides, this pretreatment allowed a high recovery of the cellulosic fraction in the solid.

1. Introduction

Due to the global energy crisis, lignocellulosic biomass has become very important as a promising raw material for obtaining second generation biofuels. Lignocellulosic materials have a high potential for ethanol production because they are abundant renewable resources, do not compete with food production, could lead to the use of large quantities of agro-industrial wastes whose disposal is problematic for the environment, and could use marginal or degraded agricultural lands for growing energy crops.

Most tropical countries, like Colombia, have a high potential, in terms of availability and variety, of lignocellulosic biomass because of their high solar radiation, diversity of climatic zones and biodiversity. These advantages allow an easy adaptation of different species and the development of energy crops such as grasses and forages. Among these, elephant grass (*Pennisetum purpureum*) has a very high production yield of dry material, i.e., 40–50 tons/acres/year [1,2], under optimal conditions of growth and management.

Because of its chemical composition, lignocellulosic biomass is very different from biomass with large content of sugars or starch which is customarily used in biofuel industry. The structure of these former materials, mainly composed by cellulose, hemicellulose and lignin, requires the process for biofuels production to be adjusted for each type of biomass, according to their component characteristics. Therefore, a previous pretreatment step must be introduced to obtain hydrolysable fractions that can be converted into sugars and subsequently fermented to ethanol.

Different kinds of pretreatment methods, under a large variety of conditions, have been studied to improve the fermentability

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and digestibility of several varieties of grasses like bermuda, switchgrass, napiergrass and silver grass [3–11]. Dilute acid pretreatment of bermudagrass at 121 °C (solid loading of 10 wt.%, sulfuric acid concentration of 1.2 wt.% and residence time of 60 min) exhibits a 70% glucan to glucose conversion with a total reducing sugar production (TRS) of 204.1 mg/g biomass at 48 h of enzymatic hydrolysis [3]. Likewise, dilute acid pretreatment of silvergrass at 121 °C for 30 min, gave a high xylan recovery (70–75%) compared with rice straw and sugarcane bagasse. Furthermore, hydrolyzed silvergrass gave a higher level of fermentability than cane bagasse because less acetic acid was formed, obtaining an ethanol yield of 64.3% of the theoretical in 48 h of fermentation [12]. The alkaline pretreatment of bermuda grass was evaluated using NaOH and $Ca(OH)_2$ to improve the recovery of fermentable sugars [13]. This study showed that at 121 °C NaOH is more efficient than $Ca(OH)_2$ to improve the reducing-sugar yield, achieving 86% of the theoretical yield (ca. 500 mg of total reducing sugars/g biomass). A recent study reported a lignin removal of 86% and a yield of total reducing sugars of 71% of the theoretical (440 mg of total reducing sugars/g biomass) under optimal pretreatment conditions of bermuda grass (15 min and 0.75 wt.% NaOH at 121 °C) without evaluation of the hydrolyzed-material fermentability [7].

Switchgrass was pretreated [6,14] by soaking it in aqueous ammonia at room temperature for 5–10 days, achieving a delignification of 40–50%, while the hemicellulose content decreased almost 50%. The pretreated material was subjected to simultaneous saccharification and fermentation (SSF) using an enzyme loading of 38.5 FPU/g cellulose (Spezyme CP) and the strain Saccharomyces cerevisiae D_5A, achieving an ethanol concentration of 22.16 g/L which corresponds to an ethanol yield of 55.4 mg/g biomass. Solid to liquid ratio and soaking time slightly affected the lignin removal but did not cause significant changes in the overall ethanol yields at sufficiently high enzyme loadings. The effect of the pretreatment with $Ca(OH)_2$ at moderates temperatures (50 °C and 21 °C) was also investigated to improve the enzymatic digestibility of switch grass [14,15]. Yields of glucose and xylose of 239.6 and 127.2 mg/g biomass, respectively, were achieved at 55 °C for 72 h, using cellulase and cellobiose loadings of 35 FPU/g biomass and 61.5 CBU/g biomass, respectively. This study evidenced that calcium ions extensively cross linked lignin molecules under alkaline conditions, which substantively decreased the lignin solubilization during the pretreatment. The high lignin content in the pretreated biomass did not affect the enzymatic digestibility. Is was also reported that the ionic-liquid pretreatment of switch grass decreased the cellulose crystallinity, increased the surface area and decreased the lignin content, significantly improving the enzymatic hydrolysis rate of the cellulosic fraction [16]. Ammonia-fiber-explosion (AFEX) pretreatment has been evaluated in Miscanthus x giganteus grass and several varieties of switchgrass to obtain fermentable sugars using enzymatic hydrolysis at 50 °C for 168 h with cellulase loadings of 15 FPU/g glucan and β-glucosidase loadings of 40 IU/g glucan, obtaining glucan conversions of 90–96% and ethanol yields of 0.2 g/g biomass in 96 h of SSF process [17–19].

So far, there are no reports that compare the effect of different pretreatments on the hydrolysis and the fermentability of elephant grass. Existing reports are specifically focused in pretreatment and fermentability of elephant grass varieties [4,20–24], but under such different experimental conditions that make difficult the comparison of the results. The enzymatic pretreatment (cellulase + esterase) of elephant grass gave 113 mg sugars/g biomass [4]. The biological delignification pretreatment of a Colombian specie of penisetum sp, using ligninolytic basidiomycetes (ganoderma spp) allowed a lignin removal of 10.7–55.9% [21]. Regarding the fermentability, ethanol yields of 45.5 mg/g biomass, using the strain Klebsiella oxytoca THLC0409, and 97–107 mg/g biomass, using the strain Saccharomyces cerevisiae D_5A, have been reported for two different genotypes of elephant grass [20]. Besides, the fermentation of pentoses and hexoses from elephant grass pretreated only by fine grinding (physical pretreatment) has been reported. After this physical pretreatment, the fermentation with Saccharomyces cerevisiae NBRC2044 during 72 h yielded 113 g ethanol/g biomass from hexoses while the fermentation with E. coli K011 during 48 h yielded 31.4 g ethanol/g biomass from pentoses [25].

The reported studies have not been carried out under comparable conditions that allow the selection of the best pretreatment. In this manuscript, the effects of the physicochemical pretreatments dilute acid, alkaline pretreatment with NaOH, alkaline peroxide, steam explosion and aqueous ammonia soaking on the hydrolysis and the ethanol fermentability of the cellulosic fraction of elephant grass are presented in a comparable way. Furthermore, the conditions for the best pretreatment found were optimized in order to maximize the ethanol yield.

2. Materials and methods

2.1. Materials

Elephant grass was grown in farms located in Antioquia (Colombia). This grass was dried in air for easy handling and transport, and then was grinded to particle size less than 3 mm and dried again to achieve a moisture content of less than 10 (wt.%). The material was characterized to determine cellulose, hemicellulose, lignin, extractives and ash contents in the solid (Table 2).

2.2. Methods

2.2.1. Pretreatments

2.2.1.1. Alkaline delignification with NaOH. 20.0 g Of material was weighed and immersed in dilute NaOH solution (1 wt.%) and a solid to liquid ratio of 1 g biomass/15 g of NaOH solution. The reactor was hermetically closed and heated to 120 °C (5 °C/min) and maintained at this temperature for 30 min. Then, the reactor was cooled to room temperature and the solid was separated from the black liquor by filtration. The solid fraction was washed with water until neutral pH and then dried and stored in a freezer.

2.2.1.2. Dilute acid hydrolysis. 100 g Of lignocellulosic material was mixed with sulfuric acid solution (2 wt.%), with a solid to liquid ratio of 1 g biomass/mL solution. The suspension was stirred at 90 °C for 90 min. The mixture was filtered to separate the black liquor and the pretreated material. The solid material was washed with water until neutral pH and then dried and stored in a freezer.

2.2.1.3. Aqueous ammonia soaking. This pretreatment consisted in soaking the lignocellulosic material in an aqueous ammonia solution (15 wt.%), using 8 mL of ammonia solution per gram of material, at 60 °C for 6 h. Then, the suspension was filtered to separate the solid and the liquid fractions. The solid fraction was washed until neutral pH, dried and stored in a freezer for subsequent hydrolysis and fermentation.

2.2.1.4. Steam explosion. Steam explosion pretreatment was performed in homemade equipment. The process was carried out by loading 150 g of the raw material in the container and putting it in contact with saturated steam at 180 °C for 5 min (severity of 3.05). After the steam explosion, the material was filtered to recover the liquid and the solid fractions. Then, the solid fraction