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Research paper

King Grass: A very promising material for the production of second generation ethanol in tropical countries

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ABSTRACT

King Grass is a fast growing, high yield, hybrid grass that grows in a variety of soil conditions and does not compete with food crops. King Grass is perennial and can be harvested several times per year. Therefore it has a high potential for ethanol production in tropical countries. The pretreatments alkaline delignification (66.6–133 °C, 30–180 min, solid to liquid ratio 1:13.3–1:20 wt and NaOH concentration 0.7–2% wt), diluted acid hydrolysis (2% wt sulfuric acid, solid to liquid ratio of 1.0 g biomass/mL solution, stirring at 90 °C for 90 min), steam explosion (at 180 °C for 5 min, i.e., severity 3.05), alkaline peroxide (2% vol. H_2O_2, liquid to solid ratio of 20:1 wt, pH 11.5, stirring at 35 °C for 3 h) and aqueous ammonia soaking (15% wt ammonia solution, 8 mL of ammonia solution per gram of material, at 60 °C for 6 h) were studied. The alkaline pretreatment with NaOH led to the highest concentration of reducing sugars, i.e., 58.6 g/L, and to the highest ethanol concentration, i.e., 17.9 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 production of ethanol from King Grass was carried out. Under pretreatment conditions of 120 °C for 1 h with 2% wt NaOH and a solid to liquid ratio of 1:20 the highest ethanol yield reported so far was obtained, i.e., 27.7 g/L (165.9 mg ethanol/g dry biomass, 92% of theoretical yield). Furthermore, this pretreatment allowed the removal of most of lignin present in King Grass, i.e., 94%, and allowed a high recovery of the cellulosic fraction in the solid (93–99%). Under typical growing conditions of King Grass in Colombia this maximum ethanol yield corresponds to 12,616 L/ha/year which is higher than that obtained from sugar cane bagasse (4602 L/ha/year).

1. Introduction

Lignocellulosic biomass is a very promising raw material for the production of second generation ethanol because it is an abundant renewable resource, does 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 like grasses. Most tropical countries, like Colombia, have a high potential in terms of availability and variety of lignocellulosic biomass because of its 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, King Grass (*Pennisetum hybridum*) has very high production yield of dry material in Colombia, i.e., 40–60 t/ha/year [1], under optimal conditions of growth and management. King Grass is a fast growing, high yield and hybrid grass that grows in a variety of soil conditions and does not compete with food crops. King Grass is perennial and can be harvested several times per year. Therefore it has a high potential for ethanol production in tropical countries.

Lignocellulosic biomass does not have large contents of sugars or starch which are customarily used in first-generation ethanol. The structure of these former materials is mainly composed by cellulose, hemicellulose and lignin. Therefore, a previous pretreatment step must be introduced to obtain hydrolysable fractions that can be converted in sugars and subsequently fermented.

Different kinds of pretreatment methods, under a wide variety of conditions, have been reported to improve the fermentability and digestibility of several varieties of grasses like bermuda, switchgrass, napiergrass and silvergrass [2–8]. Dilute acid

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pretreatment of bermuda grass 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 [9]. Likewise, dilute acid pretreatment of silvergrass at 121 °C for 30 min, gave a high xylan recovery (70–75%) compared with rice straw and sugar cane 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 [10]. The alkaline pretreatment of bermuda grass was evaluated using NaOH and $Ca(OH)_2$ to improve the recovery of fermentable sugars [11]. 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 [12].

Switchgrass was pretreated [5,13] 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 (Filter Paper Unit)/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 switchgrass [14]. 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 cellobiase 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. It was also reported that the ionic-liquid pretreatment of switchgrass decreased the cellulose crystallinity, increased the surface area and decreased the lignin content, significantly improving the enzymatic hydrolysis rate of the cellulosic fraction [15]. Ammonia-fiber-explosion (AFEX) pretreatment has been evaluated in Miscanthus × 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 [16–18].

So far, there are no reports that compare the effect of different pretreatments on the hydrolysis and the fermentability of King Grass. Existing reports are specifically focused in pretreatment and fermentability of elephant grass varieties [4,19–21], but under such a 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 [2]. 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 [22]. 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 [19].

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 King Grass are presented in a comparable way to choose the best method. Furthermore, the conditions for the best pretreatment found were optimized in order to maximize the ethanol yield.

2. Material and methods

2.1. Material

King Grass was grown in a farm located in Antioquia (Colombia). This grass was dried in air for easy handling and transport, and then was grinded to particle size smaller 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.

2.2.1.2. Dilute acid hydrolysis. 100 g of lignocellulosic material was mixed with 2% (wt) sulfuric acid solution, with a solid to liquid ratio of 1.0 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.

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.

2.2.1.4. Steam explosion. Steam explosion pretreatment was performed in a 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 3.05). After the steam explosion, the material was filtered to recover the liquid and solid fractions.

2.2.1.5. Alkaline peroxide. 50 g of solid material was treated with aqueous H_2O_2 (2% vol) and with a liquid to solid ratio of 20:1 (wt). The pH was adjusted to 11.5 with 10 M NaOH. The reaction was carried out at 35 °C for 3 h with continuous stirring. The liquid and solid fractions were separated by filtering and the solid was washed and neutralized with HCl (37% vol).

The solid fractions obtained after the different pretreatments were washed until neutral pH, dried and stored in a freezer for