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Effects of NH_4Cl and $MgCl_2$ on pretreatment and xylan hydrolysis of miscanthus straw

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ABSTRACT

This study investigated the effects of NH_4Cl and $MgCl_2$ on pretreatment and xylan hydrolysis of miscanthus straw for biofuels production. It was observed that increasing the pretreatment temperature decreased the remaining solid, increased the enzymatic digestibility, and increased the xylan removal. When 0.2–5.0% NH_4Cl and $MgCl_2$ were employed in pretreatments, increasing the inorganic salt concentration slightly diminished the remaining solid, though the enzymatic digestibility was enhanced. Under the higher-than-2% condition, no xylan remained in the solid residues after pretreatment. With pretreatment time, the remaining solid slightly decreased, but the enzymatic digestibility was increased. Moreover, xylan removal was linearly increased to 15 min, after which it was completely hydrolyzed. Overall, these results indicated that pretreatment by 2% NH_4Cl or $MgCl_2$ at 185 °C for 15 min completely hydrolyzes the xylan of miscanthus straw. In scanning electron microscopy (SEM) images, the physical surface of the miscanthus straw showed an apparently damaged surface area and exposure of the internal structure after pretreatment with NH_4Cl and $MgCl_2$ by SEM.

1. Introduction

The depletion of fossil-fuel resources and global warming are spurring the development of renewable, for example lignocellulosic and marine, and energy resources (Badger, 2002; Jeong & Park, 2010; Mosier et al., 2005). Miscanthus, as confirmed by years of research in the E.U. and U.S., is one of the most promising biomasses for energy-production purposes. It can be utilized to produce animal bedding, industrial materials, heat, electricity, and a range of liquid fuels (Lewandowski, Clifton-Brown, Scurlock, & Huisman, 2000; van den Heuvel, 1994). It also is both economically profitable and environmentally friendly (Jones & Walsh, 2001; Lewandowski et al., 2000; van den Heuvel, 1994). Miscanthus is a genus of about 15 species of perennial grasses native to subtropical and tropical regions of Africa and southern Asia, with one species, *Miscanthus sinensis*, a woody rhizomatous C4 perennial that grows rapidly to high yields per hectare, extending north into temperate eastern Asia (Miscanthus, 2012). Miscanthus has several specific advantages as an energy crop, including a long production life-time of 10–15 years, a low requirement for water and fertilizer, a low susceptibility to pests and diseases, and a low moisture content at harvest (Jones & Walsh, 2001; Lewandowski et al., 2000; van den Heuvel, 1994). Moreover, it is non-invasive plant and easily reclaimed for production of crops such as corn or soy bean (Pyter, Voigt, Dohleman, & Long, 2007).

The major sugar components of miscanthus are cellulose and hemicellulose. These can be converted to mono-sugars (C6 and C5) by biological and chemical conversion processes. Typical C6 and C5 sugars are glucose and xylose, respectively. These can be converted to biofuels and valuable chemicals and other materials by thermochemical or fermentation processes (Badger, 2002; Jeong & Park, 2010; Meinita, Hong, & Jeong, 2012). To increase the bioconversion yield of mono-sugars from lignocellulosic biomasses, a pretreatment process, which can eliminate structural and compositional obstacles to hydrolysis, is applied to the lignocellulosic biomass conversion process (Badger, 2002; Castro et al., 2011; Mosier et al., 2005).

Nevertheless, even under the same conditions, pretreatment processes present different patterns of hemicellulose or lignin removal and of enzymatic digestibility increase, according to biomass kind. Recently, inorganic salts have been applied to the pretreatment of lignocellulosic biomass for ethanol production.

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Some of these have been shown to increase the rate and yield of the hydrolysis of cellulose or hemicellulose (Liu & Wyman, 2006; Liu et al., 2009; Yu et al., 2011). Inorganic salts are classified, according to their group in the periodic table and their activity, as alkaline metal chlorides (NaCl and KCl), alkaline earth metal chlorides ($MgCl_2$ and $CaCl_2$), and transition metal chlorides ($CuCl_2$, $FeCl_2$ and $FeCl_3$) (Yu et al., 2011). In pretreatment, they act in the form of sulfate, phosphate or chloride, greatly affecting biomass structure and composition thereby (Yu et al., 2011). Inorganic salts are particularly advantageous in two respects: they are less corrosive than inorganic acids, and they are recyclable (Liu et al., 2009).

Some studies have reported that inorganic salt pretreatment enhances hemicellulose degradation and enzymatic digestibility (Chen, Dong, Qin, & Xiao, 2010; Liu & Wyman, 2006; Liu et al., 2009; Marcotullio, Krisanti, Giuntoli, & de Jong, 2011; Sun, Lu, Zhang, Zhang, & Wang, 2011; Yu et al., 2011; Zhao, Zhang, Zheng, Lin, & Huang, 2011). $FeCl_3$, notably, has a highly positive effect on these two processes. According to Liu et al. (2009), $FeCl_3$, as applied to the pretreatment of corn stover, significantly increased the hemicellulose degradation, with high xylose recovery and low cellulose removal, in the 140–200 °C temperature range. Liu and Wyman (2006) found that 0.8% $FeCl_3$ at 180 °C, compared with pressurized hot water, significantly increased the degradation rates of xylose (6-fold) and xylotriose (49-fold). In wheat straw pretreatments, almost complete removal of hemicellulose was achieved with 200 mM $FeCl_3$ solutions at 120 °C (Marcotullio et al., 2011). Chen et al. (2010) reported 100% removal of hemicellulose after pretreatment with 0.1% $FeCl_2$. Yu et al. (2011) attempted to facilitate the conversion of hemicellulose to organic acids by treatment with $FeCl_2$. In corn stover pretreatment with aqueous $FeSO_4$ solution, the hemicellulose degradation between 140 and 200 °C was significantly increased with high xylose recovery and low cellulose removal (Zhao et al., 2011). Sun et al. (2011) noted that trivalent salt showed a stronger catalytic activity than di- or mono-valent salts in lignocellulose hydrolysis of silage.

In the present study, miscanthus straw was pretreated with aqueous NH_4Cl and $MgCl_2$ solutions, and the effects of several pretreatment conditions (temperature, time, and inorganic salt concentration) on the removal of xylan and the enzymatic digestibility of the biomass were evaluated.

2. Materials and methods

2.1. Materials

Miscanthus straw was collected from the banks of the Yeongsan River in Gwangju, Korea. It was washed with distilled water to remove dust, dried at 30 °C for 3 days, subsequently milled and fractionated to a particle size of 0.71–1.40 mm by sieves, and finally stored in a plastic container at room temperature. The straw, based on its dry weight, was composed of 44.5% cellulose, 26.2% hemicellulose, 26.5% lignin and 3.0% ash, as determined by the NREL LAP procedure (Sluiter et al., 2008). Cellulclast® 1.5 L (cellulase, 98 FPU/mL, Novozymes A/S, Denmark) and Novozyme 188 (β-glucosidase, 430 CBU/mL, Sigma–Aldrich Co. Ltd., USA) were used for enzymatic hydrolysis. Magnesium chloride ($MgCl_2{\cdot}6H_2O$) and ammonium chloride (NH_4Cl), both of extra pure grade, also were employed.

2.2. Pretreatment with inorganic salts

For inorganic salts pretreatment of the miscanthus straw, a high-pressure stainless steel reactor of 100 mL total volume was utilized. Five grams of miscanthus straw and 0–5% (w/w) inorganic salt solutions were mixed together in the reactor at a solid-to-liquid ratio of 1:10. The reactor was preheated for 10 min to the setting temperature, and then heated, for 5–30 min in an oil bath, to the target temperature of 150–200 °C. After the pretreatment, the solid residues were separated using filter paper (coarse, Fisherbrand®), and then washed with deionized water until a neutral pH was reached. The pretreated biomass was dried at 30 °C for 2 days, after which it was stored in a plastic container for later use.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of the biomass, in accordance with the standard NREL chemical analysis and testing procedures (Sluiter et al., 2008), was performed at 50 °C and pH 4.8 (0.05 M sodium citrate buffer) on a shaking incubator at 150 rpm. The enzyme loading amounts were 30 FPU of cellulase and 30 CBU of β-glucosidase. The initial concentration of glucan was 1 g (1%, w/v), based on 100 mL of total liquid and solid in the enzymatic hydrolysis. Enzymatic-hydrolyzed samples were taken at 72 h and analyzed for glucose concentration (Kang, Jeong, Sunwoo, & Park, 2012; Kang, Jeong, & Park, 2012).

2.4. Analytical methods

The miscanthus straw was composition-analyzed according to the NREL LAP procedure (Selig, Weiss, & Ji, 2008). The sugar concentration was measured by HPLC (Agilent 1200, USA) equipped with a refractive index detector (RID-10A, Shimadzu Corp., Japan) and SUPELCOGEL™ Pb columns (SUPELCO™ Analytical, USA). The analysis conditions were 85 °C oven temperature and 0.6 mL/min flow rate (with deionized water). The enzymatic digestibility was calculated as follows (Kang, Jeong, Sunwoo, et al., 2012; Kang, Jeong, & Park, 2012).

$$\text{Enzymatic digestibility (\%)} = \frac{\text{g cellulose digested}}{\text{g cellulose added}} \times 100 \quad (1)$$

2.5. Scanning electron microscopy of biomass

Scanning electron microscopy (SEM; JSM-5400, JEOL Ltd., Japan) was utilized to investigate the physical surface characteristics of both the raw and pretreated miscanthus straw samples. Preparatory to the analysis, the samples were coated with a thin layer of gold (Kang, Jeong, & Park, 2012; Kang, Jeong, Sunwoo, et al., 2012).

3. Results and discussion

Two kinds of inorganic salt, mono-valent salt (NH_4Cl), and divalent salt ($MgCl_2$), were employed in the pretreatment of the miscanthus straw. The effects of pretreatment conditions (i.e. pretreatment temperature, pretreatment time, concentration of inorganic salts) on the pretreatment and xylan hydrolysis of the straw were investigated.

3.1. Effect of pretreatment temperature

Miscanthus straw was preliminarily treated with 0.5% (w/w) NH_4Cl and $MgCl_2$ solutions at 150–200 °C for 15 min. The straw was then tested to determine the effects of pretreatment temperature. Table 1 lists the results. For the pretreatment with NH_4Cl, the remaining solid ranged from 64.2% to 92.6%, and the enzymatic digestibility ranged from 6.7% to 45.2%. Under the 150 °C condition, 92.6% of remaining solid and 97.9% of glucan were recovered, whereas the enzymatic digestibility was a low 6.7%. Under the 200 °C condition, 64.2% remaining solid, 90.8% of glucan and 45.2% of enzymatic digestibility were the values obtained. As shown in