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

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A B S T R A C T

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.

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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).

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

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

Table 1
Effect of pretreatment temperature on pretreatment of miscanthus straw by inorganic salts.

Pretreatment condition		Composition of pretreated biomass				Glucan recovery (%)	Enzymatic digestibility (%)
Inorganic salt	Temp. (°C)	Solid remaining (%)	Glucan (%)	Xylan (%)	Lignin (%)		
NH_4Cl	150	92.6	40.3	25.6	23.8	90.6	6.7
	175	86.0	39.4	18.0	22.2	88.5	11.3
	185	72.9	40.6	11.8	21.4	91.1	15.4
	200	64.2	40.5	4.0	22.0	90.8	45.2
$MgCl_2$	150	92.5	42.2	25.3	24.4	94.8	6.9
	175	85.9	39.9	19.7	23.2	89.5	10.2
	185	73.3	40.9	12.5	21.9	91.8	16.9
	200	64.7	39.4	2.2	21.9	88.4	44.6

Note: The biomass were pretreated with 0.5% inorganic salts solution for 15 min and preheated for 10 min.

Table 1, by increasing the pretreatment temperature, the remaining solid was decreased, but the enzymatic digestibility was increased.

In the $MgCl_2$ pretreatment, 64.7–92.5% remaining solid and 6.9–44.6% enzymatic digestibility were observed. Under the 200 °C condition, $MgCl_2$ presented 39.8% enzymatic digestibility. The other patterns of remaining solid and enzymatic digestibility were similar to those observed for $MgCl_2$ pretreatment. With rising pretreatment temperature, there was decreasing remaining solid and enhanced enzymatic digestibility.

Comparably, Liu et al. (2009) reported that enzymatic hydrolysis of corn stover pretreated with $FeCl_3$ at 160 °C for 20 min resulted in an optimum yield of 98.0%. However, the sugar recovery was lower (only 49.8%) at 180 °C than at lower temperatures (140 and 160 °C). Also, pretreatment with $FeSO_4$ was significantly correlated with high xylose recovery and low cellulose removal between 140 and 200 °C. Under the 0.1 M $FeSO_4$ at 180 °C for 20 min condition, the cellulose conversion was 36.4% higher than that for pretreatment with hot water (Zhao et al., 2011). These results indicated that at high pretreatment temperature, the dissolution of lignocellulose rapidly occurred, and also resulted in sugar degradation (Liu et al., 2009).

As shown in Table 1, the increasing of temperature in the inorganic salt pretreatments removed xylan from the biomass. When the biomass was treated at 200 °C, the xylan content remaining was only 4% and 2.2% for NH_4Cl and $MgCl_2$, respectively. Also, it was found that the pretreated biomass contained low xylan and manifested a high enzymatic digestibility in each inorganic salt pretreatment.

Fig. 1 shows the effect of pretreatment temperature on xylan removal from miscanthus straw. The patterns of xylan removal for NH_4Cl and $MgCl_2$ were similar to the pretreatment temperature ones. With rising pretreatment temperature, the xylan removal was linearly increased. Fully 90.2 and 94.6% of xylan was removed by 0.5% NH_4Cl and $MgCl_2$ solutions at 200 °C, respectively. Under the 185 °C condition, 67.2 and 65.0% of xylan removal was observed for 0.5% NH_4Cl and $MgCl_2$ solutions, respectively. These results reflected the fact that pretreatment by 0.5% NH_4Cl and $MgCl_2$ solutions removed the xylan and enhanced the enzymatic digestibility. Liu et al. (2009) found that high temperature could remove 100% hemicellulose in pretreatment of corn stover with the inorganic salts $FeCl_3$, $FeCl_2$, $FeSO_4$, $Fe_2(SO_4)_3$, and $CaCl_2$. However, high pretreatment temperature accelerated not only cellulose solubilization, but also glucose degradation (Liu et al., 2009).

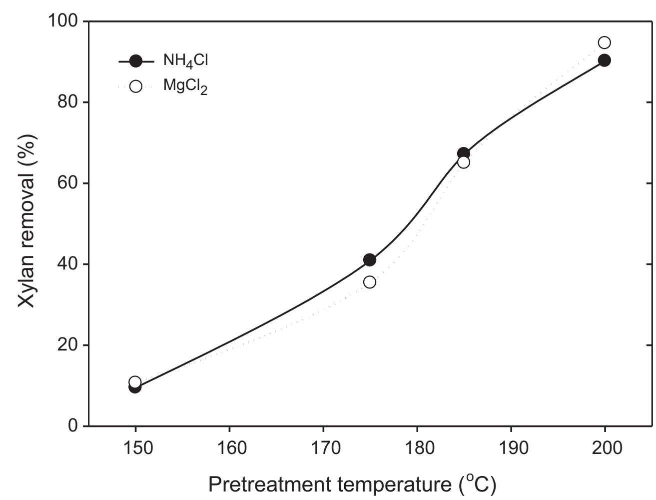

Fig. 1. Effect of pretreatment temperature on xylan removal from miscanthus straw. The biomass were pretreated with 0.5% inorganic salts solution for 15 min.

3.2. *Effect of inorganic salt concentration*

To investigate the effects of NH_4Cl and $MgCl_2$ concentration on inorganic salt pretreatment, miscanthus straw was treated with 0.2–5.0% (w/w) NH_4Cl and $MgCl_2$ solution under the 185 °C for 15 min condition. Table 2 lists the results. When 0.2–5.0% NH_4Cl solutions were employed, the remaining solid was 66.9–72.9%, the glucan recovery 91.1–93.7%, and the enzymatic digestibility,

Table 2
Effect of inorganic salt concentration on pretreatment of miscanthus straw by inorganic salts.

Pretreatment condition		Composition of pretreated biomass				Glucan recovery (%)	Enzymatic digestibility (%)
Inorganic salt	Conc. (%)	Solid remaining (%)	Glucan (%)	Xylan (%)	Lignin (%)		
NH_4Cl	0.5	72.9	40.6	11.8	21.4	91.1	15.4
	1.0	69.5	41.8	5.1	23.5	93.9	28.0
	2.0	69.2	42.1	0.0	18.8	94.4	39.7
	5.0	66.9	41.7	0.0	18.8	93.7	42.4
$MgCl_2$	0.5	73.3	40.9	12.5	21.9	91.8	16.9
	1.0	69.2	40.0	5.0	21.1	89.8	27.9
	2.0	70.9	42.3	0.0	19.2	95.0	29.9
	5.0	69.0	42.4	0.0	20.2	95.2	40.6

Note: The biomass were pretreated at 185 °C for 15 min and preheated for 10 min.

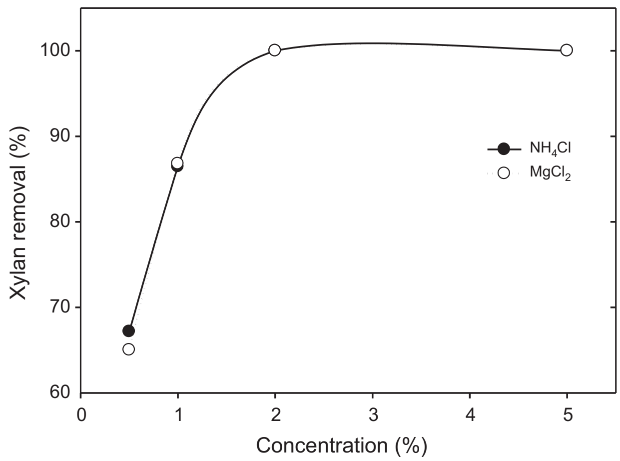

Fig. 2. Effect of pretreatment time on xylan removal from miscanthus straw. The biomass were pretreated with 2% inorganic salts solution at 185 °C for 15 min.

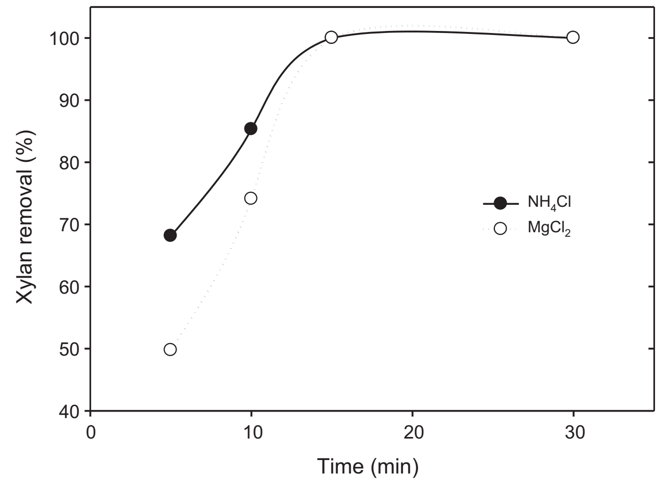

Fig. 3. Effects of NH_4Cl and $MgCl_2$ concentrations on xylan removal from miscanthus straw. The biomass were pretreated at 185 °C for 15 min.

15.4–42.4%. Under the 0.5% NH_4Cl condition, there was 72.9% remaining solid and a low (15.4%) enzymatic digestibility. Under the 2% NH_4Cl condition, 69.2% remaining solid and 39.7% enzymatic digestibility were obtained. By increasing the NH_4Cl concentration in the pretreatment, the remaining solid was slightly diminished, though the enzymatic digestibility was enhanced.

In $MgCl_2$ pretreatment of miscanthus straw, the results were similar to those of NH_4Cl pretreatment: the increase of $MgCl_2$ concentration afforded a slight decrease of remaining solid, with enhanced enzymatic digestibility. Under pretreatment with 0.2–5.0% $MgCl_2$ solutions, the remaining solid was 69.0–73.3%, the glucan recovery 89.8–95.2%, and the enzymatic digestibility, 16.9–40.6%. Under the condition of 0.5% $MgCl_2$, 73.3% remaining solid and 16.9% enzymatic digestibility were obtained. Under 2% $MgCl_2$, 70.9% remaining solid and 29.9% enzymatic digestibility were obtained.

As shown in Table 2, increasing the concentration of NH_4Cl and $MgCl_2$ in the pretreatment of miscanthus straw linearly decreased the xylan content in the biomass. After pretreatment with 0.5% NH_4Cl or $MgCl_2$ solution, the xylan contents of the solid residues were 11.8 and 12.5%, respectively. Under the higher-than-2%-concentration conditions, no xylan remained in the solid residues after pretreatment with NH_4Cl or $MgCl_2$ solution.

Fig. 2 shows the effects of NH_4Cl and $MgCl_2$ concentrations on xylan removal from the miscanthus straw under the 185 °C for 15 min condition. Similar patterns were shown. By increasing the inorganic salt concentration to 2%, the xylan removal was linearly increased; at over-2% inorganic salt concentrations, the xylan was completely removed from the solid residues. These results indicated that pretreatment by 2% NH_4Cl and $MgCl_2$ solutions at 185 °C removed the xylan from the biomass. For further work, the concentration of NH_4Cl and $MgCl_2$ will be fixed at 2.0%. Similarly, Marcotullio et al. (2011) reported almost complete removal of hemicellulose under the 200 mM $FeCl_3$, 120 °C wheat straw pretreatment condition. For corncob pretreatment, they reported that the hemicellulose removal attained 100% efficiency under the 0.1% $FeCl_2$ solution condition (Chen et al., 2010).

3.3. Effect of pretreatment time

Table 3 shows the effects of pretreatment time on the 2% NH_4Cl and $MgCl_2$ pretreatments of miscanthus straw at 185 °C. When NH_4Cl solutions were employed, 69.5–80.3% remaining solid, 93.9–101.1% glucan recovery and 11.6–49.1% enzymatic digestibility were obtained. As time passed, the remaining solid slightly decreased, though the enzymatic digestibility was increased. Increasing pretreatment time under the 2% NH_4Cl and 185 °C condition increased the xylan removal from the solid residues. After 5 and 10 min of pretreatment, the xylan contents in the solid residues were 10.4 and 5.1%, respectively. After 15 min, there was no xylan remaining in the solid residues.

In the $MgCl_2$ pretreatment of miscanthus straw, the increase of pretreatment time showed a slight decrease of remaining solid; however, the enzymatic digestibility was enhanced. As time passed under the 2% NH_4Cl at 185 °C condition, the rate of xylan removal from the solid residues increased. After 5 and 10 min of pretreatment, there remained about 16.9 and 9.0% xylan in the solid residues, respectively. Also, similarly to NH_4Cl pretreatment, there was no xylan remaining in the solid residues after 15 min.

Fig. 3 shows the effect of pretreatment time on xylan removal from the miscanthus straw. The patterns of xylan removal for

Table 3
Effect of pretreatment time on pretreatment of miscanthus straw by inorganic salts.

Pretreatment condition		Composition of pretreated biomass				Glucan recovery (%)	Enzymatic digestibility (%)
Inorganic salt	Time (min)	Solid remaining (%)	Glucan (%)	Xylan (%)	Lignin (%)		
NH_4Cl	5	80.3	45.0	10.4	23.0	101.1	11.6
	10	75.5	41.6	5.1	21.2	93.3	25.6
	15	69.2	42.1	0.0	18.8	94.4	39.7
	30	69.5	41.8	0.0	20.1	93.9	49.1
$MgCl_2$	5	77.9	46.3	16.9	20.8	104.0	21.2
	10	75.4	42.5	9.0	21.5	95.3	33.9
	15	69.0	42.4	0.0	20.2	95.2	40.6
	30	69.2	40.0	0.0	21.9	89.8	51.2

Note: The biomass were pretreated with 2% inorganic salts solution at 185 °C for 15 min and preheated for 10 min.

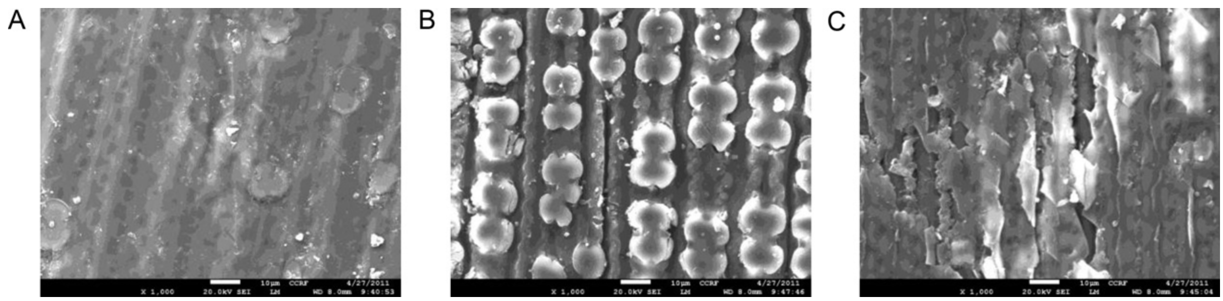

Fig. 4. Scanning electron microscopy (SEM) images of raw and pretreated miscanthus straw. (A) Raw miscanthus straw, (B) NH_4Cl-treated miscanthus straw, and (C) $MgCl_2$-treated miscanthus straw.

NH_4Cl and $MgCl_2$ were similar to that of the pretreatment time. As the pretreatment time increased, the xylan removal was linearly increased to 15 min; after 15 min, the xylan was completely hydrolyzed. Overall, these results indicated that pretreatment by aqueous 2% NH_4Cl and $MgCl_2$ solutions at 185 °C for 15 min completely hydrolyzed the xylan of the miscanthus straw. NH_4Cl and $MgCl_2$ had similar effects on xylan removal and enzymatic digestibility.

Similarly, it was reported that for pretreatment of corn stover with 0.8% $FeCl_3$ at 180 °C, almost 90% of the xylose was removed after 20 min (Liu & Wyman, 2006). Elsewhere, the decomposition of xylose and xylotriose increased in pretreatment of corn stover using different salt solutions (Liu et al., 2009). Nevertheless, the mechanisms of xylan removal remain unclear; further work is needed to reveal the pathway for enhancement of xylose and xylotriose degradation by inorganic salts (Liu & Wyman, 2006).

3.4. Physical surface change of miscanthus straw by NH_4Cl and $MgCl_2$ pretreatments

Physical surface changes of miscanthus straw after pretreatment with the inorganic salts NH_4Cl and $MgCl_2$ were compared by SEM (Fig. 4). In Fig. 4a, the raw miscanthus straw shows a compact structure. SEM photos (Fig. 4b and c) of the pretreated miscanthus straw, by contrast, present an apparently damaged surface area and exposure of the internal structure. This might make for easy enzyme access to cellulose in enzymatic hydrolysis (Kang, Jeong, & Park, 2012; Kang, Jeong, Sunwoo, et al., 2012; Mosier et al., 2005). However, the surface morphologies of biomasses pretreated by NH_4Cl and $MgCl_2$ differ. An additional investigation undertaken to determine the cause of the differential morphology was inconclusive. It is suggested that subsequent studies be conducted to solve this mystery.

4. Conclusions

In this study, two kinds of inorganic salt, mono-valent salt (NH_4Cl) and divalent salt ($MgCl_2$), were introduced to the pretreatment of miscanthus straw. In 0.5% $MgCl_2$ or NH_4Cl pretreatment, increasing the pretreatment temperature (150–200 °C), decreased the remaining solid, but with an increase of enzymatic digestibility. Also, the removal of xylan from the biomass was linearly increased. Fully 90.2% and 94.6% of xylan in the miscanthus straw was removed by 0.5% NH_4Cl or $MgCl_2$ solution at 200 °C, respectively. When 0.2–5.0% NH_4Cl or $MgCl_2$ solution was used, increasing the inorganic salt concentration resulted in remaining solid, enzymatic digestibility and xylan removal patterns similar to that of pretreatment temperature. Under the higher-than-2%-concentration condition, no xylan remained in the solid residues after pretreatment with NH_4Cl or $MgCl_2$ solution. In the xylan removal according to pretreatment time, NH_4Cl and $MgCl_2$ showed similar patterns. As the pretreatment time increased, the xylan removal was linearly increased to 15 min; after 15 min, the xylan was completely hydrolyzed. Under the 2% NH_4Cl at 200 °C for 15 min condition, 100% of xylan was removed, 94.4% of glucan was recovered, and the enzymatic digestibility was 39.7%. Under the same $MgCl_2$ condition, 100% xylan removal, 95.0% glucan recovery, and 29.9% enzymatic digestibility were shown, considerably lower values than for NH_4Cl. In the overall results, inorganic salts reduced the hemicellulose content in miscanthus straw. NH_4Cl and $MgCl_2$ showed strong, almost 100% xylan-removal effects under the same conditions.

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