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Pretreatment of *Miscanthus giganteus* with Lime and Oxidants for Biofuels

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ABSTRACT: To make biomass more accessible for enzymatic hydrolysis, lime pretreatment of *Miscanthus giganteus* with oxidants was explored from 100 to 150 °C. Composition data for the recovered solid were obtained to determine the effects of the reaction time, lime dosage, oxidant loading, and temperature on sugar production efficiency. Under selected conditions (0.2 g of lime/g of biomass, 200 psig O₂, and 150 °C for 1 h), delignification was 64.7%. The pretreated biomass was then followed by enzymatic hydrolysis. The yield of cellulose in the recovered solid to glucose was 91.7% and hemicellulose to xylose was 67.3%, 7.1 and 18.2 times larger than those obtained from raw biomass, respectively. Pretreatment with oxidants substantially raised delignification of raw *M. giganteus*, thereby enhancing enzymatic hydrolysis to sugars, while results were not improved when pretreatment included ammonium molybdate.

1. INTRODUCTION

Because of the rising consumption of non-renewable resources and anthropogenic production of greenhouse gases, lignocellulosic biomass for making biofuel has attracted increasing attention. Development of green processing for lignocellulosic biomass is essential for sustainability and environmental protection. *Miscanthus giganteus* is a promising energy crop because of minimal requirements for obtaining a high yield per acre.¹ Table 1 gives the composition of dried raw *M. giganteus*; it is primarily composed of cellulose, hemicellulose, and lignin. In a typical biomass-to-biofuel process, carbohydrates are hydrolyzed to sugars using chemical or biochemical methods; the sugars are then fermented to bioalcohols.² Owing to its high lignin content, large cellulose crystallinity, low surface area/pore volume, and hemicellulose acetylation, the enzymatic hydrolysis of raw biomass is low. Therefore, pretreatment is required to break down the biomass matrix to release the polysaccharides.³

Several pretreatment methods have been developed for enhancing the efficiency of enzymatic hydrolysis. Alkaline pretreatment has several potential advantages compared to other pretreatment processes: low operation cost, reduced degradation of polysaccharides, and compatibility with oxidants.^{4,5} Because the reactor required for alkali is not subject to corrosion as is the reactor for acid pretreatment, it will be cheaper. There are few degradation products from sugar during the alkali process, e.g., furfural and 5-(hydroxymethyl)-furfural, that inhibit the subsequent fermentation. Alkali pretreatment removes acetate groups from hemicellulose; hence, steric hindrance for hydrolytic enzymes is reduced, enhancing carbohydrate digestibility. The common widely used alkalis are sodium hydroxide, ammonia, and calcium hydroxide.

Sodium hydroxide effectively raises lignocellulose digestibility, but it is expensive, requires safety precautions, and is difficult to recover.⁶ Ammonia pretreatment has also received much attention because ammonia is easy to recover, but it is moderately expensive and requires careful handling to avoid safety problems.^{7,8} In comparison to sodium hydroxide and ammonia, calcium hydroxide (lime) is cheaper and safer and can be recovered by reacting with CO₂ to produce water-insoluble CaCO₃. Lime is a weak alkali, poorly soluble in water; at 100 °C, the solubility is 0.071 g/100 g of saturated solution.⁹ To make a lime process as efficient as possible, optimum pretreatment conditions need to be identified.

Lime without oxidants is effective for delignification from low-lignin biomass, but oxidants are required for lignin removal from biomass with a high lignin content.³ For switchgrass, crop residue bagasse, wheat straw, corn stover, and polar wood, several studies have been reported for lime pretreatment processes with and without oxygen;^{10–17} however, little attention has been given to using lime with hydrogen peroxide. Hydrogen peroxide is a bleaching reagent in the pulp and paper industry; its oxidation power is likely to help delignification. Moreover, as shown by Verma et al., a pretreatment process at 140 °C can be improved when using ammonium molybdate activated by hydrogen peroxide.¹⁸

In this work, we investigate lime pretreatment of *M. giganteus* with and without oxygen or hydrogen peroxide and with and without ammonium molybdate at temperatures ranging from 100 to 150 °C.

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Table 1. Composition of Dried Raw *M. giganteus*

biomass	composition (wt %)				
	cellulose	hemicellulose	lignin	ash	extractable
<i>M. giganteus</i>	43.1 ± 0.2	23.6 ± 0.2	26.3 ± 0.5	3.0 ± 0.5	4.0 ± 0.3

Table 2. Lime Pretreatment of *M. giganteus* with and without Oxidants at 150 °C for 3 h

pretreatment condition ^a			pretreatment results (%) ^b			post-pretreatment
lime loading (g/g of biomass)	O ₂ (psig)	H ₂ O ₂ (wt %)	cellulose recovered	hemicellulose recovered	lignin removal	lime consumption (g/g of biomass)
0	0	0	90.9 ± 1.1	66.5 ± 1.3	23.1 ± 0.8	0
0.13	0	0	90.4 ± 0.9	60.8 ± 1.1	35.2 ± 0.7	0.056
0.20	0	0	89.5 ± 1.3	60.2 ± 0.3	35.4 ± 1.6	0.072
0.13	200	0	88.5 ± 1.2	50.8 ± 0.5	42.5 ± 1.3	0.125
0.20	200	0	89.4 ± 0.8	50.4 ± 1.1	66.9 ± 1.2	0.200
0.20	0	2.0	87.9 ± 1.5	51.9 ± 0.9	53.2 ± 0.8	0.200

^aThe solid/liquid ratio is 1:8. ^bRecovered and removal results are calculated on the basis of the mass in the raw biomass.

2. EXPERIMENTAL SECTION

2.1. Materials. *M. giganteus* was provided by the Energy Biosciences Institute, University of Illinois at Urbana–Champaign, Champaign, IL. A Retsch grinder and a 4 mm sieve produced 4 mm particles. Hydrogen peroxide solution (30 wt %) and citric acid were purchased from Sigma-Aldrich (St. Louis, MO). Ammonium molybdate was purchased from Acros (Morris Plains, NJ). Calcium hydroxide powder was purchased from Mallinckrode Baker, Inc. (Phillipsburg, NJ). Sulfuric acid, hydrochloric acid, sodium citrate, and sodium hydroxide were purchased from Fisher Scientific (Fair Lawn, NJ). Enzymes Cellic CTec 2 and Cellic HTec 2 were purchased from Novozymes. Sodium azide aqueous solution (0.5%, w/v) was purchased from Ricca Chemical Company (Arlington, TX). All reagents were used without further purification. Nanopure water (18.2 MΩ) was used to prepare the solutions and for washing the recovered solid.

2.2. Procedures. Hydrogen peroxide or ammonium molybdate was weighed using analytical balances (Mettler Toledo, model AB204-S and XS6002S) and added to a bottle with Nanopure water to obtain the desirable concentration. The chemicals were dissolved in the bottle with stirring using a magnetic hot plate stirrer (IKA RCT Basic IKAMAG). Dried *M. giganteus*, lime, and prepared solution were weighed to fix a solid/liquid weight ratio of 1:8. The solid/liquid mixture was placed in a stainless-steel pressure reactor with stirring. If oxygen is used in pretreatment, the reactor was initially purged with oxygen. The reactor was submerged in a silicon–oil bath at a preset temperature. After a fixed reaction time, the reactor was taken from the oil bath and cooled to 70 °C using an ice–water bath. After cooling, the pulp was neutralized with hydrochloric acid until the pH was between 6 and 7 and then filtered to separate solid from liquid. The recovered solid was washed several times with Nanopure water. A small solid sample was dried overnight in a 105 °C oven prior to determine its composition. The residual (not dried) recovered solid was subjected to enzymatic hydrolysis. The composition of the liquid phase was not analyzed, because in the liquid, the concentrations of cellulose, hemicellulose, and sugars were much too low for use in an industrial process.

2.3. Composition Analysis of the Recovered Solid. The composition of the recovered solid *M. giganteus* was determined by the analytical procedure proposed by the National Renewable Energy Laboratory (NREL).¹⁹ Details are given in a previous publication.⁸ Each sample was analyzed in triplicate.

2.4. Enzymatic Hydrolysis of Pretreated Materials. For hydrolysis, the pretreated *M. giganteus* was not dried to prevent irreversible pore collapse. Enzymatic hydrolysis was carried out following the NREL protocol.⁹ The recovered solid was mixed with a solution containing citrate buffer and enzymes at 20 filter paper units (FPU)/g of cellulose and 20 cellobiase units (CBU)/g of hemicellulose. The 0.1 M citrate buffer was made by mixing citric acid and

sodium citrate to maintain the hydrolysis solution at pH 4.8. To prevent microbial growth, 2 mL of 0.5% (w/v) sodium azide/g of cellulose was added. Aliquots of supernatant liquid were withdrawn to measure the concentration of glucose and xylose using Shimadzu high-performance liquid chromatography (HPLC) at 50 °C with an Aminex HPX 87H column (300 × 7.8 mm) and a refractive index detector. The flow rate of 0.01 N sulfuric acid eluent was 0.6 mL/min.

3. RESULTS AND DISCUSSION

3.1. Pretreatment of *M. giganteus*. **3.1.1. Effect of Lime Loading.** The primary aim of pretreatment is to decompose and remove lignin to open the crystalline structure in cellulose, making the biomass more accessible to enzymes for hydrolysis while minimizing the loss of polysaccharides. With the goal of developing a cost-effective pretreatment process for bioalcohol production, it is necessary to study the effect of lime dosage to optimize lime loading for enhancing hydrolysis to sugar. Therefore, lime loading was investigated first, while the temperature was constant at 150 °C and the reaction time was 3 h according to previous studies.^{8,11,12}

Table 2 shows compositions of pretreated *M. giganteus* at 150 °C for 3 h. When no lime or oxidants were loaded into the reactor, 23.1% of the original lignin was removed. However, 35.2% was removed when the reactor contained 0.13 g of lime/g of biomass. When the lime loading increased to 0.20 g/g of biomass, delignification was nearly the same (35.4%), indicating that, in the absence of oxidants, raising lime loading beyond 0.13 g/g of biomass is not helpful. However, when O₂ or H₂O₂ was added to the lime solution, results improved remarkably. Delignification was raised from 35.2 to 42.5% when the lime loading was 0.13 g/g of biomass augmented by 200 psig O₂. Delignification rose from 35.4 to 66.9% when the lime loading was 0.20 g/g of biomass augmented by 200 psig O₂ and to 53.2% when the lime loading was augmented by 2 wt % H₂O₂. Regrettably, with an oxidant, 10% more hemicellulose was dissolved and lost. However, with an oxidant, the recovery of cellulose was good, near 90%.

Previous studies have shown that an increase in lime loading has a limited effect on lignin removal when lime loading is above 10 wt %/weight of the dry weight of the biomass.⁵ In our work, when no oxidants are used, increasing lime loading has no effect; however, with an oxidant, increased lime loading raises delignification. When lime loading increased from 0.13 to 0.2 g/g of biomass, delignification increased from 42.5 to 66.9%.

Table 3. Lime Pretreatment of *M. giganteus* Using 0.2 g of Lime/g of Biomass with Oxidants at 100, 120, and 140 °C for 3 h

pretreatment condition ^a			pretreatment results (%) ^b			post-pretreatment
T (°C)	O ₂ (psig)	H ₂ O ₂ (wt %)	cellulose recovered	hemicellulose recovered	lignin removal	lime consumption (g/g of biomass)
100	200	0	92.8 ± 0.5	59.7 ± 0.5	50.3 ± 1.1	0.108
100	0	2.0	94.7 ± 0.9	71.0 ± 0.1	40.9 ± 0.3	0.095
120	200	0	92.5 ± 0.6	58.9 ± 0.7	55.4 ± 0.4	0.154
120	0	2.0	94.1 ± 0.8	66.0 ± 0.6	50.1 ± 0.9	0.163
140	200	0	92.6 ± 1.1	57.1 ± 0.5	70.7 ± 1.3	0.200
140	0	2.0	92.4 ± 0.7	63.4 ± 0.9	52.4 ± 0.5	0.200

^aThe solid/liquid ratio is 1:8. ^bRecovered and removal results are calculated on the basis of the mass in the raw biomass.

Table 4. Lime Pretreatment of *M. giganteus* Using 0.2 g of Lime/g of Biomass with Oxidants at 100, 120, 140, and 150 °C for 1 h

pretreatment condition ^a			pretreatment results (%) ^b			post-pretreatment
T (°C)	O ₂ (psig)	H ₂ O ₂ (wt %)	cellulose recovered	hemicellulose recovered	lignin removal	lime consumption (g/g of biomass)
100	200	0	95.0 ± 0.9	66.1 ± 1.3	45.6 ± 1.3	0.124
100	0	2.0	93.7 ± 0.7	74.4 ± 1.2	32.2 ± 0.6	0.084
120	200	0	93.7 ± 1.1	61.7 ± 1.5	51.4 ± 0.7	0.126
120	0	2.0	94.4 ± 0.5	68.0 ± 0.9	42.5 ± 0.5	0.118
140	200	0	93.8 ± 0.8	59.0 ± 0.8	59.8 ± 1.2	0.125
140	0	2.0	91.9 ± 0.9	66.2 ± 1.0	45.4 ± 1.3	0.145
150	200	0	93.6 ± 1.3	56.7 ± 1.2	64.7 ± 1.5	0.200
150	0	2.0	91.2 ± 1.8	62.9 ± 1.5	46.9 ± 1.1	0.182

^aThe solid/liquid ratio is 1:8. ^bRecovered and removal results are calculated on the basis of the mass in the raw biomass.

Table 5. Lime Pretreatment of *M. giganteus* Using 2.0 wt % H₂O₂ and Ammonium Molybdate with and without Lime at 140 °C

pretreatment condition ^a				pretreatment results (%) ^b			post-pretreatment
lime loading (g/g of biomass)	H ₂ O ₂ (wt %)	ammonium molybdate (wt %)	time (h)	cellulose recovered	hemicellulose recovered	lignin removal	lime consumption (g/g of biomass)
0	2.0	0.02	0.5	87.7 ± 0.3	49.2 ± 1.1	46.7 ± 0.3	0
0	2.0	0.10	0.5	88.0 ± 0.8	41.5 ± 1.2	45.7 ± 0.7	0
0	2.0	0	1.0	87.4 ± 0.9	25.0 ± 0.8	45.7 ± 0.8	0
0	2.0	0.02	1.0	86.8 ± 1.3	23.2 ± 0.9	48.6 ± 1.1	0
0	2.0	0.10	1.0	85.9 ± 1.1	24.2 ± 1.0	46.2 ± 0.5	0
0.20	2.0	0.10	3.0	92.7 ± 1.5	64.1 ± 0.5	52.8 ± 0.7	0.200

^aThe solid/liquid ratio is 1:8. ^bRecovered and removal results are calculated on the basis of the mass in the raw biomass.

Because lime is only slightly soluble in water, increasing lime loading produces a suspension. The suspended lime particles have a very high surface area; therefore, lime particles dissolve quickly to replace the dissolved lime that was consumed during the pretreatment reaction that forms calcium–lignin linkages.²⁰ With the help of oxidants, hydroxide ions react with chromophoric and reactive groups in the lignin to break aryl ether bonds and other linkages between lignin and carbohydrates.⁸

Therefore, 0.2 g of lime/g of biomass with oxidants was selected for further investigation.

3.1.2. Pretreatment Conditions. Table 3 shows pretreatment results at several conditions. Because previous studies showed that solution loading had little effect on pretreatment, the solid/liquid ratio of 1:8 was retained.^{8,10} At mild conditions, increasing the temperature does not significantly improve pretreatment, unlike at severe conditions.^{8,10,11} Therefore, at a low temperature, we use a 20 °C gradient, and at a high temperature, we use a 10 °C gradient. The reaction was at 100, 120, 140, and 150 °C. A solution containing 0.2 g of lime/g of biomass was used to pretreat *M. giganteus* with oxidants for 3 h. Toward better understanding the effect of the reaction time, we also conducted the reaction for 1 h at 100–150 °C, with results shown in Table 4.

At 150 °C, with 200 psig O₂, delignification for 3 h was 66.9%, while for 1 h, it was nearly the same, 64.7%. At 150 °C, with 2 wt % H₂O₂, for 3 h, it was 53.2% and, for 1 h, it was 46.9%. At 140 °C, with 200 psig O₂, delignification for 3 h was 70.7%, while for 1 h, it was 59.8%. At 140 °C, with 2 wt % H₂O₂, for 3 h, it was 52.4% and, for 1 h, it was 45.4%. At these conditions, 3 h of reaction time showed little benefit compared to 1 h.

With oxidants, delignification increased with raising the reaction temperature. However, using O₂ for 3 h, raising the temperature from 140 to 150 °C, did not produce higher delignification. It has been suggested that, because the low solubility of lime decreases with the rising temperature, at a high temperature, the alkali is converted to irrecoverable salts or is incorporated as salt into the biomass.⁴ More hemicellulose was removed when the temperature is 150 °C. The highest delignification was achieved at 140 °C using 0.2 g of lime/g of biomass with 200 psig O₂ for 3 h. Because of its poor solubility in water, lime is a weak base. However, pretreatment with lime is more successful when augmented by oxidants.

Several studies have shown that molybdate ions could be activated by hydrogen peroxide to improve delignification.^{18,21,22} In this work, we pretreated the biomass with hydrogen peroxide and ammonium molybdate; results are

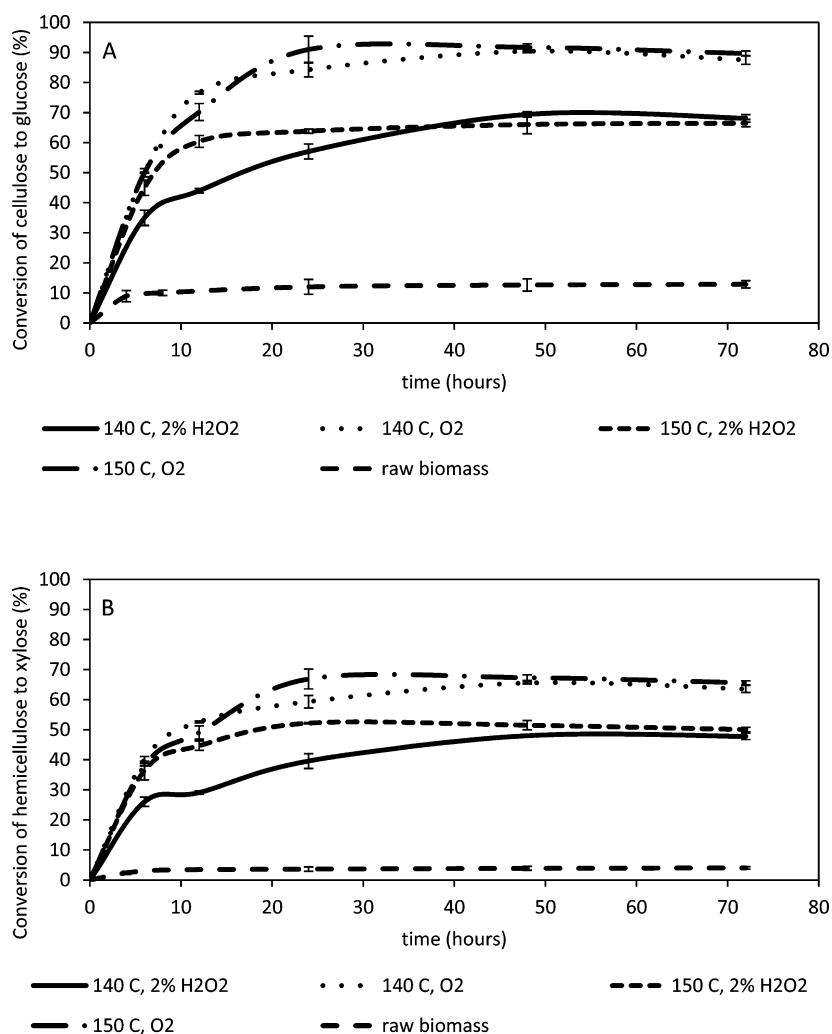


Figure 1. Enzymatic hydrolysis of *M. giganteus* pretreated with 0.2 g of lime/g of biomass and 200 psig O_2 or 2 wt % H_2O_2 at 140 or 150 °C for 1 h: (A) hydrolysis of cellulose to glucose and (B) hydrolysis of hemicellulose to xylose. The solid/liquid ratio is 1:8.

shown in Table 5. At 140 °C for 1 h using 0.02% molybdate without lime, delignification was 48.6%; it was 45.7% without lime or molybdate; and 76.8% hemicellulose was removed using 0.02% molybdate without lime. However, when lime was present with molybdate and hydrogen peroxide, the removal of lignin was similar to that without lime, whereas more hemicellulose was removed with molybdate. It appears that molybdate with hydrogen peroxide has little additional benefit for delignification, while it has a negative benefit on the recovery of polysaccharides because more hemicellulose was lost.

3.1.3. Lime Consumption. After reaction, the lime-treated biomass slurry was neutralized by 5.0 wt % hydrochloric acid. Knowing the mass of HCl required to bring the solution to pH 7.0 provides the lime consumption shown in Tables 2–5. Lime consumption ranged from 0.056 to 0.200 g/g of biomass. Increasing the temperature may raise consumption of lime.

3.1.4. Interaction between Lignin and Calcium Ions. With a rise in lime loading from 0.13 to 0.20 g/g of biomass, delignification increased from 42.5 to 66.9% at 150 °C for 3 h using 200 psig O_2 . With oxidants, increasing lime loading produces a significant increase in lignin removal. However, more polysaccharide was removed. Because hemicellulose is covalently linked to lignin, when lignin is degraded, it takes

hemicellulose with it.³ Several studies showed that divalent calcium ions have high affinity for lignin.²³ The divalent calcium ions are negatively charged under alkaline conditions because of the ionization of functional groups (i.e., carboxyl, methoxy, and hydroxyl). Calcium ions tend to cross-link lignin molecules to form a calcium–lignin complex. Calcium ions promote formation of covalent bonds. Thus, hydroxide ions react with biomass and continuously consume.

3.2. Enzymatic Hydrolysis of the Pretreated Biomass.

The goal of biomass pretreatment is to increase the enzymatic hydrolysis of lignocellulosic biomass. When the bonds holding the biomass components together are removed by alkaline attack, the biomass structure is loosened; the enzymes can then access the carbohydrates even in the presence of decreased lignin.²³ In this work, hydrolysis was for 72 h, but results show that hydrolysis is nearly complete in much less time. To choose an optimum pretreatment condition for enzymatic hydrolysis (reaction temperature, time, and lime loading), the determining criteria are overall yields of cellulose to glucose and hemicellulose to xylose. Yield is defined as

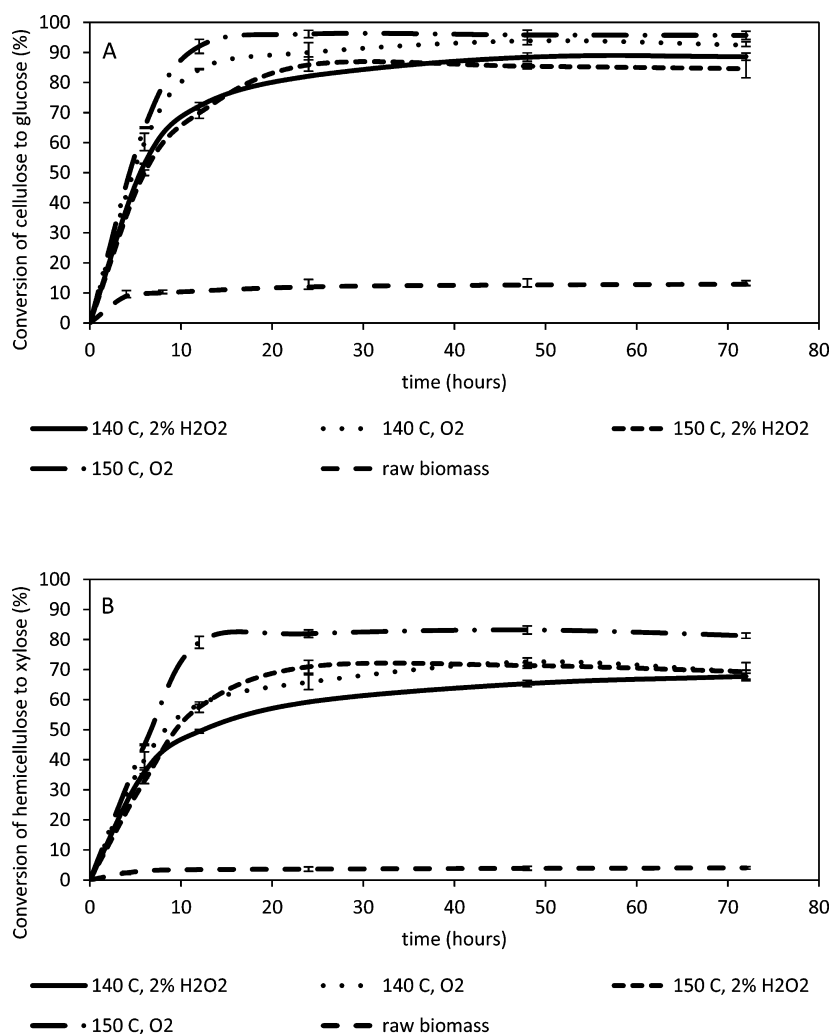


Figure 2. Enzymatic hydrolysis of *M. giganteus* pretreated with 0.2 g of lime/g of biomass and with 200 psig O₂ or 2 wt % H₂O₂ at 140 or 150 °C for 3 h: (A) hydrolysis of cellulose to glucose and (B) hydrolysis of hemicellulose to xylose. The solid/liquid ratio is 1:8.

$$\text{overall yield (\%)} = \left(\frac{\text{equiv glucose (xylose)}}{\text{mass} \times \text{correction factor}} \right) / \text{cellulose (hemicellulose)} \\ \text{in raw biomass} \times 100 \quad (1)$$

Considering the loss of polysaccharides during pretreatment, the enzymatic yield is calculated as

$$\text{enzymatic yield (\%)} = \left(\frac{\text{equiv glucose (xylose)}}{\text{mass} \times \text{correction factor}} \right) / \text{cellulose (hemicellulose)} \\ \text{in pretreated biomass} \times 100 \quad (2)$$

The correction factor for C₆ sugars (glucose, galactose, and mannose) is 0.90 (162/180), and the correction factor for C₅ sugars (xylose and arabinose) is 0.88 (132/150), as suggested by Sluiter et al.¹⁹

Figure 1 shows results from enzymatic hydrolysis of pretreated *M. giganteus* using lime with 200 psig O₂ or 2 wt % H₂O₂ at 140 and 150 °C for 1 h. For the raw biomass, the yields of converting cellulose to glucose and hemicellulose to xylose are 12.9 and 3.7%, respectively. Hydrolysis results for biomass treated for 1 h at 140 and 150 °C with oxidants indicate that using 200 psig O₂ gives yields higher than those using 2 wt % H₂O₂. These results are consistent with those for

delignification. At 150 °C for 1 h, the yield of cellulose to glucose was 91.7% and the yield of hemicellulose to xylose was 67.3% when using O₂, where delignification was 64.7%. It appears that using lime pretreatment with H₂O₂ for 1 h is not able to achieve a good enzymatic yield.

Figure 2 shows results for the enzymatic hydrolysis of pretreated biomass using lime with 200 psig O₂ or 2 wt % H₂O₂ at 140 and 150 °C for 3 h. The conversion was 93.9% for cellulose to glucose and 72.6% for hemicellulose to xylose at 140 °C for 3 h with O₂, where delignification was 70.7%. At 150 °C with O₂, the conversion of cellulose to glucose was 95.8% and the conversion of hemicellulose to xylose was 83.3%, where delignification was 66.9%.

At 140 °C for 3 h with 2 wt % H₂O₂, delignification was 52.4%. The enzymatic yield of cellulose to glucose is 88.6%, and the enzymatic yield of hemicellulose to xylose is 67.7%.

Figure 3 shows results for enzymatic hydrolysis of pretreated biomass with 2 wt % H₂O₂ and 0.02 wt % ammonium molybdate at 140 °C for 0.5 and 1 h without lime. When pretreated for 0.5 h, the hydrolysis yield of cellulose to glucose was 26.9% and the hydrolysis yield of hemicellulose to xylose was 36.5%, while the conversion of cellulose to glucose was 35.7% and the conversion of hemicellulose to xylose was 30.8% when pretreated for 1 h.

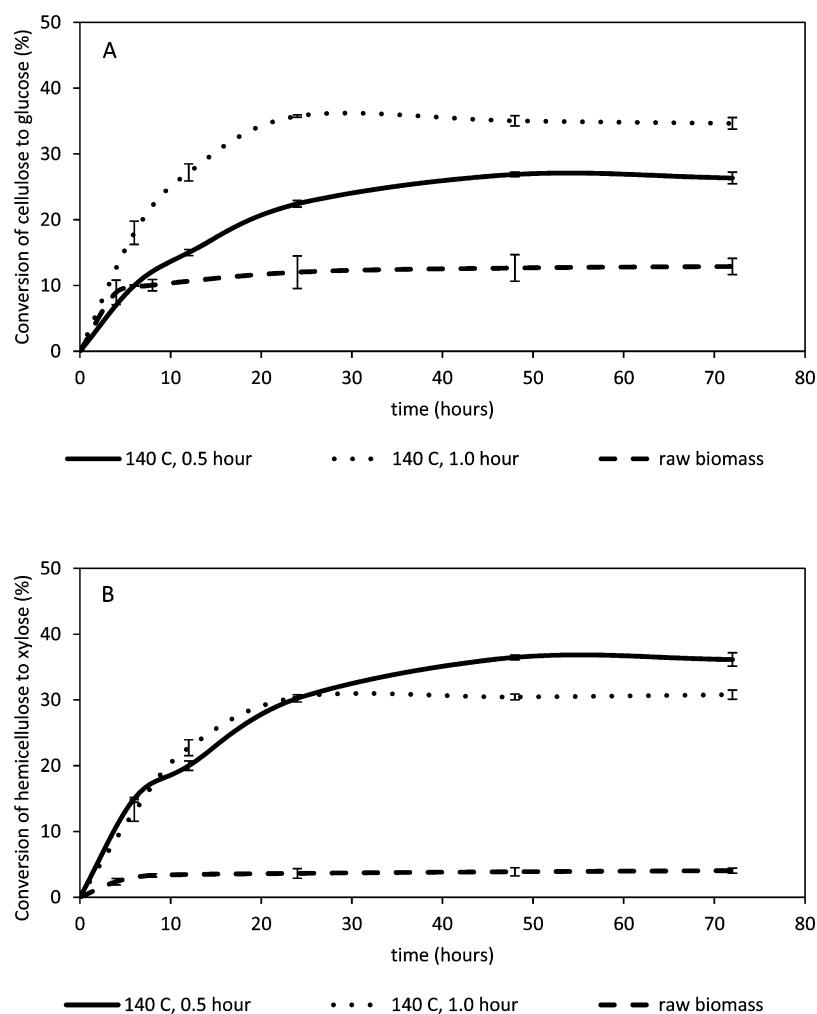


Figure 3. Enzymatic hydrolysis of *M. giganteus* pretreated with 2 wt % H_2O_2 and 0.02 wt % ammonium molybdate at 140 °C for 0.5 and 1 h: (A) hydrolysis of cellulose to glucose and (B) hydrolysis of hemicellulose to xylose.

Table 6. Enzymatic Hydrolysis Yields of Pretreated *M. giganteus* Using 0.2 g of Lime/g of Biomass

T (°C)	pretreatment condition ^a				enzymatic yield (%)		overall yield (%)	
	O ₂ (psig)	H ₂ O ₂ (wt %)	ammonium molybdate (wt %)	time (h)	glucose	xylose	glucose	xylose
0	0	0	0	0	12.9 ± 1.2	3.7 ± 0.4	12.9	3.7
140	0	2	0	1	69.4 ± 1.3	48.0 ± 0.9	63.8	31.8
140	200	0	0	1	90.4 ± 1.5	65.5 ± 1.2	84.8	38.6
150	0	2	0	1	66.5 ± 1.2	52.2 ± 0.8	60.6	32.8
150	200	0	0	1	91.7 ± 0.8	67.3 ± 0.7	85.8	38.2
140	0	2	0	3	88.6 ± 1.2	67.7 ± 1.0	81.9	42.9
140	200	0	0	3	93.9 ± 1.1	72.6 ± 0.5	87.0	41.5
150	0	2	0	3	85.9 ± 2.8	71.4 ± 3.0	75.5	37.1
150	200	0	0	3	95.8 ± 1.4	83.3 ± 0.9	85.6	42.0
140	0	2	0.02	0.5	26.9 ± 0.9	36.5 ± 1.0	23.6	18.0
140	0	2	0.02	1	35.7 ± 0.9	30.8 ± 0.7	31.0	7.2

^aThe solid/liquid ratio is 1:8.

Table 6 shows the enzymatic hydrolysis yields of pretreated biomass as well as the overall yields of cellulose to glucose and hemicellulose to xylose based on the raw biomass. The highest overall yield was achieved at 140 °C for 3 h with O₂. However, to reduce the input energy, 1 h of reaction time was selected and the recommended conditions are 150 °C for 1 h with 200 psig O₂. If the lignin content in the pretreated biomass is 14–16%, a significant increase in the enzymatic hydrolysis yield is

expected. H₂O₂ is a promising addition for lime pretreatment; it is effective for delignification and enhancing conversion of cellulose and hemicellulose to sugars by enzymatic hydrolysis. It is likely that, if more H₂O₂ is added for the pretreatment, better overall enzymatic yields can be obtained. Table 7 indicates that the pretreatment conditions and results obtained in this work are comparable to those in the literature.^{10,13–16,23–25}

Table 7. Lime Pretreatment Conditions and Results

biomass	pretreatment condition	delignification (%)	cellulose enzymatic yield (%)	reference
<i>M. giganteus</i>	0.2 g of lime/g of biomass at 150 °C for 1 h with 200 psig O ₂	64.7	91.7	this work
poplar wood	0.4 g of lime/g of biomass at 140 °C for 2 h with 21.7 bar (absolute) O ₂	60	99.6	Sierra et al. ¹⁶
poplar wood	0.1 g of lime/g of biomass at 150 °C for 6 h with 14 bar (absolute) O ₂	78	77	Chang et al. ²⁴
Alamo switchgrass	1.0 g of lime/g of biomass at 110 °C for 4 h with 6.89 bar (absolute) O ₂	51.8	~93	Falls et al. ¹³
Dacotah switchgrass	0.3 g of lime/g of biomass at 120 °C for 4 h with 6.89 bar (absolute) O ₂	60	~85.2	Falls et al. ¹⁴
sugar cane bagasse	0.4 g of lime/g of dry biomass at 60 °C for 36 h	NA ^a	86.4	Rabelo et al. ²⁵
switchgrass	0.1 g of lime/g of dry biomass at 120 °C for 2 h	29	58.0	Chang et al. ¹⁰
corn stover	0.5 g of lime/g of dry biomass at 55 °C for 4 weeks	87.5	91.3	Kim et al. ¹⁵
switchgrass	0.1 g of lime/g of dry biomass at 50 °C for 24 h	16.7–35.5	67.4	Xu et al. ²³

^aNA = not available.

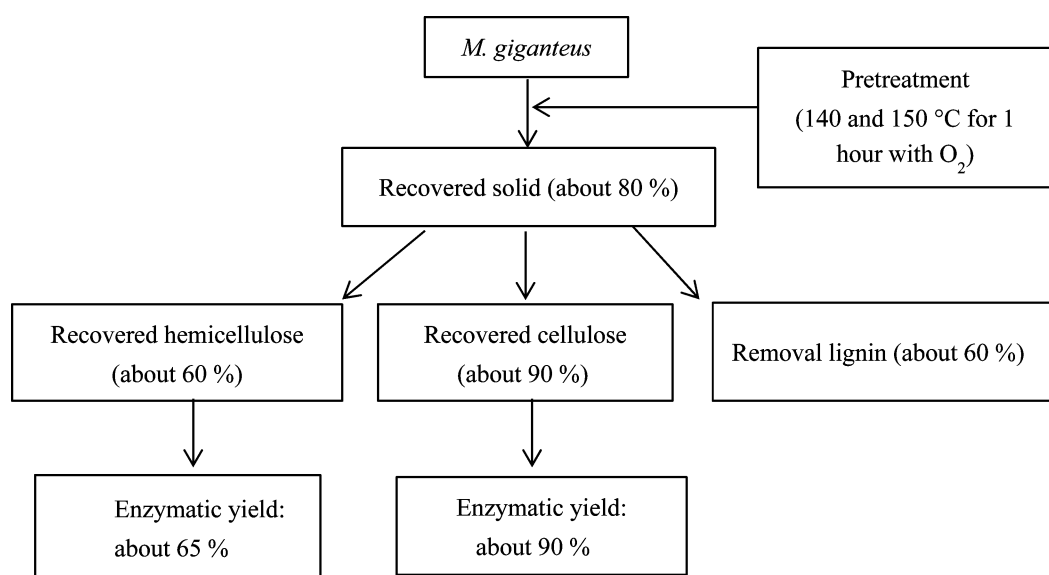


Figure 4. Mass balances (recovered and removal results are calculated on the basis of the mass in the raw biomass).

In summary, *M. giganteus* optimum conditions for pretreatment with lime are 0.2 g of lime/g of dry biomass, 1 h, 150 °C, and 200 psig O₂.

3.3. Mass Balances. Figure 4 presents mass balances for the pretreatment of *M. giganteus*. The balances were obtained for the biomass pretreated under selected conditions (140 and 150 °C for 1 h with O₂). The dried biomass was weighed before and after pretreatment to calculate the total solid recovery. The contents of the pretreated biomass components (cellulose, hemicellulose, lignin, ash, and others) were determined; they are recorded as the recovered and removal compositions based on the mass in raw biomass. At selected pretreatment conditions, total solid recovery was about 80%. Balances for lignin, cellulose, and hemicellulose show that nearly 90% of cellulose remained in the pretreated solid, indicating a good overall preservation of cellulose after lime pretreatment. However, lignin and hemicellulose were not retained as well as cellulose; lime pretreatment produced a relatively low recovery for lignin (about 40%) and hemicellulose (about 60%). The residual fraction of hemicellulose in the biomass solid is correlated with the extent of delignification during lime pretreatment because hemicellulose is covalently linked to lignin.³ The total sugar yield in the hydrolyzates shows that high conversion of carbohydrates after enzymatic hydrolysis can be achieved without entire delignification, consistent with the previous study.¹¹ The lignin content and degree of crystallinity

have a major impact on biomass digestibility, while the acetyl content has a minor impact; however, a low lignin content is sufficient to obtain high digestibility, regardless of crystallinity or acetyl content.¹¹

4. CONCLUSION

For *M. giganteus*, lime pretreatment with oxidants effectively improves the efficiency of enzymatic hydrolysis. Delignification is 64.7% when using pretreatment with 0.2 g of lime/g of biomass at 150 °C for 1 h with 200 psig O₂. The conversion of cellulose to glucose is 91.7% for hydrolysis of the pretreated biomass, while the overall yield is 85.8%. The conversion of hemicellulose to xylose is 67.3%, but the overall yield is only 38.2%. Oxygen and hydrogen peroxide are promising additions for lime pretreatment; they are effective for enhancing enzymatic hydrolysis. However, using ammonium molybdate, with and without oxidants, is not useful for delignification.

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Heaton, E. A.; Dohleman, F. G.; Fernando Miguez, A.; Juvik, J. A.; Lozovaya, V.; Widholm, J.; Zabolina, O. A.; McIsaac, G. F.; David, M. B.; Voigt, T. B. Miscanthus: A promising biomass crop. *Adv. Bot. Res.* **2010**, *56* (10), 75–137.
- (2) McMillan, J. D. Pretreatment of lignocellulosic biomass. In *Enzymatic Conversion of Biomass for Fuels Production*; Himmel, M. E., Baker, J. O., Overend, R. P., Eds.; American Chemical Society (ACS): Washington, D.C., 1994; ACS Symposium Series, Vol. 566, Chapter 15, pp 292–324.
- (3) Sierra, R.; Granda, C.; Holtzapfle, M. Lime pretreatment. In *Biofuels*; Mielenz, J. R., Ed.; Humana Press: New York, 2009; Vol. 581, pp 115–124.
- (4) Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y. Y.; Holtzapfle, M.; Ladisch, M. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* **2005**, *96* (6), 673–686.
- (5) Cheng, Y.-S.; Zheng, Y.; Yu, C.; Dooley, T.; Jenkins, B.; VanderGheynst, J. Evaluation of high solids alkaline pretreatment of rice straw. *Appl. Biochem. Biotechnol.* **2010**, *162* (6), 1768–1784.
- (6) Liu, Z.; Padmanabhan, S.; Cheng, K.; Xie, H.; Gokhale, A.; Afzal, W.; Na, H.; Pauly, M.; Bell, A. T.; Prausnitz, J. M. Two-step delignification of Miscanthus to enhance enzymatic hydrolysis: Aqueous ammonia followed by sodium hydroxide and oxidants. *Energy Fuels* **2013**, *28* (1), 542–548.
- (7) Liu, Z.; Padmanabhan, S.; Cheng, K.; Schwyter, P.; Pauly, M.; Bell, A. T.; Prausnitz, J. M. Aqueous-ammonia delignification of miscanthus followed by enzymatic hydrolysis to sugars. *Bioresour. Technol.* **2013**, *135*, 23–29.
- (8) Yu, G.; Afzal, W.; Yang, F.; Padmanabhan, S.; Liu, Z.; Xie, H.; Shafy, M. A.; Bell, A. T.; Prausnitz, J. M. Pretreatment of *Miscanthus × giganteus* using aqueous ammonia with hydrogen peroxide to increase enzymatic hydrolysis to sugars. *J. Chem. Technol. Biotechnol.* **2014**, *89* (5), 698–706.
- (9) National Lime Association (NLA). *Lime Physical & Chemical Properties*; NLA: Arlington, VA, 2015.
- (10) Chang, V.; Burr, B.; Holtzapfle, M. Lime pretreatment of switchgrass. *Appl. Biochem. Biotechnol.* **1997**, *63–65* (1), 3–19.
- (11) Chang, V.; Holtzapfle, M. Fundamental factors affecting biomass enzymatic reactivity. *Appl. Biochem. Biotechnol.* **2000**, *84–86* (1–9), 5–37.
- (12) Chang, V.; Nagwani, M.; Holtzapfle, M. Lime pretreatment of crop residues bagasse and wheat straw. *Appl. Biochem. Biotechnol.* **1998**, *74* (3), 135–159.
- (13) Falls, M.; Holtzapfle, M. Oxidative lime pretreatment of Alamo switchgrass. *Appl. Biochem. Biotechnol.* **2011**, *165* (2), 506–522.
- (14) Falls, M.; Sierra-Ramirez, R.; Holtzapfle, M. Oxidative lime pretreatment of Dacotah switchgrass. *Appl. Biochem. Biotechnol.* **2011**, *165* (1), 243–259.
- (15) Kim, S.; Holtzapfle, M. T. Lime pretreatment and enzymatic hydrolysis of corn stover. *Bioresour. Technol.* **2005**, *96* (18), 1994–2006.
- (16) Sierra, R.; Granda, C.; Holtzapfle, M. T. Short-term lime pretreatment of poplar wood. *Biotechnol. Prog.* **2009**, *25* (2), 323–332.
- (17) Sierra, R.; Holtzapfle, M. T.; Granda, C. B. Long-term lime pretreatment of poplar wood. *AIChE J.* **2011**, *57* (5), 1320–1328.
- (18) Verma, P.; Watanabe, T.; Honda, Y.; Watanabe, T. Microwave-assisted pretreatment of woody biomass with ammonium molybdate activated by H₂O₂. *Bioresour. Technol.* **2011**, *102* (4), 3941–3945.
- (19) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. *Determination of Structural Carbohydrates and Lignin in Biomass*; National Renewable Energy Laboratory (NREL): Golden, CO, 2008; NREL/TP-510-42618.
- (20) Wang, Z.; Cheng, J. J. Lime pretreatment of coastal bermudagrass for bioethanol production. *Energy Fuels* **2011**, *25* (4), 1830–1836.
- (21) Suchy, M.; Argyropoulos, D. S. Catalysis and activation of oxygen and peroxide delignification of chemical pulps: A review. In *Oxidative Delignification Chemistry*; Argyropoulos, D. S., Ed.; American Chemical Society (ACS): Washington, D.C., 2001; ACS Symposium Series, Vol. 785, Chapter 1, pp 2–43.
- (22) Nardello, V.; Marko, J.; Vermeersch, G.; Aubry, J. M. ⁹⁰Mo NMR and kinetic studies of peroxomolybdic intermediates involved in the catalytic disproportionation of hydrogen peroxide by molybdate ions. *Inorg. Chem.* **1995**, *34* (20), 4950–4957.
- (23) Xu, J.; Cheng, J. J.; Sharma-Shivappa, R. R.; Burns, J. C. Lime pretreatment of switchgrass at mild temperatures for ethanol production. *Bioresour. Technol.* **2010**, *101* (8), 2900–2903.
- (24) Chang, V.; Nagwani, M.; Kim, C.-H.; Holtzapfle, M. Oxidative lime pretreatment of high-lignin biomass. *Appl. Biochem. Biotechnol.* **2001**, *94* (1), 1–28.
- (25) Rabelo, S. C.; Maciel Filho, R.; Costa, A. C. A comparison between lime and alkaline hydrogen peroxide pretreatments of sugarcane bagasse for ethanol production. *Appl. Biochem. Biotechnol.* **2008**, *148* (1–3), 45–58.