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Pretreatment of Miscanthus giganteus with Lime and Oxidants for Biofuels

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Complete List of Authors:	Yang, Fuxin; University of California, Berkeley, Dept of Chemical and Biomolecular Engineering Liu, Zhongguo; University of California, Berkeley, Azfal, Waheed; University of California, Berkeley, Bell, Alexis; University of California, Berkeley, Prasunitz, John; University of California, Berkeley,

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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 reaction time, lime loading, oxidant loading and temperature on sugar-production efficiency. Following enzymatic hydrolysis, overall yields of biomass-derived sugars were obtained. Under a selected condition (0.2 g lime/g biomass, 200 psig O₂, 150 °C for 1 hour), delignification was 64.7 %. Based on the recovered solid, the yields of glucose and xylose were 91.7 % and 67.3 % respectively, 7.1 and 18.2 times larger than those obtained from raw (untreated) biomass. Based on raw biomass, the overall yields of glucose and xylose were 85.8 % and 38.2 %. Pretreatment with oxidants substantially raised delignification of raw *Miscanthus giganteus* thereby enhancing enzymatic hydrolysis to sugars. Results were not improved when pretreatment included ammonium molybdate.

1. Introduction

Because of rising consumption of non-renewable resources, and because of 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 for environmental protection. *Miscanthus giganteus* (*M. giganteus*) is a promising energy crop due to minimal requirements for obtaining high yield per acre¹. Table 1 gives the composition of

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4 1 dried raw *M. giganteus*; it is primarily composed of cellulose, hemicellulose and
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6 2 lignin. Owing to its high lignin content, large cellulose crystallinity, low surface
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9 3 area/pore volume, and hemicellulose acetylation, pretreatment is required to break
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11 4 down the biomass matrix to release the polysaccharides ². In a typical
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14 5 biomass-to-biofuel process, carbohydrates are hydrolyzed to sugars using chemical or
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16 6 biochemical methods; the sugars are then fermented to bio-alcohols ³.
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19 7 Dilute-acid pretreatment methods have been extensively investigated ⁴. Using only
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21 8 dilute acid without enzymes provides a promising chemical method to hydrolyze
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24 9 biomass to sugars. However, in an acid process, sugar is often degraded and the total
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26 10 sugar yield is low.
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29 11 Alkaline pretreatment has several potential advantages compared to other
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31 12 pretreatment processes: low operation cost, reduced degradation of polysaccharides,
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34 13 compatibility with oxidants ⁵. Because alkali removes acetate groups from
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36 14 hemicellulose, steric hindrance for hydrolytic enzymes is reduced, enhancing
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39 15 carbohydrate digestibility.
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41 16 Sodium hydroxide effectively raises lignocellulose digestibility, but it is expensive,
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44 17 requires safety precautions and is difficult to recover ⁶. Ammonia pretreatment has
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46 18 also received much attention because ammonia is easy to recover, but it is moderately
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49 19 expensive and requires careful handling to avoid safety problems ^{7,8}. Compared to
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51 20 sodium hydroxide and ammonia, calcium hydroxide (lime) is cheaper, safer, and can
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54 21 be recovered by reacting with CO₂ to produce water-insoluble CaCO₃. Lime is a weak
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56 22 alkali, poorly soluble in water; at 100 °C, the solubility is 0.071 g per 100 g saturated
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1 solution ⁹. To make a lime process as efficient as possible, optimum pretreatment
2 conditions need to be identified.

3 Lime without oxidants is effective for delignification from low-lignin biomass, but
4 oxidants are required for lignin removal from biomass with high lignin content ². For
5 switchgrass, crop residue bagasse, wheat straw, corn stover and polar wood, several
6 studies have been reported for lime pretreatment processes with and without oxygen
7 ¹⁰⁻¹⁷; however, little attention has been given to using lime with hydrogen peroxide.
8 Moreover, as shown by Verma et al., a pretreatment process at 140 °C can be
9 improved when using ammonium molybdate activated by hydrogen peroxide ¹⁸.

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

13 **2. Experimental**

14 **2.1 Materials**

15 *M. giganteus* was provided by the Energy Biosciences Institute, University of Illinois
16 at Urbana-Champaign. A Retsch grinder and a 4-mm sieve produced 4-mm particles.
17 30 wt. % hydrogen peroxide solution and citric acid were purchased from
18 Sigma-Aldrich (St. Louis, MO, USA); ammonium molybdate from Acros (New Jersey,
19 USA); calcium hydroxide powder from Mallinckrode Baker Inc. (New Jersey, USA);
20 sulfuric acid, hydrochloric acid, sodium citrate and sodium hydroxide from Fisher
21 Scientific (New Jersey, USA); enzymes Cellic CTec 2 and Cellic HTec 2 from

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4 1 Novozymes; 0.5 % (w/v) sodium azide aqueous solution from Ricca chemical
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6 2 company (Texas, USA). All reagents were used without further purification.
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9 3 Nanopure water (18.2 MΩ) was used to prepare the solutions and for washing the
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11 4 recovered solid.

5 **2.2 Procedures**

6 Dried *M. giganteus* and aqueous-lime solution with/without hydrogen peroxide were
7 weighed to fix a solid-to-liquid weight ratio 1:8. The solid/liquid mixture was placed
8 into a stainless-steel pressure reactor with stirring. If oxygen is used in pretreatment,
9 the reactor was initially purged with oxygen. The reactor was submerged into a
10 silicon-oil bath at a pre-set temperature. After a fixed reaction time, the reactor was
11 taken from the oil bath and cooled to 70 °C using an ice-water bath. After cooling, the
12 pulp was neutralized with hydrochloric acid until the pH was between 6 and 7, and
13 then filtered to separate solid from liquid. The recovered solid was washed several
14 times with Nanopure water. A small solid sample was dried overnight in a
15 105 °C-oven prior to determine its composition. The residual (not dried) recovered
16 solid was subjected to enzymatic hydrolysis. The composition of the liquid phase was
17 not analyzed, because in the liquid, the concentrations of cellulose, hemicellulose and
18 sugars were much too low for use in an industrial process.

19 **2.3 Composition analysis of the recovered solid**

20 The composition of the recovered solid *M. giganteus* was determined by the analytical
21 procedure proposed by the National Renewable Energy Laboratory (NREL) ¹⁹.

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1 Details are given in a previous publication ⁸.

2 **2.4 Enzymatic hydrolysis of pretreated materials**

3 For hydrolysis, the pretreated *M. giganteus* was not dried to prevent irreversible pore
4 collapse. Enzymatic hydrolysis was carried out following the NREL protocol ⁹. The
5 recovered solid was mixed with a solution containing citrate buffer and enzymes at 20
6 FPU/g cellulose and 20 CBU/g hemicellulose. The 0.1 M citrate buffer was made by
7 mixing citric acid and sodium citrate to maintain the hydrolysis solution at pH 4.8. To
8 prevent microbial growth, 2 ml 0.5 % (w/v) sodium azide/g cellulose was added.
9 Aliquots of supernatant liquid were withdrawn to measure the concentration of
10 glucose and xylose using a Shimadzu high-performance liquid chromatograph (HPLC)
11 at 50 °C with an Aminex HPX 87H column (300×7.8 mm) and a refractive-index
12 detector. The flow rate of 0.01N sulfuric-acid eluent was 0.6 mL/min.

13 **3. Results and Discussion**

14 **3.1 Effect of lime loading**

15 The primary aim of pretreatment is to decompose and remove lignin to open the
16 crystalline structure in cellulose. Removing lignin makes the biomass more accessible
17 to enzymes for hydrolysis, while minimizing the loss of polysaccharides. With the
18 goal of developing a cost-effective pretreatment process for alcohol production, it is
19 necessary to study the effect of lime dosage to optimize lime loading for enhancing
20 hydrolysis to sugar. Therefore, lime loading was investigated first, while the
21 temperature was constant at 150 °C and the reaction time was 3 hours.

1 Table 2 shows compositions of pretreated *M. giganteus* at 150 °C for 3 hours. When
2 no lime or oxidants were loaded into the reactor, 23.1 % of the original lignin was
3 removed. However, 35.2 % was removed when the reactor contained 0.13 g lime/g
4 biomass. When the lime loading increased to 0.20 g/g biomass, delignification was
5 nearly the same (35.4 %), indicating that in the absence of oxidants, raising lime
6 loading beyond 0.13 g/g biomass is not helpful. However, when O₂ or H₂O₂ was
7 added to the lime solution, results improved remarkably. Delignification was raised
8 from 35.2 % to 42.5 % when the lime loading was 0.13 g/g biomass augmented by
9 200 psig O₂. Delignification rose from 35.4 % to 66.9 % when the lime loading was
10 0.20 g/g biomass augmented by 200 psig O₂; and to 53.2 % when the lime loading
11 was augmented by 2 wt. % H₂O₂. Regrettably, with an oxidant, 10 % more
12 hemicellulose was dissolved and lost. However, with an oxidant, the recovery of
13 cellulose was good, near 90 %.

14 Previous studies have shown that an increase in lime loading has a limited effect on
15 lignin removal when lime loading is above 10 % wt./wt. of the dry weight of the
16 biomass⁵. In our work, when no oxidants are used, increasing lime loading has no
17 effect; however, with an oxidant, increased lime loading raises delignification. When
18 lime loading increased from 0.13 to 0.2 g/g biomass, delignification increased from
19 42.5 % to 66.9 %. Because lime is only slightly soluble in water, increasing lime
20 loading produces a suspension. The suspended lime particles have a very high surface
21 area; therefore, lime particles dissolve quickly to replace the dissolved lime that was
22 consumed during the pretreatment reaction that forms calcium-lignin linkages²⁰. With

1 the help of oxidants, hydroxide ions react with chromophoric and reactive groups in
2 the lignin to break aryl ether bonds and other linkages between lignin and
3 carbohydrates⁸.

4 Because hydrogen peroxide is a bleaching reagent in the pulp-and-paper industry, its
5 oxidation power is likely to help in delignification. Therefore, 0.2 g lime/g biomass
6 with oxidants was selected for further investigation.

7 **3.2 Pretreatment conditions**

8 Table 3 shows pretreatment results at various conditions. Because previous studies
9 showed that solution loading had little effect on pretreatment, the solid-to-liquid ratio
10 1:8 was retained^{8,10}. A solution containing 0.2 g lime/g biomass was used to pretreat
11 *M. giganteus* with oxidants at 100, 120, 140 and 150 °C for 3 hours. Toward better
12 understanding for the effect of reaction time, we also conducted the reaction for 1
13 hour at 100 to 150 °C, with results shown in Table 4. At 150 °C with 200 psig O₂,
14 delignification for 3 hours was 66.9 %, while for 1 hour it was nearly the same,
15 64.7 %. With 2 wt. % H₂O₂, for 3 hours it was 53.2 % and for 1 hour it was 46.9 %.
16 At 140 °C with 200 psig O₂, delignification for 3 hours was 70.7 %, while for 1 hour
17 it was 59.8 %; with 2 wt. % H₂O₂, for 3 hours it was 52.4 % and for 1 hour it was
18 45.4 %. At these conditions, 3 hours of reaction time showed a little benefit compared
19 to 1 hour.

20 With oxidants, delignification increased with raising reaction temperature. However,
21 using O₂ for 3 hours, raising the temperature from 140 to 150 °C, did not produce
22 higher delignification. It has been suggested that because the low solubility of lime

1 decreases with rising temperature, and at high temperature, the alkali is converted to
2 irrecoverable salts or it is incorporated as salt into the biomass⁴. More hemicellulose
3 was removed when the temperature is 150 °C. Highest delignification was achieved at
4 140 °C using 0.2 g lime/g biomass with 200 psig O₂ for 3 hours.

5 Several studies have shown that molybdate ions could be activated by hydrogen
6 peroxide to improve delignification^{18, 21, 22}. In this work, we pretreated the biomass
7 with hydrogen peroxide and ammonium molybdate; results are shown in Table 5. At
8 140 °C for 1 hour using 0.02 % molybdate without lime, delignification was 48.6 %;
9 it was 45.7 % without lime or molybdate; 76.8 % hemicellulose was removed using
10 0.02 % molybdate without lime. However, when lime was present with molybdate and
11 hydrogen peroxide, the removal of lignin was similar to that without lime, whereas
12 more hemicellulose was removed with molybdate. It appears that molybdate with
13 hydrogen peroxide has little additional benefit for delignification, while it has a
14 negative benefit on the recovery of polysaccharides because more hemicellulose was
15 lost.

16 **3.3 Interaction between lignin and calcium ions**

17 With a rise in lime loading from 0.13 to 0.20 g/g biomass, delignification increased
18 from 42.5 % to 66.9 % at 150 °C for 3 hours using 200 psig O₂. With oxidants,
19 increasing lime loading produces a significant increase in lignin removal. However,
20 more polysaccharide was removed. Because hemicellulose is covalently linked to
21 lignin, when lignin is degraded, it takes hemicellulose with it². Several studies
22 showed that divalent calcium ions have high affinity for lignin; calcium ion may

1 overall yields of cellulose to glucose and hemicellulose to xylose.

2 Figure 1 and Table 6 show results from enzymatic hydrolysis of pretreated *M.*

3 *giganteus* using lime with 200 psig O₂ or 2 wt. % H₂O₂ at 140 and 150 °C for 1 hour.

4 For the raw (untreated) biomass, the yields of converting cellulose to glucose and

5 hemicellulose to xylose are 12.9 % and 3.7 %, respectively. Hydrolysis results for

6 biomass treated for 1 hour at 140 and 150 °C with oxidants, indicate that using 200

7 psig O₂ gives yields higher than those using 2 wt. % H₂O₂. These results are consistent

8 with those for delignification. At 150 °C for 1 hour, the yield of cellulose to glucose

9 was 91.7 % and 67.3 % for hemicellulose to xylose when using O₂ where

10 delignification was 64.7 %. It appears that using lime pretreatment with H₂O₂ for one

11 hour is not able to achieve a good enzymatic yield.

12 Figure 2 and Table 6 show results for the enzymatic hydrolysis of pretreated biomass

13 using lime with 200 psig O₂ or 2 wt. % H₂O₂ at 140 and 150 °C for 3 hours.

14 The conversion of cellulose to glucose was 93.9 % and 72.6 % for hemicellulose to

15 xylose at 140 °C for 3 hours with O₂ where delignification was 70.7 %. At 150 °C, the

16 conversion of cellulose to glucose was 95.8 % and 83.3 % for hemicellulose to xylose

17 where delignification was 66.9 %.

18 Figure 3 shows results for enzymatic hydrolysis of pretreated biomass with 2 wt. %

19 H₂O₂ and 0.02 wt. % ammonium molybdate at 140 °C for 0.5 and for 1 hour without

20 lime. When pretreated for 0.5 hour, the hydrolysis yield of cellulose to glucose was

21 26.9 % and 36.5% for hemicellulose to xylose, while the conversion of cellulose to

22 glucose was 35.7 % and 30.8 % for hemicellulose to xylose when pretreated for 1

1 hour.

2 Table 6 shows the enzymatic hydrolysis yields of pretreated biomass, as well as the
3 overall yields of cellulose to glucose and hemicellulose to xylose based on the raw
4 (untreated) biomass. The highest overall yield was achieved at 140 °C for 3 hours
5 with O₂. However, to reduce the input energy, 1 hour of reaction time was selected
6 and the recommended conditions are: 150 °C for 1 hour with 200 psig O₂. If the lignin
7 content in the pretreated biomass is 14-16 %, a significant increase in enzymatic
8 hydrolysis yield is expected. For H₂O₂, it is likely that if more H₂O₂ is added for the
9 pretreatment, better overall enzymatic yields can be obtained.

10 In summary, for *M. giganteus* optimum condition for pretreatment with lime is: 0.2 g
11 lime/g dry biomass, 1 hour, 150 °C with 200 psig O₂.

12 **3.6 Material balance**

13 Material balances were obtained for the biomass pretreated under selected conditions
14 (140 and 150 °C for 1 hour with O₂). The dried biomass was weighed before and after
15 pretreatment to calculate the total solid recovery. The contents of the pretreated
16 biomass components (cellulose, hemicellulose, lignin, ash and others) were
17 determined; they are recorded as the recovered and removal compositions based on
18 the mass in raw (untreated) biomass. At selected pretreatment conditions, total solid
19 recovery was about 80 %. Balances for lignin, cellulose, and hemicellulose show that
20 nearly 90 % of cellulose remained in the pretreated solid, indicating a good overall
21 preservation of cellulose after lime pretreatment. However, lignin and hemicellulose
22 were not retained as well as cellulose; lime pretreatment produced a relatively low

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4 1 recovery for lignin (14-16 %) and for hemicellulose (about 60 %). The residual
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6 2 fraction of hemicellulose in the biomass solid is correlated with the extent of
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9 3 delignification during lime pretreatment because hemicellulose is covalently linked to
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11 4 lignin ². Total sugar yield in the hydrolyzates shows that high conversion of
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14 5 carbohydrates after enzymatic hydrolysis can be achieved even without extensive
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16 6 delignification. The lignin content and the degree of crystallinity have greatest impact
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19 7 on biomass digestibility while acetyl content has a minor impact, however, a low
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21 8 lignin content is sufficient to obtain high digestibility regardless of crystallinity or
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24 9 acetyl content ¹¹.

10 4. Conclusions

11 For *M. giganteus*, lime pretreatment with oxidants effectively improves the efficiency
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13 12 of enzymatic hydrolysis. Delignification is 64.7 % when using pretreatment with 0.2 g
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16 13 lime/g biomass at 150 °C for 1 hour with 200 psig O₂; the conversion of cellulose to
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19 14 glucose is 91.7 % for hydrolysis of the pretreated biomass while the overall yield is
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22 15 85.8 %. The conversion of hemicellulose to xylose is 67.3 % for hydrolysis of the
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25 16 pretreated biomass but the overall yield is only 38.2 %. Hydrogen peroxide is a
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28 17 promising addition for lime pretreatment; it is effective for delignification and for
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31 18 enhancing conversion to sugars by enzymatic hydrolysis. At 140 °C for 3 hours with 2
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34 19 wt. % H₂O₂, delignification was 52.4 %, conversion of cellulose to glucose is 88.6 %
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37 20 for hydrolysis of the pretreated biomass and 81.9 % for the overall yield; conversion
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40 21 of hemicellulose to xylose is 67.7 % and 42.9 % for the overall yield. Using
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43 22 ammonium molybdate, with and without oxidants, is not useful for delignification.
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1 **Table**2 Table 1 Composition of dried raw *M. giganteus*3
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Biomass	Composition (wt. %)				
	Cellulose	Hemicellulose	Lignin	Ash	Extractable
<i>M. giganteus</i>	43.1	23.6	26.3	3.0	4.0

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1 Table 2 Lime pretreatment of *M. giganteus* with and without oxidants at 150 °C for 3
 2 hours

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Pretreatment condition ^a			Pretreatment results (%) ^b			Post-pretreatment
Lime loading (g/ g biomass)	O ₂ (psig)	H ₂ O ₂ (wt. %)	Cellulose recovered	Hemicellulose recovered	Lignin removal	Lime consumption (g/g biomass)
0	0	0	90.9	66.5	23.1	0
0.13	0	0	90.4	60.8	35.2	0.056
0.20	0	0	89.5	60.2	35.4	0.072
0.13	200	0	88.5	50.8	42.5	0.125
0.20	200	0	89.4	50.4	66.9	0.200
0.20	0	2.0	87.9	51.9	53.2	0.200

4 ^a: The solid-to-liquid ratio is 1:8.

5 ^b: Recovered and removal results are calculated based on the mass in the raw
 6 (untreated) biomass.

1 Table 3 Lime pretreatment of *M. giganteus* using 0.2 g lime/ g biomass with oxidants
 2 at 100, 120 and 140 °C for 3 hours
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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 biomass)
100	200	0	92.8	59.7	50.3	0.108
100	0	2.0	94.7	71.0	40.9	0.095
120	200	0	92.5	58.9	55.4	0.154
120	0	2.0	94.1	66.0	50.1	0.163
140	200	0	92.6	57.1	70.7	0.200
140	0	2.0	92.4	63.4	52.4	0.200

4 ^a: The solid-to-liquid ratio is 1:8.

5 ^b: Recovered and removal results are calculated based on the mass in the raw
 6 (untreated) biomass.

1 Table 4 Lime pretreatment of *M. giganteus* using 0.2 g lime/ g biomass with oxidants
 2 at 100, 120, 140 and 150 °C for 1 hour
 3

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 biomass)
100	200	0	95.0	66.1	45.6	0.124
100	0	2.0	93.7	74.4	32.2	0.084
120	200	0	93.7	61.7	51.4	0.126
120	0	2.0	94.4	68.0	42.5	0.118
140	200	0	93.8	59.0	59.8	0.125
140	0	2.0	91.9	66.2	45.4	0.145
150	200	0	93.6	56.7	64.7	0.200
150	0	2.0	91.2	62.9	46.9	0.182

4 ^a: The solid-to-liquid ratio is 1:8.

5 ^b: Recovered and removal results are calculated based on the mass in the raw
 6 (untreated) biomass.

1 Table 5 Lime pretreatment of *M. giganteus* using 2.0 wt. % H₂O₂, and ammonium
 2 molybdate with and without lime at 140 °C
 3

Pretreatment condition ^a				Pretreatment results (%) ^b			Post-pretreatment
Lime loading (g/ g biomass)	H ₂ O ₂ (wt. %)	Ammonium molybdate (wt. %)	Time (hours)	Cellulose recovered	Hemicellulose recovered	Lignin removal	Lime consumption (g/g biomass)
0	2.0	0.02	0.5	87.7	49.2	46.7	0
0	2.0	0.10	0.5	88.0	41.5	45.7	0
0	2.0	0	1.0	87.4	25.0	45.7	0
0	2.0	0.02	1.0	86.8	23.2	48.6	0
0	2.0	0.10	1.0	85.9	24.2	46.2	0
0.20	2.0	0.10	3.0	92.7	64.1	52.8	0.200

4 ^a: The solid-to-liquid ratio is 1:8.

5 ^b: Recovered and removal results are calculated based on the mass in the raw
 6 (untreated) biomass.

1 Table 6 Enzymatic hydrolysis yields of pretreated *M. giganteus* using 0.2 g lime/g
 2 biomass

3

Pretreatment condition ^a					Enzymatic yield (%)		Overall yield (%)	
T (°C)	O ₂ (psig)	H ₂ O (wt. %)	Ammonium molybdate (wt. %)	Time (hours)	Glucose	Xylose	Glucose	Xylose
0	0	0	0	0	12.9	3.7	12.9	3.7
140	0	2	0	1	69.4	48.0	63.8	31.8
140	200	0	0	1	90.4	65.5	84.8	38.6
150	0	2	0	1	66.5	52.2	60.6	32.8
150	200	0	0	1	91.7	67.3	85.8	38.2
140	0	2	0	3	88.6	67.7	81.9	42.9
140	200	0	0	3	93.9	72.6	87.0	41.5
150	0	2	0	3	85.9	71.4	75.5	37.1
150	200	0	0	3	95.8	83.3	85.6	42.0
140	0	2	0.02	0.5	26.9	36.5	23.6	18.0
140	0	2	0.02	1	35.7	30.8	31.0	7.2

4 ^a: The solid-to-liquid ratio is 1:8.

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4 **1 Figure Captions**
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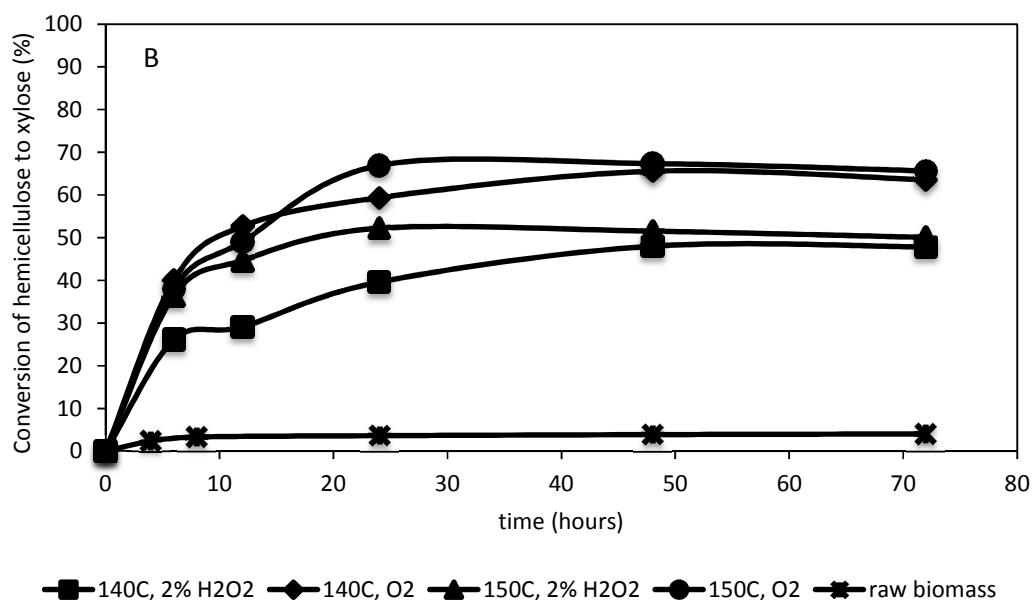
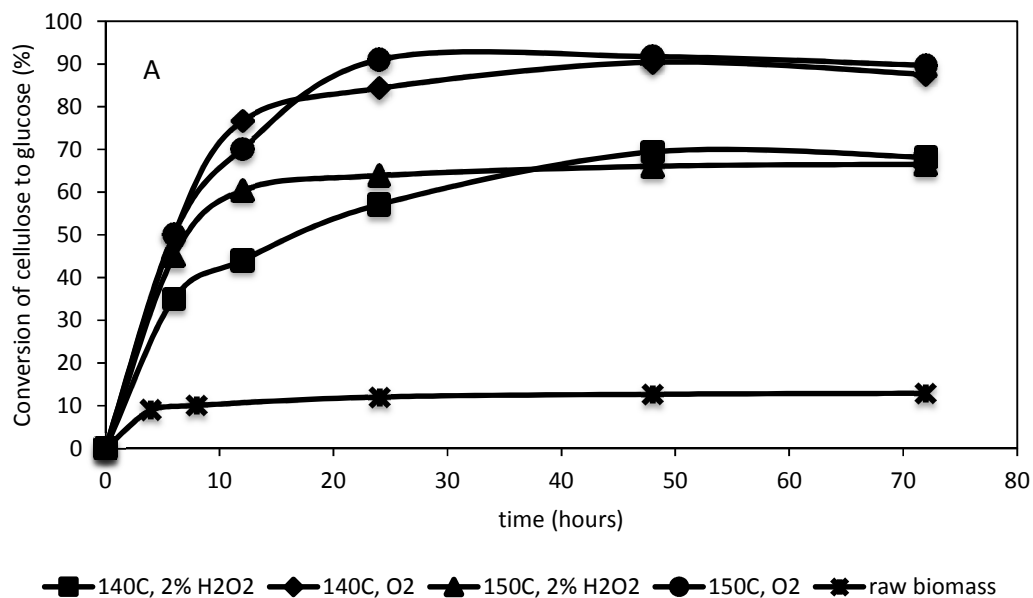
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9 3 Figure 1 Enzymatic hydrolysis of *M. giganteus* pretreated with 0.2 g lime/g biomass
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12 4 and with 200 psig O₂ or 2 wt. % H₂O₂ at 140 and 150 °C for 1 hour. The
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14 5 solid-to-liquid ratio is 1:8.
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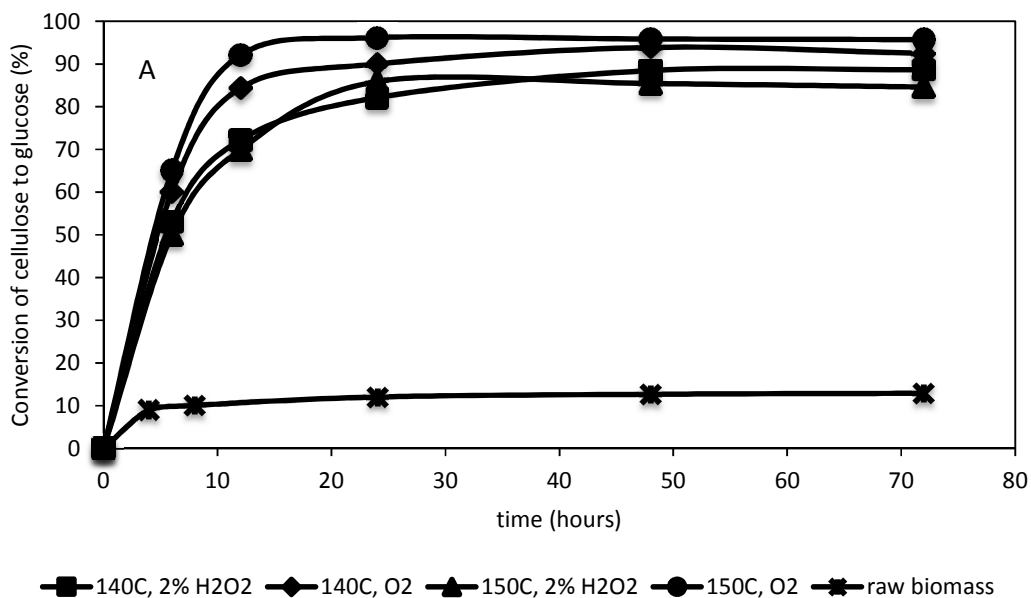
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19 7 Figure 2 Enzymatic hydrolysis of *M. giganteus* pretreated with 0.2 g lime/g biomass
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22 8 and with 200 psig O₂ or 2 wt. % H₂O₂ at 140 and 150 °C for 3 hours. The
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24 9 solid-to-liquid ratio is 1:8.
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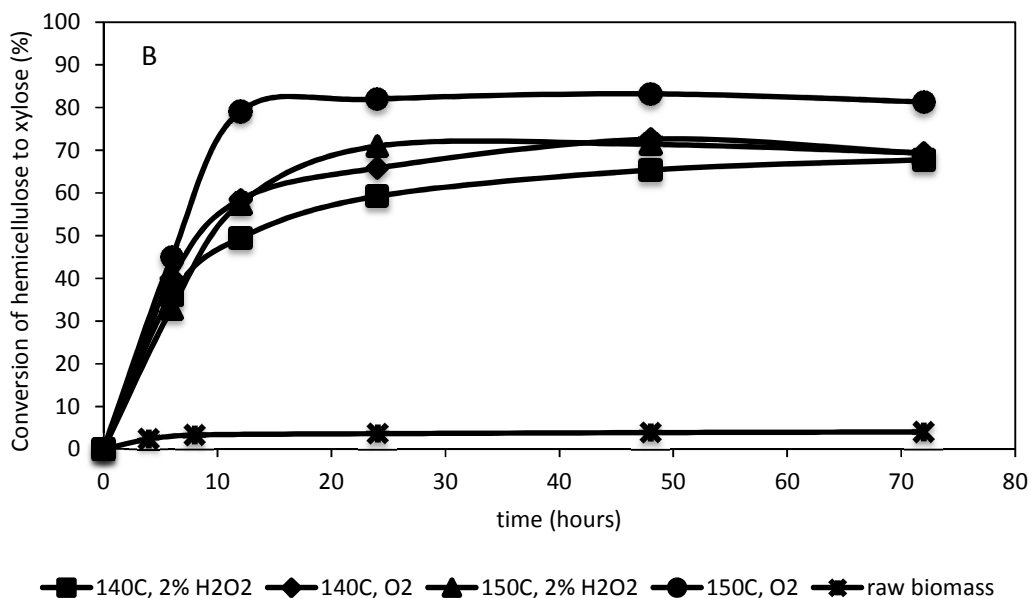
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29 11 Figure 3 Enzymatic hydrolysis of *M. giganteus* pretreated with 2 wt. % H₂O₂ and 0.02
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32 12 wt. % ammonium molybdate at 140 °C for 0.5 and 1 hour.
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3 Figure 1 Enzymatic hydrolysis of *M. giganteus* pretreated with 0.2 g lime/g biomass
4 and with 200 psig O₂ or 2 wt. % H₂O₂ at 140 or 150 °C for 1 hour. The solid-to-liquid
5 ratio is 1:8. (A) hydrolysis of cellulose to glucose; (B) hydrolysis of hemicellulose to
6 xylose

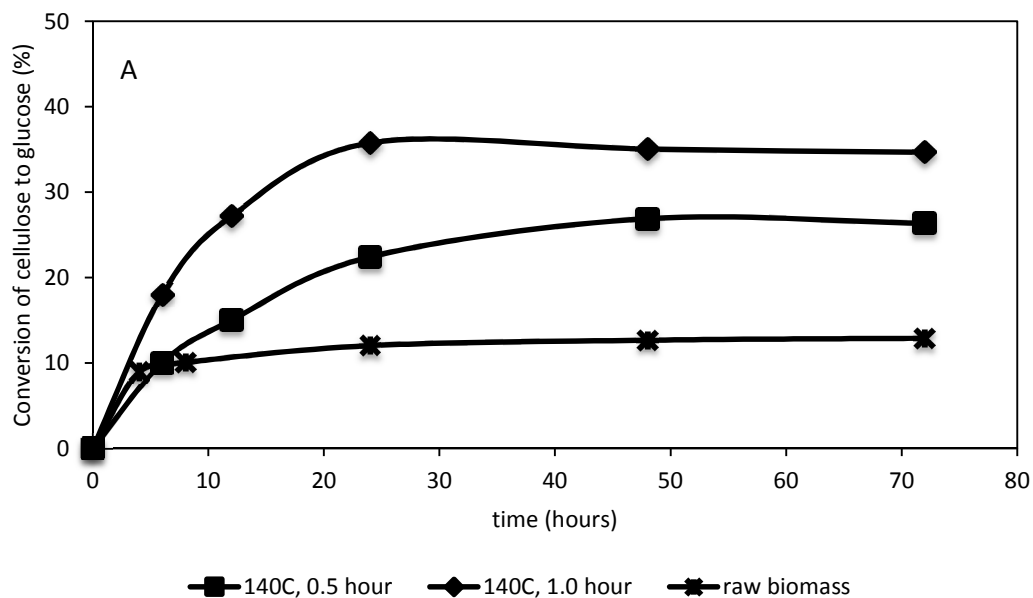


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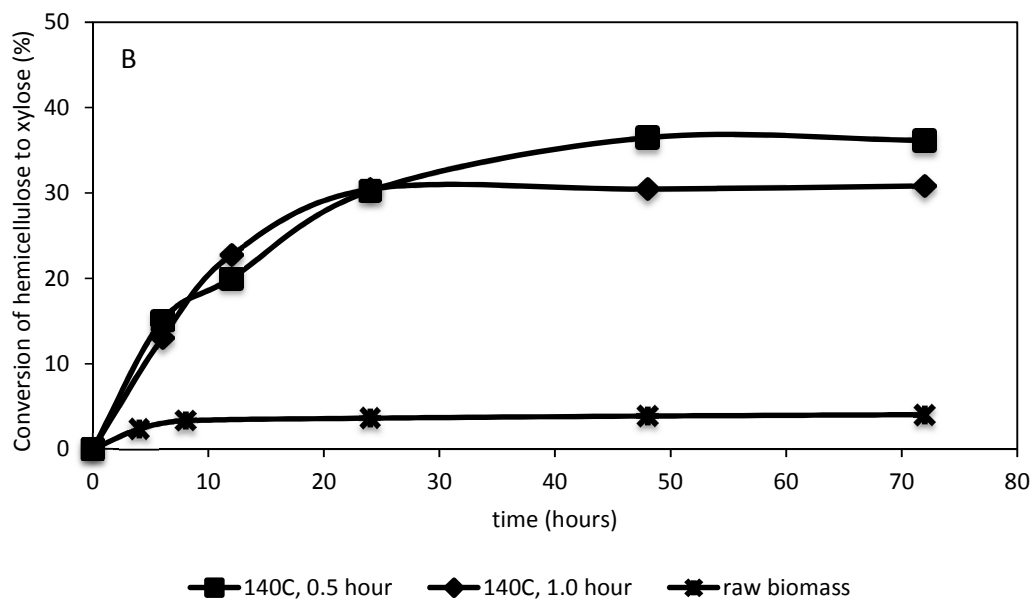


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3 Figure 2 Enzymatic hydrolysis of *M. giganteus* pretreated with 0.2 g lime/g biomass
 4 and with 200 psig O₂ or 2 wt. % H₂O₂ at 140 or 150 °C for 3 hours. The
 5 solid-to-liquid ratio is 1:8. (A) hydrolysis of cellulose to glucose; (B) hydrolysis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



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3 Figure 3 Enzymatic hydrolysis of *M. giganteus* pretreated with 2 wt. % H₂O₂ and 0.02
4 wt. % ammonium molybdate at 140 °C for 0.5 and 1 hour. (A) hydrolysis of cellulose
5 to glucose; (B) hydrolysis of hemicellulose to xylose

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