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Oxidative Lime Pretreatment of Dacotah Switchgrass

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Abstract Oxidative lime pretreatment increases the enzymatic digestibility of lignocellulosic biomass primarily by removing lignin. In this study, recommended pretreatment conditions (reaction temperature, oxygen pressure, lime loading, and time) were determined for Dacotah switchgrass. Glucan and xylan overall hydrolysis yields (72 h, 15 FPU/g raw glucan) were measured for 105 different reaction conditions involving three different reactor configurations (very short term, short term, and long term). The short-term reactor was the most productive. At the recommended pretreatment condition (120 °C, 6.89 bar O_2, 240 min), it achieved an overall glucan hydrolysis yield of 85.2 g glucan hydrolyzed/100 g raw glucan and an overall xylan yield of 50.1 g xylan hydrolyzed/100 g raw xylan. At this condition, glucan oligomers (1.80 g glucan recovered/100 g glucan in raw biomass) and xylan oligomers (25.20 g xylan recovered/100 g xylan in raw biomass) were recovered from the pretreatment liquor, which compensate for low pretreatment yields.

Keywords Switchgrass · Pretreatment · Lime · Enzymatic digestion · Biofuels

Introduction

Petroleum is currently responsible for almost 40% of US energy consumption, with renewable energy accounting for only 8%. Because of limited domestic petroleum reserves, approximately 70% of US petroleum consumption is imported [1]. Dependence on foreign oil has led to military conflicts and fluctuating oil prices, resulting in economic instability [2]. Environmental issues (e.g., groundwater contamination, acid deposition, air pollution,

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and oil spills) and human health effects have increased desire to develop sustainable alternatives [3, 4]. Greenhouse gas emissions from burning fossil fuels have been linked to climate change as well.

The current transportation infrastructure is built on liquid fuels, so renewable liquid biofuels are a promising solution. Current commercial biofuel technology uses starch from corn, or sucrose from sugarcane, to produce ethanol fuel. However, limited feedstock availability and feed vs. fuel pressures prevent these processes from producing the necessary quantities to make a meaningful impact [5]. Alternatively, lignocellulosic biomass is very abundant and is comprised of many feedstocks: high-yield energy crops, forestry residues, agricultural waste, municipal solid waste, and industrial waste [6, 7].

The biological conversion of lignocellulosic biomass to ethanol has four primary steps: (a) pretreatment to increase cellulose accessibility and enzymatic reactivity, (b) enzymatic hydrolysis of carbohydrate polymers to free sugars, (c) fermentation of sugars to ethanol, and (d) ethanol recovery [8]. Several factors inhibit the hydrolysis of cellulose and hemicellulose to fermentable carbohydrates including high lignin content, presence of acetyl groups on hemicellulose, cellulose crystallinity, degree of cellulose polymerization, and limited surface area [9–11]. The goal of biomass pretreatment, which is responsible for a large percentage of the overall process cost, is to minimize these barriers through chemical or mechanical processes [12]. Common pretreatment methods include alkali (lime, ammonia fiber expansion, soaking in aqueous ammonia), acid (dilute sulfuric acid, sulfur dioxide), and hot water [13]. Alkaline pretreatments are highly effective at removing lignin, which improves enzyme effectiveness by increasing cellulose accessibility and eliminating non-productive adsorption sites [14]. It has also been shown that alkaline pretreatments remove acetyl groups from hemicellulose, which lowers steric hindrances of enzymes and improves carbohydrate digestibility [15]. Advantages of using lime as the alkaline agent include low cost, compatibility with oxidants, ease of recovery, and ease of use [16].

This work is part of a collaboration with the Consortium for Applied Fundamentals and Innovation (CAFI). The CAFI team is a devoted group of academic and industry partners who observed an important need for consistent research and reporting of pretreatment studies [17, 18]. The feedstocks used by the collaboration were harvested, milled, divided, and then distributed to the individual research laboratories. Common enzymes, washing procedures, analytical methods, and reporting guidelines were used. CAFI 1 and CAFI 2 investigated the effect of different pretreatment methods on corn stover [19] and poplar wood [20], respectively. This work was performed as part of the CAFI 3 project, which focused on increasing the enzymatic digestibility of multiple varieties of switchgrass (*Panicum virgatum*).

The Department of Energy has chosen switchgrass as a model biomass feedstock because of its adaptability, high yields on marginal lands, drought resistance, low nutrient inputs, and high pest resistance [21, 22]. It is a native, perennial, warm-season prairie grass that can grow in most of the eastern two thirds of the USA, as well as Mexico and Canada [3]. Average yields of 13.4 Mg/(ha·year) have been achieved [23].

In the past, lime pretreatment of several different feedstocks has been studied including sugarcane bagasse [8, 24], corn stover [25], and poplar wood [26]. The goal of this study was to determine the reaction temperature, time, lime loading, and oxygen pressure that produced the most enzymatically digestible lime-pretreated switchgrass. To determine the most effective treatment conditions, pretreatment yield, carbohydrate yield, and enzymatic yield were considered.

Materials and Methods

Substrate and Enzymes

The feedstock used in this study was the Dacotah variety of switchgrass (*P. virgatum*) kindly provided by Ceres, Inc. This variety was planted on December 6, 1999 in Pierre, SD and harvested on March 1, 2008 after the plot stood over the winter. The bales were stored indoors until shipped to Hazen Research, Inc. (Golden, CO, USA) where they were ground by a hammer mill equipped with a 0.25-in. screen. The material was then mixed using the cone-and-quartering technique, separated into 5-kg sub-lots, and delivered to the Texas A&M laboratory. The composition determined by Ceres, Inc. was 35.0% glucan, 21.8% xylan, 3.5% arabanin, 21.4% lignin, 2.8% acetyl, and 8.1% extractives.

Cellulase was Spezyme CP® (lot 301-04075-054, 82 mg protein/mL, 59 FPU/mL), kindly provided by Genencor International, Inc.®. The β-glucosidase was Novozyme 188® (67 mg protein/mL, 600 CBU/mL) and was obtained from Sigma Aldrich (St. Louis, MO, USA). The protein concentration of each enzyme was measured using TCA precipitation.

Pretreatment Methods

Very Short Term

The very short-term reactions were conducted in a 304 stainless steel pipe reactor (7-in. long, 1.25-in. ID). One end of the reactor was sealed with a temperature gauge and the other sealed by a 1.25-in. stainless steel plate. Three fast-heat conduction bands (Tutco 400 W Better Band, 6 in.×2 in.) wrapped around the reactor produced the desired reaction temperature. The reactor was attached to a sieve shaker (Combustion Engineering Model RX-86), which provided the shaking action. The reactor was loaded with 8 g dry switchgrass, excess calcium hydroxide (1 g $Ca(OH)_2$/g dry biomass), and water (15 g/g dry biomass). Constant-pressure pure oxygen was supplied through 0.25-in. stainless steel tubing from an oxygen cylinder. Reaction time did not include the initial heat-up time, which was typically about 5 min. After the desired reaction time, the heating elements and oxygen supply were turned off, the reactor was cooled by blowing compressed air over the exterior, and the reactor contents were transferred to a 1-L plastic centrifuge bottle. The post-pretreatment conditioning procedure was then performed on the resulting slurry.

Short Term

Short-term lime pretreatment was conducted in a pair of 304 stainless steel pipe reactors (5-in. long, 1.5-in. ID) with 1.5-in. 304 stainless steel caps. The reactors were sealed using Teflon tape. Reactors were loaded with 8 g dry switchgrass each and excess calcium hydroxide (1 g $Ca(OH)_2$/g dry biomass) and water (15 g/g dry biomass). Constant-pressure pure oxygen was supplied to a manifold through a flexible stainless steel hose attached to an oxygen tank. The reactors were connected to a swing arm to provide constant stirring and placed in a preheated temperature-controlled oven set at the desired reaction temperature. Initial heat-up time of the reaction contents was included in the overall reaction time. Upon completing the desired reaction time, reactors were removed from the oven and immediately placed in an ice bath to quench the reaction. Once cooled, the reactors were opened slowly to relieve pressure, and the contents were transferred to a 1-L

plastic centrifuge bottle using distilled water. The reaction contents underwent the post-pretreatment conditioning procedure.

Long Term

Long-term pretreatment was conducted in plastic 450-mL bottles. The bottles were loaded with 16 g dry switchgrass each and excess calcium hydroxide (1 g $Ca(OH)_2$/g dry biomass). Water was added at a ratio of 15 g/g dry biomass. Compressed air was supplied through a manifold and bubbled into each bottle at 1.01 bar pressure. The bottles were placed in a temperature-controlled oven set at 65 °C. Stirring was performed manually twice per day using stainless steel spatulas. The water level of each bottle was checked regularly and additional water was added when necessary. Reaction time was 1, 2, 7, 14, and 28 days, after which the post-pretreatment conditioning procedure was performed.

Post-Pretreatment Conditioning

The lime-treated biomass slurry was neutralized using 5 N HCl to a pH of approximately 4.0 to solubilize any residual lime, and then underwent several washings with distilled water until the pH of the slurry rose to approximately 6.0. The final slurry was vacuum filtered and the filtrate was collected for carbohydrate analysis. Moisture content and final solid weight were recorded to obtain pretreatment yield and the solids were stored in the freezer until compositional analysis and enzymatic hydrolysis were performed.

Lime Consumption

As part of the post-pretreatment conditioning, the lime-treated biomass slurry was neutralized using 5 N HCl. The volume of 5 N HCl required to titrate the solution to an end point of pH 7.0 was recorded and used to calculate the amount of un-reacted excess lime present in the pretreatment slurry. Using this value and the known initial quantity of lime, the amount of lime consumed was calculated.

Compositional Analysis

Compositional analysis was performed on the raw and pretreated samples. The material was prepared by air drying to a measured moisture content of less than 10%. The composition was analyzed using an NREL acid hydrolysis procedure [27]. The sample (0.3 g) was weighed into a glass test tube followed by adding 3 mL of 72 wt.% sulfuric acid. The test tubes were placed in a 30 °C water bath and stirred regularly for 1 h. The contents of the test tube were quantitatively transferred to glass autoclave bottles using 84 mL distilled water, capped, sealed, and steam autoclaved for 1 h. Samples were cooled, opened, and filtered through glass filtering crucibles, which were placed in a 105 °C oven to dry. The filtrate was neutralized and then analyzed for carbohydrates using HPLC Analysis (Bio-Rad Aminex HPX-87P column, HPLC-grade water mobile phase, 0.6 mL/min, 80 °C column temperature). The weight of the dried, filtered solids minus their ash weight was recorded and used to calculate lignin content. Ash content was determined by heating samples in a 575 °C furnace until completion. The extractives were determined by extracting the biomass with 95% ethanol for 24 h in a Soxhlet apparatus. The measured compositions for both the raw and pretreated materials were used in the enzymatic hydrolysis loading calculations.

Sugar Analysis in the Pretreatment Liquor

Prior to neutralizing the lime-treated biomass slurry, a 10-mL aliquot of pretreatment liquor was obtained using vacuum filtration. The monomeric sugar content of the pretreatment liquor was quantified using HPLC analysis (Bio-Rad Aminex HPX-87P column, HPLC-grade water mobile phase, 0.6 mL/min, 80 °C column temperature).

The oligomeric sugar content of the pretreatment liquor was quantified by subjecting the pretreatment liquor to acid hydrolysis with 4% sulfuric acid using an autoclave at 121 °C for 1 h. HPLC analysis was used to measure the glucose and xylose concentrations of each sample, which were then recalculated as equivalent glucan and xylan concentrations.

Enzymatic Hydrolysis

The enzymatic hydrolysis procedure for both glucan and xylan closely followed the enzymatic saccharification procedure provided by NREL [28]. Hydrolysis samples were prepared in 50-mL plastic centrifuge tubes. Pretreated biomass loading weight was calculated based on moisture content and glucan composition to yield 0.1 g glucan per sample. Sodium citrate buffer (5 mL, 0.1 M, pH 4.8), 0.04 mL tetracycline (10 mg/mL in 70% ethanol), 0.04 mL cycloheximine (10 mg/mL in distilled water), 1 mL of each enzyme dilution (cellulase, β-glucosidase), and an appropriate volume of water were added to bring the total working volume to 10 mL. The enzyme dilutions were calculated on a raw glucan basis using the enzyme activity and a desired enzyme loading. The cellulase enzyme loading was 15 FPU/g raw glucan, and β-glucosidase was loaded in excess at a loading of 60 CBU/g raw glucan. Hydrolysis occurred in a shaking incubator (100 rpm) at 50 °C for 72 h. To quench the hydrolysis, the samples were placed in a 105 °C oven for 5 min and then cooled in an ice bath. Samples were stored in a freezer until HPLC analysis. HPLC analysis (Bio-Rad Aminex HPX-87P column, HPLC-grade water mobile phase, 0.6 mL/min, 80 °C column temperature) was used to measure the glucose and xylose concentrations of each sample. These concentrations were then recalculated as equivalent glucan and xylan concentrations to report digestibility yields.

Experimental Design

The goal of this work was to determine the set of pretreatment conditions (reaction time, lime loading, temperature, O_2 pressure) that resulted in the most digestible switchgrass (Fig. 1). Table 1 shows the full list of conditions. The very short-term reactions involved a full-factorial experimental design of five temperatures (150, 160, 170, 190, and 200 °C), six reaction times (5, 10, 15, 20, 25, and 30 min), and two O_2 pressures (3.45 and 6.89 bar absolute O_2). The short-term reactions involved five temperatures (100, 110, 120, 140, and 150 °C), four reaction times (60, 120, 180, and 240 min), and two O_2 pressures (3.45 and 6.89 bar absolute O_2). The long-term reactions were conducted at 65 °C and 1.01 bar pressure for 1, 2, 7, 14, and 28 days. Lime consumption, pretreatment yields, and overall enzymatic yields were measured. Overall enzymatic yields were obtained using a 72-h enzymatic hydrolysis with a cellulase loading of 15 FPU/g glucan in raw biomass and an excess β-glucosidase loading of 60 CBU/g glucan in raw biomass.

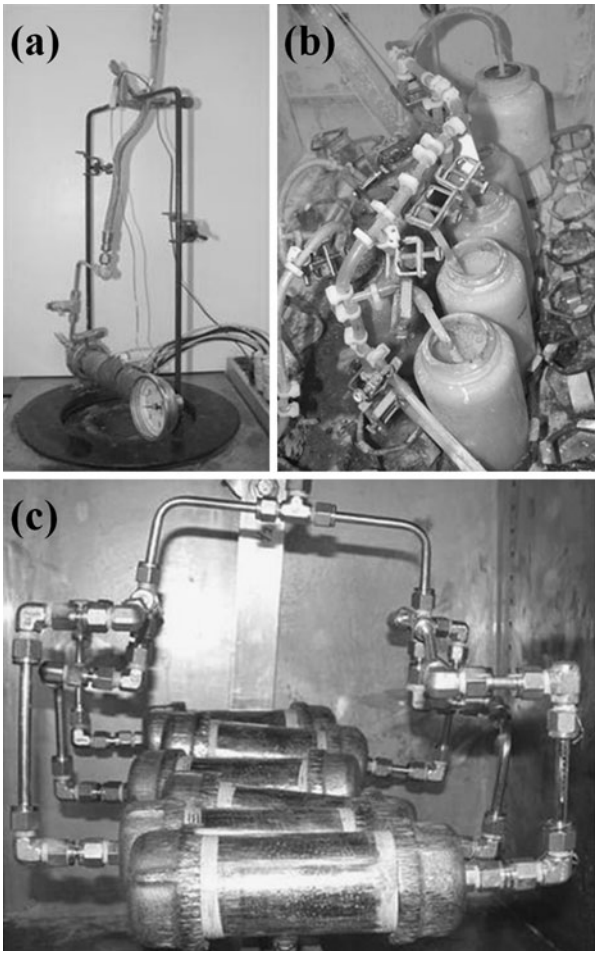

Fig. 1 Pretreatment reactors. **a** Very short-term pretreatment reactor. **b** Long-term pretreatment reactor. **c** Short-term pretreatment reactor

Results and Discussion

Lime Consumption

Figure 2 shows lignin removal as a function of lime consumed for both the short-term and very short-term pretreatment conditions. Lime consumption ranged from 0.07 to 0.42 g lime consumed/g raw biomass (Table 2). At the recommended pretreatment condition

Table 1 List of pretreatment conditions

	Time	Pressure	Temperature
Very short term	5, 10, 15, 20, 25, 30 min	3.45, 6.89 bar O_2	150, 160, 170, 180, 200 °C
Short term	60, 120, 180, 240 min	3.45, 6.89 bar O_2	100, 110, 120, 140, 150 °C
Long term	1, 2, 7, 14, 28 days	1.01 bar, bubbling air	65 °C

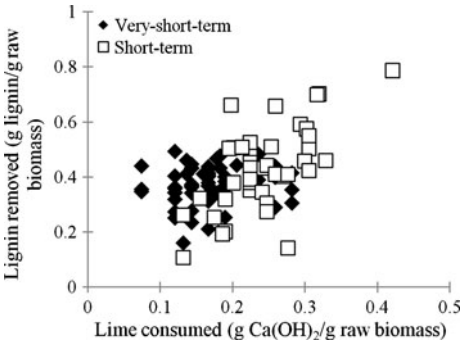

Fig. 2 Correlation of lignin removal with lime consumption

(120 °C, 6.89 bar O_2, 240 min), lime consumption was 0.30 g lime consumed/g raw biomass.

Sugars Recovered from Pretreatment Liquor

Analysis of the pretreatment liquor revealed the absence of monomeric glucose and xylose; however, small concentrations of glucan oligomers and more substantial concentrations of xylan oligomers were present. Table 3 shows the amount of glucan and xylan recovered in the pretreatment liquor for several representative pretreatment conditions. From the four conditions examined, the highest glucan recovery (g glucan recovered/100 g glucan in raw biomass) was 9.75 (200 °C, 6.89 bar O_2, 5 min). Xylan recovery (g xylan recovered/100 g xylan in raw biomass) was significant in three of the four samples: 19.58 (200 °C, 6.89 bar O_2, 5 min), 21.76 (110 °C, 6.89 bar O_2, 240 min), and 25.20 (120 °C, 6.89 bar O_2, 240 min). The high amounts of xylan recovered in the pretreatment liquor compensate for the lower xylan yields shown in the pretreatment yields.

Table 2 Lime consumption (g $Ca(OH)_2$/g raw biomass) of very short and short-term lime pretreatments

	3.45 bar O_2					6.89 bar O_2				
Very short term	150 °C	160 °C	170 °C	180 °C	200 °C	150 °C	160 °C	170 °C	180 °C	200 °C
5 min	0.13	0.19	0.17	0.28	0.14	0.21	0.07	0.18	0.14	0.14
10 min	0.17	0.17	0.12	0.17	0.12	0.19	0.14	0.17	0.16	0.17
15 min	0.12	0.14	0.12	0.19	0.07	0.26	0.17	0.17	0.18	0.19
20 min	0.12	0.12	0.17	0.17	0.24	0.14	0.14	0.17	0.14	0.18
25 min	0.12	0.14	0.19	0.19	0.28	0.14	0.28	0.17	0.18	0.26
30 min	0.12	0.07	0.26	0.17	0.17	0.12	0.19	0.14	0.17	0.24
Short term	100 °C	110 °C	120 °C	140 °C	150 °C	100 °C	110 °C	120 °C	140 °C	150 °C
60 min	0.28	0.19	0.19	0.27	0.22	0.13	0.25	0.25	0.19	0.28
120 min	0.20	0.13	0.22	0.22	0.30	0.17	0.31	0.26	0.31	0.26
180 min	0.24	0.15	0.20	0.22	0.33	0.24	0.22	0.21	0.32	0.20
240 min	0.22	0.31	0.22	0.20	0.25	0.25	0.29	0.30	0.42	0.32

Table 3 Sugars recovered from pretreatment liquor

Pretreatment conditions			Sugars recovered[a]	
Temperature (°C)	Pressure (bar O_2)	Time (min)	Glucan	Xylan
110	6.89	60	2.24	4.24
110	6.89	240	3.42	21.76
120	6.89	240	1.80	25.20
200	6.89	5	9.75	19.58

[a] Grams of component recovered/100 g component in raw biomass

Pretreatment Yields

When comparing the effectiveness of each pretreatment condition, it is important to consider degradation of three main components present in the biomass (glucan, xylan, and lignin). Pretreatment yields of the solid material were calculated using the following definition:

$$Y_i = \frac{C_i Y_t}{C_{i0}}$$

where

- i component (lignin L, glucan G, xylan X)
- Y_i pretreatment yield of component i at time t (g residual component i/g component i in raw biomass)
- C_{i0} Component i content at time zero (g component i in raw biomass/g raw biomass)
- C_i Component i in time t (g residual component i/g residual biomass)
- Y_t total solids pretreatment yield at time t (g residual biomass/g raw biomass)

The primary goal of lime pretreatment is to achieve low lignin pretreatment yields (i.e., high lignin removal) while maintaining high glucan and xylan pretreatment yields.

Very Short Term

The very short-term pretreatments (Fig. 3) resulted in very low glucan degradation. The glucan pretreatment yields (g glucan recovered/100 g glucan in raw biomass) were typically greater than 80. For the 3.45-bar O_2 samples, the highest glucan pretreatment yields were 99.2 (5 min, 200 °C), 99.1 (5 min, 150 °C), and 98.0 (5 min, 180 °C). The lowest glucan pretreatment yields were 78.5 (30 min, 160 °C) and 79.9 (25 min, 160 °C), with the remaining glucan pretreatment yields greater than 80. Increased pressure (6.89 bar O_2) lowered glucan pretreatment yields with the maximum glucan pretreatment yields being 99.7 (5 min, 180 °C) and 96 (10 min, 180 °C). The remaining glucan pretreatment yields ranged from 80 to 95.

Xylan pretreatment yields (g xylan recovered/100 g xylan in raw biomass) showed significantly more degradation than glucan. For the low-pressure case (3.45 bar O_2), xylan pretreatment yields were as high as 77.6 (5 min, 150 °C) and as low as 49.2 (30 min, 200 °C). The majority of the samples showed a xylan pretreatment yield between 65 and 75. The highest xylan yields at 6.89 bar O_2 were 73.8 (10 min, 160 °C), 72.7 (15 min, 150 °C and 5 min, 160 °C), and 72.6 (5 min, 150 °C). Xylan pretreatment yields were as low as 52.1 (30 min, 200 °C) and 52.7 (5 min, 170 °C); however, the majority were 60–70.

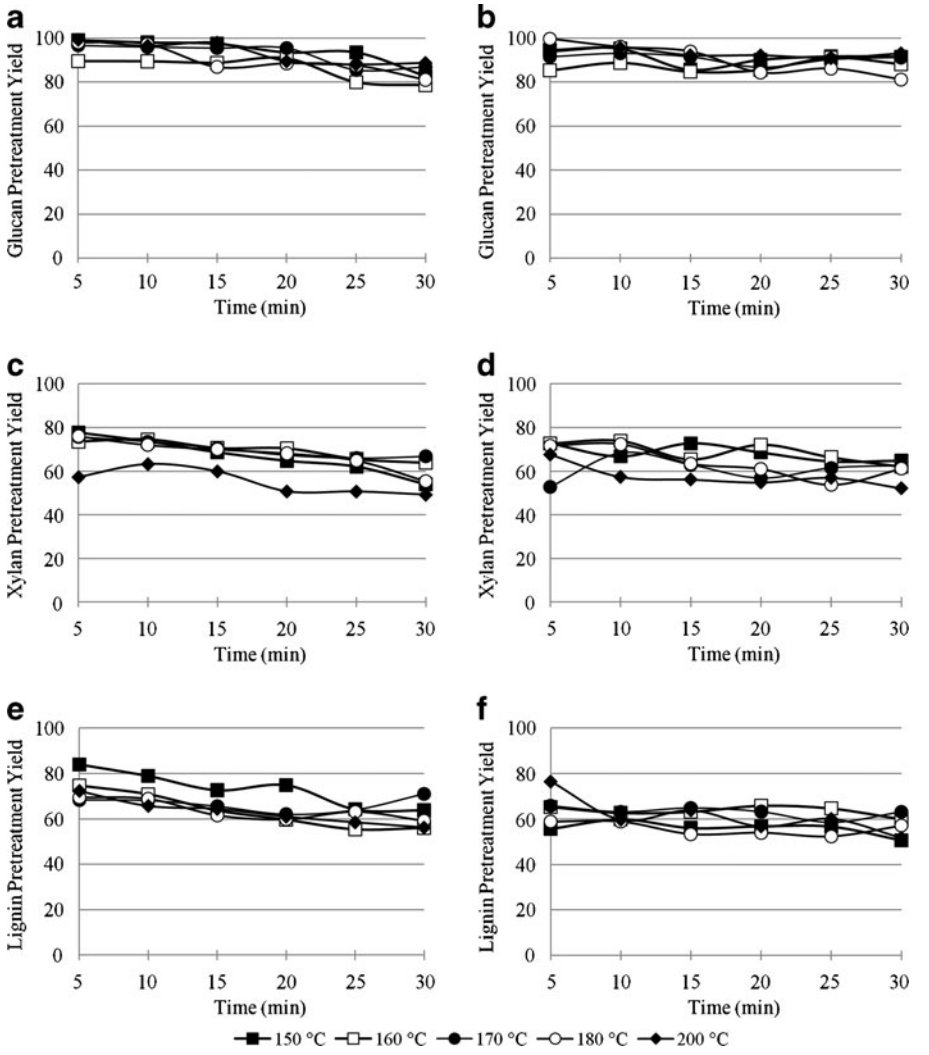

Fig. 3 Very short-term pretreatment yields. **a** Glucan, 3.45 bar O_2; **b** glucan, 6.89 bar O_2; **c** xylan, 3.45 bar O_2; **d** xylan, 6.89 bar O_2; **e** lignin, 3.45 bar O_2; **f** lignin, 6.89 bar O_2 [Note: all pretreatment yields are expressed as g component recovered/100 g component in raw biomass.]

Lignin pretreatment yields (g lignin recovered/100 g lignin in raw biomass) of the 3.45-bar O_2 samples were inconsistent. Although the goal of lime pretreatment is to significantly reduce lignin content, in many cases xylan degradation was more significant than lignin degradation. Lignin pretreatment yields ranged from 84.0 (5 min, 150 °C) to 55.4 (25 min, 160 °C). However, the 6.89-bar O_2 samples consistently showed lower lignin pretreatment yields than either xylan or glucan pretreatment yields. Lignin pretreatment yields were observed as low as 50.7 (30 min, 150 °C) and 51.8 (30 min, 200 °C).

For the very short-term pretreatments, the average glucan pretreatment yields were 91.2 (3.45 bar O_2) and 90.5 (6.89 bar O_2). Xylan showed a little more degradation with average pretreatment yields of 65.9 (3.45 bar O_2) and 63.9 (6.89 bar O_2). Average lignin

pretreatment yields were 66.0 (3.45 bar O_2) and 60.0 (6.89 bar O_2), which is similar to xylan.

Short Term

Overall, the short-term pretreatments were more successful in selectively degrading lignin while maintaining high glucan and moderate xylan pretreatment yields (Fig. 4). Glucan pretreatment yields were typically greater than 80, with certain conditions maintaining glucan pretreatment yields of almost 100. For the 3.45-bar O_2 case, glucan pretreatment yields were 98.4 (60 min, 120 °C and 60 min, 140 °C) and 98.2 (60 min, 100 °C). With

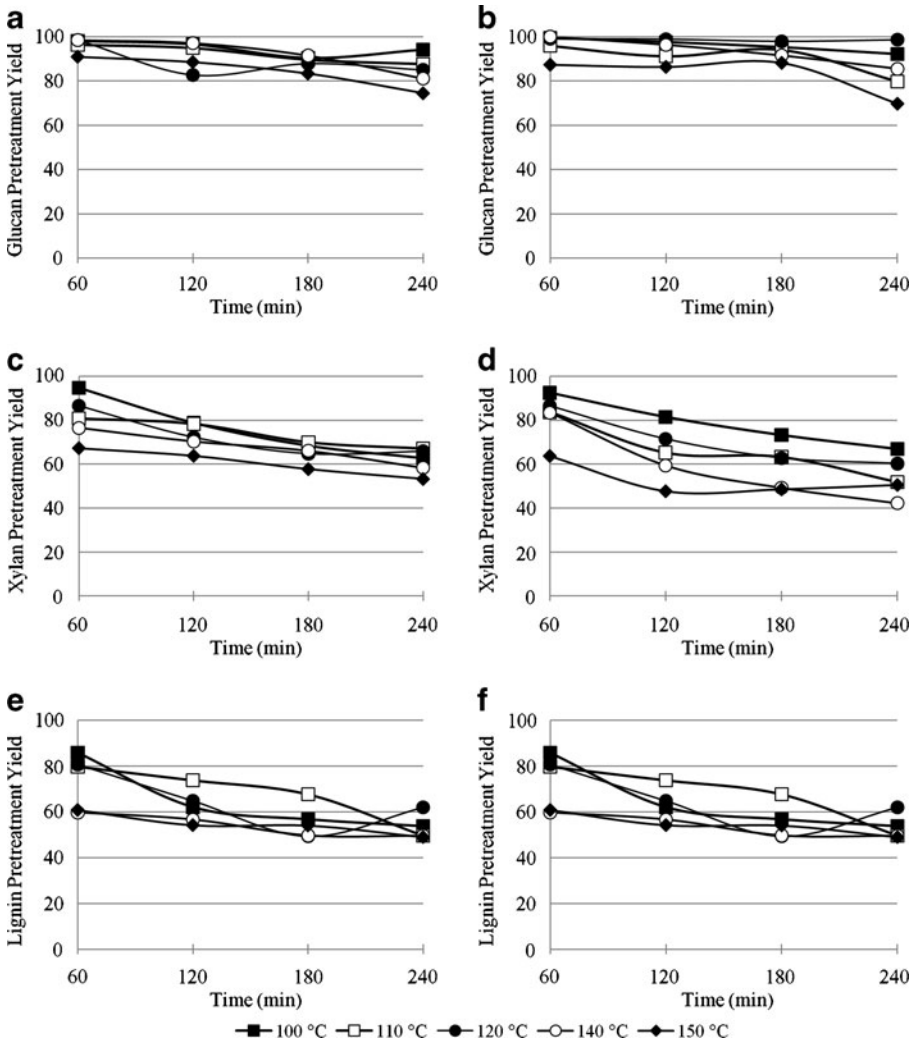

Fig. 4 Short-term pretreatment yields. **a** Glucan, 3.45 bar O_2; **b** glucan, 6.89 bar O_2; **c** xylan, 3.45 bar O_2; **d** xylan, 6.89 bar O_2; **e** lignin, 3.45 bar O_2; **f** lignin, 6.89 bar O_2 [Note: all pretreatment yields are expressed as grams of component recovered/100 g component in raw biomass.]

increased reaction time, glucan pretreatment yields fell as low as 74.5 (240 min, 150 °C) and 81.1 (240 min, 140 °C). At 6.89 bar O_2, almost all of the glucan (>99) was conserved for the 60-min samples at 100, 120, and 140 °C. Again, with increased reaction time, glucan recovery decreased with pretreatment yields as low as 69.8 (240 min, 150 °C) and 79.8 (240 min, 110 °C).

At 3.45 bar O_2, the maximum xylan pretreatment yields were 94.8 (60 min, 100 °C) and 86.6 (60 min, 120 °C). The 150 °C samples showed the lowest xylan pretreatment yields of 57.8 (180 min) and 53.3 (240 min). Compared to the 3.45-bar O_2 samples, the 6.89-bar O_2 samples showed slightly more xylan degradation. The highest xylan pretreatment yields observed were 92.5 (60 min, 100 °C) and 86.7 (60 min, 120 °C), with the lowest being 42.3 (240 min, 140 °C), 47.7 (120 min, 150 °C), and 48.6 (180 min, 150 °C).

The short-term lime pretreatments showed significantly greater lignin degradation than either glucan or xylan degradation. At the lower pressure (3.45 bar O_2), lignin pretreatment yields ranged from 85.8 (60 min, 100 °C) to as low as 49.0 (240 min, 150 °C), with the majority in the range of 50–70. Increasing the pressure to 6.89 bar O_2 strongly improved the degree of lignin degradation. Lignin pretreatment yields were 21.3 (240 min, 140 °C), 29.8 (180 min, 140 °C), 30.1 (240 min, 150 °C), and 33.9 (180 min, 150 °C). Only a single sample (60 min, 100 °C) showed very slight lignin degradation with a lignin pretreatment yield of 89.2.

For the short-term pretreatments, glucan pretreatment yields decreased with increased severity of conditions. Glucan was typically conserved with average pretreatment yields of 90.4 (3.45 bar O_2) and 92.2 (6.45 bar O_2). Xylan degradation was slightly more severe with average pretreatment yields of 70.2 (3.45 bar O_2) and 65.2 (6.45 bar O_2). Lignin degradation was the most severe with average lignin pretreatment yields of 61.0 (3.45 bar O_2) and 52.3 (6.45 bar O_2). From these averages, it is clear that increasing oxygen pressure significantly improves lignin degradation, with the negative side effect of also removing additional xylan. The data also demonstrate that increasing the severity of conditions (increasing temperature or time) helps improve lignin degradation with only a slight increase in glucan degradation.

Long Term

The long-term pretreatment samples all maintained high glucan pretreatment yields (>95). Xylan pretreatment yields were lower and decreased with time. The 1-day pretreatment had a xylan pretreatment yield of 84.5, which decreased to 66.1 for the 28-day pretreatment. Lignin degradation was promising with lignin pretreatment yields starting at 72.9 (1 and 2 days), decreasing to 58.0 after 7 days, and reaching a minimum of 55.0 after 28 days. Table 4 shows the complete set of results.

Enzymatic Yields

The primary goal of this study was to determine the set of pretreatment conditions (reaction time, lime loading, temperature, and pressure) that resulted in the most digestible switchgrass. This study used a 72-h enzymatic hydrolysis with a cellulase loading of 15 FPU/g glucan in raw biomass and an excess loading of β-glucosidase (60 CBU/g glucan in raw biomass). The primary factor in choosing the best-performing pretreatment condition was overall yield of glucan and xylan. Overall yield (Y_{oi}) is defined as the amount of glucan or xylan enzymatically hydrolyzed after pretreatment per unit of glucan or xylan in the raw feedstock.

Table 4 Long-term pretreatment yields

Reaction time (days)	Pretreatment yields[a] 65°, air		
	Glucan	Xylan	Lignin
1	99.9	84.5	72.9
2	97.3	86.8	72.9
7	95.9	72.6	58.0
14	97.0	72.1	56.8
28	96.3	66.1	55.0

[a] Grams of component recovered/100 g component in raw biomass

$$Y_{oi} = Y_i \times Y_{ei}$$

where

i component (glucan G or xylan X)
Y_{oi} overall yield of component i (g hydrolyzed component i/g component i in raw biomass)
Y_i pretreatment yield of component i (g residual component i/g component i in raw biomass)
Y_{ei} enzymatic yield of component i (g hydrolyzed component i/g component i in pretreated biomass)

Very Short Term

Overall, the very short-term pretreatments did not effectively increase glucan overall yield. Results (Fig. 5) were inconsistent making it difficult to derive any meaningful conclusions from the data.

At 3.45 bar O_2, overall glucan yields (g glucan hydrolyzed/100 g glucan in raw biomass) ranged from 26.9 to 45.0. In general, the most successful temperature was 160 °C, with overall glucan yields of 38.4, 44.8, 44.5, 41.0, and 40.7 for reaction times of 5, 10, 15, 20, 25, and 30 min, respectively. It is apparent that although lignin degradation increases with reaction time, overall pretreatment yield decreases; therefore, there is a delicate balance between reaction time and overall glucan yield. Overall xylan yields (g xylan hydrolyzed/100 g xylan in raw biomass) were also inconsistent, and were between 22.3 (30 min, 200 °C) and 47.4 (25 min, 150 °C). In most cases, overall xylan yield had similar trends as overall glucan yield.

In the very short-term reactor, pretreating the switchgrass at 6.89 bar O_2 proved slightly more successful, although still inconsistent. Overall glucan yields of 54.7 (5 min, 200 °C) and 50.2 (10 min, 150 °C) were achieved with the highest overall glucan yields obtained at short reaction times. Some pretreatments were highly unsuccessful, with overall glucan yields as low as 12.7 (5 min, 180 °C) and several others below 25 (15 min, 150 °C; 25 min, 150 °C; 30 min, 150 °C; 5 min, 160 °C; 25 min, 180 °C). Although a few high-pressure samples showed improved overall yields compared to the low-pressure samples, most of the

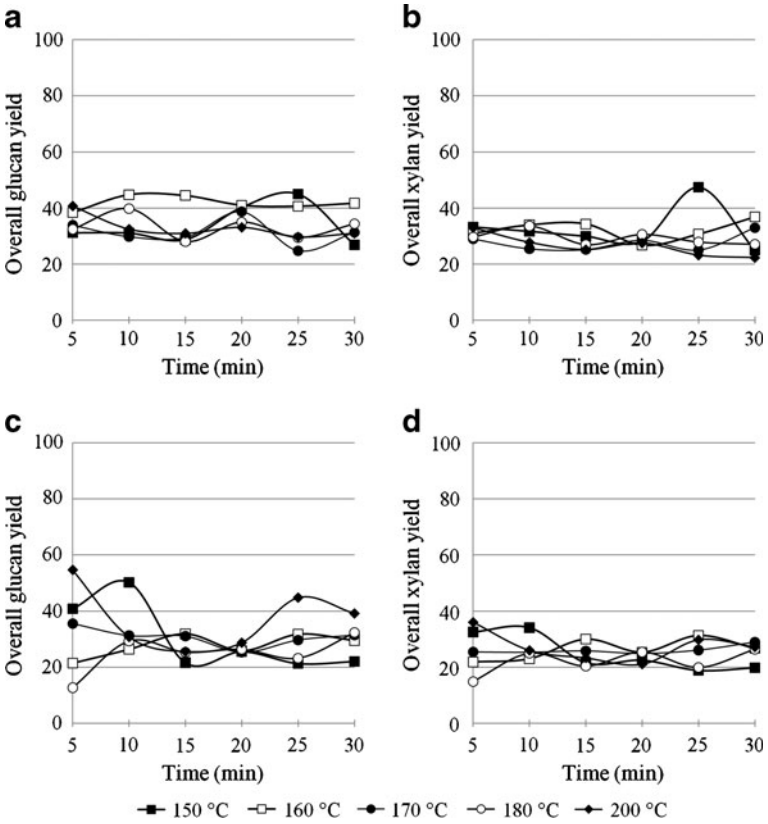

Fig. 5 Overall enzymatic yield results for very short-term pretreatments. Enzymatic hydrolysis was performed for 72 h with a cellulase enzyme loading of 15 FPU/g glucan in raw biomass. **a** Overall glucan yield, 3.45 bar O_2. **b** Overall xylan yield, 3.45 bar O_2. **c** Overall glucan yield, 6.89 bar O_2. **d** Overall xylan yield, 6.89 bar O_2 [Note: all overall enzymatic yields are expressed as grams of component hydrolyzed/100 g raw component.]

high-pressure samples did considerably worse. Overall xylan yields were also quite low, with values ranging from 14.9 (5 min, 180 °C) to 36.0 (5 min, 200 °C).

The average overall glucan yields for the very short-term reactor were 34.7 (3.45 bar O_2) and 30.2 (6.89 bar O_2), clearly demonstrating the ineffectiveness of the very short-term reactor. With the poor performance of the very short-term reactor, it appears that reaction times were too short to obtain a highly digestible substrate.

Short Term

Although the very short-term pretreatment proved unsuccessful at producing highly digestible switchgrass, the short-term pretreatment demonstrated that oxidative lime pretreatment is a promising approach (Fig. 6).

At the lower pressure (3.45 bar O_2), overall glucan yields were moderate and similar to the very short-term pretreatment. A reaction time of 180 min consistently produced the

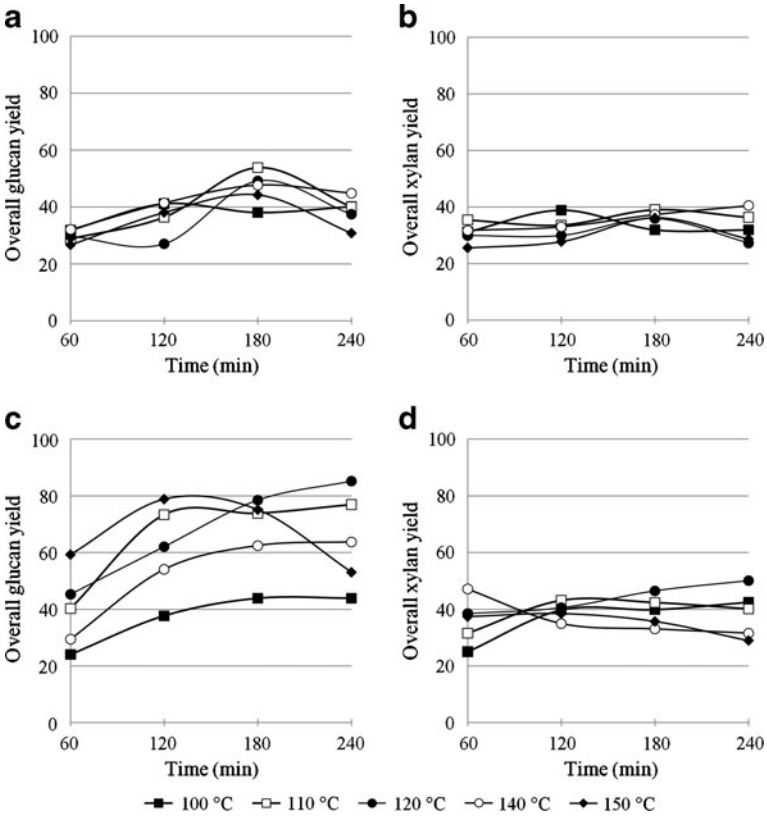

Fig. 6 Overall enzymatic yield results for short-term pretreatments. Enzymatic hydrolysis was performed for 72 h with a cellulase enzyme loading of 15 FPU/g glucan in raw biomass. **a** Overall glucan yield, 3.45 bar O_2. **b** Overall xylan yield, 3.45 bar O_2. **c** Overall glucan yield, 6.89 bar O_2. **d** Overall xylan yield, 6.89 bar O_2 [Note: all overall enzymatic yields are expressed as grams of component hydrolyzed/100 g raw component.]

highest overall glucan yields of 53.9 (100 °C), 49.2 (120 °C), 47.7 (140 °C), and 44.3 (150 °C). Additionally, overall glucan yields improved with time up to 180 min. In all cases except for 100 °C, a reaction time of 240 min led to low pretreatment yields, which negatively affected overall glucan yields. Overall xylan yields were relatively low as well, with a maximum yield of 40.5 (240 min, 140 °C) and a minimum yield of 25.5 (60 min, 150 °C). The majority of the overall xylan yields were 27–37.

In this short-term study, the most promising results occurred at 6.89 bar O_2. At 100 °C, overall glucan yields were low. The 60-min sample had an overall glucan yield of 24.0, which improved to 43.9 for the 180- and 240-min samples. Increasing the temperature to 110 °C resulted in overall glucan yields of 43.9 (60 min) to 73.9 (240 min). The most successful temperature of the study was 120 °C, with overall glucan yields of 45.2 (60 min), 62.1 (120 min), 78.5 (180 min), and 85.2 (240 min). The overall glucan yield of 85.2 (6.89 bar O_2, 120 °C, 240 min) was the highest yield observed in this

Table 5 Long-term enzymatic overall yields

Reaction time (days)	Enzymatic overall yield[a] 15 FPU/g raw glucan	
	Glucan	Xylan
1	30.1	29.0
2	38.7	30.2
7	53.5	31.6
14	63.9	44.4
28	54.5	37.1

72-h hydrolysis

[a] Grams of component digested/100 g raw component

study; therefore, this set of conditions was chosen as the recommended oxidative lime pretreatment condition for switchgrass. Increased temperatures (140 °C and 150 °C) had low pretreatment yields, which decreased overall glucan yields. For these temperatures, overall glucan yields were 29.5 (60 min, 140 °C) to 78.8 (120 min, 150 °C). Overall xylan yields also improved at the higher pressure, with several samples having overall xylan yields greater than 40. The recommended pretreatment condition (6.89 bar O_2, 120 °C, 240 min) had an overall xylan yield of 50.1, which was also the highest observed.

Particularly at the higher pressure, the average overall glucan yields of the short-term reactor were clearly better than the very short-term reactor (38.0, 3.45 bar O_2 and 58.0, 6.89 bar O_2). Average overall xylan yields of the short-term reactor (38.3, 6.89 bar O_2) also showed significant improvement over the very short-term reactor (25.3, 6.89 bar O_2).

Long Term

The long-term lime pretreatment had similar trends as the shorter pretreatments (Table 5). With increased time, overall glucan yield increased from 30.1 (1 day) to 63.9 (14 days). At 28 days, overall glucan yield decreased to 54.5, showing the importance of maintaining a high pretreatment yield. Overall xylan yields showed the same trend, increasing from 29.0 (1 day) to 44.4 (14 days), before decreasing to 37.1 (28 days).

Conclusions

For Dacotah switchgrass, the recommended oxidative lime pretreatment conditions are 120 °C, 6.89 bar O_2, and 240 min. At these conditions, lime consumption was 0.30 g Ca $(OH)_2$/g raw biomass, overall glucan yield was 85.2 g glucan digested/100 g glucan in raw biomass, and overall xylan yield was 50.1 g xylan digested/100 g xylan in raw biomass. Also, significant xylan oligomers (25.20 g xylan recovered/100 g xylan in raw biomass) were recovered in the pretreatment liquor. In general, the short-term reactions performed at 6.89 bar O_2 were the only successful results. The long-term reactor achieved moderate results, whereas the very short-term reactor was not productive.

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