

# Oxidative Lime Pretreatment of High-Lignin Biomass

*Poplar Wood and Newspaper*

VINCENT S. CHANG, MURLIDHAR NAGWANI,  
CHUL-HO KIM, AND MARK T. HOLTZAPPLE\*

*Department of Chemical Engineering, Texas A&M University,  
College Station, TX 77843-3122, E-mail: m-holtzapple@tamu.edu*

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## Abstract

Lime ( $\text{Ca}[\text{OH}]_2$ ) and oxygen ( $\text{O}_2$ ) were used to enhance the enzymatic digestibility of two kinds of high-lignin biomass: poplar wood and newspaper. The recommended pretreatment conditions for poplar wood are  $150^\circ\text{C}$ , 6 h, 0.1 g of  $\text{Ca}(\text{OH})_2/\text{g}$  of dry biomass, 9 mL of water/g of dry biomass, 14.0 bar absolute oxygen, and a particle size of  $-10$  mesh. Under these conditions, the 3-d reducing sugar yield of poplar wood using a cellulase loading of 5 filter paper units (FPU)/g of raw dry biomass increased from 62 to 565 mg of eq. glucose/g of raw dry biomass, and the 3-d total sugar (glucose + xylose) conversion increased from 6 to 77% of raw total sugars. At high cellulase loadings (e.g., 75 FPU/g of raw dry biomass), the 3-d total sugar conversion reached 97%. In a trial run with newspaper, using conditions of  $140^\circ\text{C}$ , 3 h, 0.3 g of  $\text{Ca}(\text{OH})_2/\text{g}$  of dry biomass, 16 mL of water/g of dry biomass, and 7.1 bar absolute oxygen, the 3-d reducing sugar yield using a cellulase loading of 5 FPU/g of raw dry biomass increased from 240 to 565 mg of eq. glucose/g of raw dry biomass. A material balance study on poplar wood shows that oxidative lime pretreatment solubilized 38% of total biomass, including 78% of lignin and 49% of xylan; no glucan was removed. Ash increased because calcium was incorporated into biomass during the pretreatment. After oxidative lime pretreatment, about 21% of added lime could be recovered by  $\text{CO}_2$  carbonation.

**Index Entries:** High-lignin biomass; pretreatment; lime; oxidative; oxygen; enzymatic digestibility; cellulase; sugar; material balance; delignification.

\*Author to whom all correspondence and reprint requests should be addressed.

## Introduction

The Intergovernmental Panel on Climate Change estimates that global surface temperatures have increased by 0.3–0.6°C since the late nineteenth century (1). Thomson (2) suggests that the increase in global temperature strongly correlates with atmospheric CO<sub>2</sub> concentration, which has increased 24% (from 290 to 360 ppm) since 1860 (1). Mitchell et al. (3) predict that average temperature will increase 1.5–4.5°C if atmospheric CO<sub>2</sub> concentration doubles. This rapid increase in atmospheric CO<sub>2</sub> results mainly from fossil fuel combustion, which currently generates about  $2.2 \times 10^{10}$  t of CO<sub>2</sub> annually (1 t = 1000 kg) (4). Therefore, to reduce net CO<sub>2</sub> emissions and global warming, fossil fuel combustion must decrease and the use of alternative, environmentally friendly energy must increase.

Lignocellulosic biomass has been considered as an alternative energy source because it captures CO<sub>2</sub> during growth, and, thus, its combustion does not release net CO<sub>2</sub> (4). It can be converted to liquid fuels, such as alcohols, using appropriate processes (5). However, the digestibility of lignocellulosic biomass is low owing to structural features such as lignin, acetyl groups, and crystallinity (6). Hence, lignocellulosic biomass must be pretreated to enhance its digestibility before conversion to liquid fuels.

The effects of pretreatments depend on process conditions and structural features of biomass. Lignin content and crystallinity have major effects on biomass enzymatic digestibility whereas acetyl content has a minor impact (6). Pretreatments that can significantly remove lignin or reduce crystallinity are particularly effective. Compared with low-lignin biomass, high-lignin biomass has lower digestibility and is less responsive to pretreatments. Tables 1 and 2 summarize the pretreatment conditions and effects for two kinds of high-lignin biomass—poplar wood and newspaper, respectively. Ball milling increases biomass digestibility by decrystallization (7–10,32,34), but it requires high-energy input and is not economically feasible (39). Peracetic acid and organosolv increase biomass digestibility by delignification (14,28,29,37), but they require expensive pretreatment agents and are not considered practical (39).

By comparison, alkaline pretreatments are less expensive and only consume low-to-moderate energy. Sodium hydroxide (NaOH), ammonia (NH<sub>3</sub>), and lime (Ca[OH]<sub>2</sub>) are the most commonly used alkalis (9,10,17–23,36,37). Table 3 compares these alkalis and shows that lime is a good pretreatment agent because it is very inexpensive (\$0.06/kg) (40), is safe, and can be recovered by carbonating wash water with CO<sub>2</sub> (41). Unfortunately, because lime is a weak base and has a low solubility, it appears to be less effective than other alkalis (42). However, under appropriate pretreatment conditions, lime substantially enhances the digestibility of moderate-lignin biomass (e.g., switchgrass, bagasse, and wheat straw) by removing 30–43% of lignin and all acetyl groups (6,41,43). For high-lignin biomass, lime alone does not remove enough lignin to significantly enhance the digestibility; an oxidant must be added.

The present study explores nonoxidative (lime alone) and oxidative (lime + oxygen) lime pretreatments using two common high-lignin lignocelluloses: poplar wood and newspaper. In addition, enzyme loading studies, enzymatic hydrolysis profiles, material balances, and lime recovery were performed on poplar wood.

## Materials and Methods

### *Substrates*

Hybrid poplar was provided by the National Renewable Energy Laboratory (NREL) (Golden, CO). Newspaper was the local newspaper *Eagle* (Bryan/College Station, TX). Both materials were ground using a Thomas-Wiley laboratory mill (Arthur H. Thomas Company, Philadelphia, PA) and then passed through a 40-mesh screen.

### *Lime Pretreatment*

#### Nonoxidative Lime Pretreatment

Poplar wood and newspaper were pretreated with lime in the presence of water under various conditions (temperature, time, lime loading, water loading, and biomass particle size). The detailed procedure was described previously (43). In the nonoxidative lime pretreatment, there was a minor amount of oxygen pressure owing to the presence of air in the reactor. At room temperature, the oxygen partial pressure was 0.21 bar absolute ( $=1.0 \text{ bar absolute} \times 21\%$ ).

#### Oxidative Lime Pretreatment

Owing to high-lignin contents (i.e., 25–30%) (6,44), poplar wood and newspaper are more recalcitrant than herbaceous biomass; therefore, oxidative lime pretreatment was used to facilitate further delignification. The oxygen pressure in the reactor was measured at room temperature prior to pretreatment. The oxygen partial pressure includes both the added oxygen and the oxygen from the air in the reactor. The detailed procedure of oxidative lime pretreatment is described by Chang (45).

### *Enzymatic Hydrolysis*

Pretreated and untreated biomass were enzymatically hydrolyzed at 50°C for 3 d in a 100-rpm air-bath shaker. Acetic acid was used to adjust the pH to 4.8 and citrate buffer (0.05 M) was used to maintain the pH throughout hydrolysis. Sodium azide (0.03%) was added to prevent microbial growth. The biomass concentration was 0.05 g/mL.

Cellulase activity was measured using a standard assay (46). The standard cellulase loading was 5 filter paper units (FPU)/g of raw dry biomass except in the enzyme loading studies, in which the cellulase loading varied. The cellobiase loading was 28.4 cellobiase units (CBU)/g of raw dry biomass throughout this study. Two batches of cellulase were used. Cytolase

Table 1  
Pretreatment Conditions and Digestions for Poplar Wood

Pretreatment	Temperature (°C)	Time	Pretreatment agent loading (g/g dry biomass) or other conditions	Untreated digestibility (%)	Treated digestibility (%)	Reference
Physical						
Mechanical						
Ball milling (vibratory)	2	5 h	—	5 <sup>b</sup>	78 <sup>b</sup>	7,8
Ball milling (vibratory)	4	2 h	—	37 <sup>c</sup>	78 <sup>c</sup>	9
Ball milling (vibratory)	—	30 min	—	11 <sup>b</sup>	56 <sup>b</sup>	10
Nonmechanical						
High-energy irradiation	—	—	Radiation = 10 <sup>8</sup> rep. <sup>a</sup>	55 <sup>c</sup>	78 <sup>c</sup>	9
Steam	165	2 h	—	23.4 <sup>c</sup>	56.6 <sup>c</sup>	11
Steam explosion	—	5 min	Pressure = 31 bar	15.2 <sup>b</sup>	98.5 <sup>b</sup>	12
Steam	190	25 min	—	—	75 <sup>b</sup>	13
Steam explosion	240	80 s	—	—	75 <sup>b</sup>	13
Hydrothermolysis	210	30 min	100	8 <sup>b</sup>	36 <sup>b</sup>	14
Steam explosion	—	5 min	Pressure = 31 bar	18 <sup>b</sup>	99 <sup>b</sup>	15
Liquid hot water	220	2 min	Pressure = 50 bar	10 <sup>d</sup>	92.3 <sup>d</sup>	16
Chemical						
Alkaline						
NaOH	—	—	15	5 <sup>c</sup>	50 <sup>c</sup>	17
NaOH	Ambient	2 h	0.06	35 <sup>e</sup>	55 <sup>e</sup>	18
NH <sub>3</sub> (gaseous)	30	8.5 h	Pressure = 10.7 bar	33 <sup>c</sup>	51 <sup>c</sup>	9
NH <sub>3</sub> (liquid)	30	1 h	—	33 <sup>e</sup>	51 <sup>e</sup>	9
NaOH	121	1.5 h	4%	18 <sup>b</sup>	72 <sup>b</sup>	19
NaOH	Ambient	2 h	0.05	41 <sup>f</sup>	52 <sup>f</sup>	20

NaOH	30	1 h	0.2	11 <sup>b</sup>	51 <sup>b</sup>	10
NH <sub>3</sub> (liquid)	30	1 h	Pressure = 10.3 bar	11 <sup>b</sup>	36 <sup>b</sup>	10
Ca(OH) <sub>2</sub>	Ambient	150 d	0.2	39.2 <sup>e</sup>	52.0 <sup>e</sup>	21
NaOH	Ambient	1 d	0.16	39.2 <sup>e</sup>	49.7 <sup>e</sup>	21
NH <sub>3</sub> (supercritical)	175	20 min	0.3 g/mL (≈16 bar)	18.9 <sup>b</sup>	100 <sup>b</sup>	22
NaOH	55	1 h	0.27	—	10 <sup>b</sup>	23
Acidic						
SO <sub>2</sub>	120	2 h	Pressure = 2 bar	9 <sup>f</sup>	63 <sup>f</sup>	24
Dilute acid (H <sub>2</sub> SO <sub>4</sub> )	200	5.8 s	0.41%	21.4 <sup>b</sup>	74.9 <sup>b</sup>	25
Dilute acid (H <sub>2</sub> SO <sub>4</sub> )	140	1 h	0.5%	14 <sup>b</sup>	80 <sup>b</sup>	26
Dilute acid (H <sub>2</sub> SO <sub>4</sub> )	180–220	7 to 8 s	1%	4 <sup>b</sup>	38 <sup>b</sup>	15
Dilute acid (H <sub>2</sub> SO <sub>4</sub> )	170	10 min	0.73%	—	79 <sup>b</sup>	27
Dilute acid (H <sub>2</sub> SO <sub>4</sub> )	121	1 h	0.05	—	8 <sup>b</sup>	23
Peracetic acid	Ambient	7 d	0.6	6.8 <sup>b</sup>	100 <sup>b</sup>	28
Other						
Organosolv (MeOH)	210	30 min	40	8 <sup>b</sup>	82 <sup>b</sup>	14
Organosolv (MeOH)	165	2.5 h	4	—	75.2 <sup>b</sup>	29
<i>n</i> -Propylamine	0	24 h	10	—	60.7 <sup>b</sup>	30

Multiple						
NH <sub>3</sub> + H <sub>2</sub> O <sub>2</sub>	170	1.5 h	NH <sub>3</sub> = 3; H <sub>2</sub> O <sub>2</sub> = 0.28	—	90 <sup>b</sup>	31
H <sub>2</sub> O <sub>2</sub> + NaOH	50	1 h	NaOH = 0.2; H <sub>2</sub> O <sub>2</sub> = 0.67	—	16 <sup>b</sup>	23
Peroxymonosulfate + NaOH	50 + 55	2 h + 1 h	—	—	96 <sup>b</sup>	23
Acetic acid + H <sub>2</sub> O <sub>2</sub>	110	2 h	Acid = 12.5; H <sub>2</sub> O <sub>2</sub> = 3.6	—	95 <sup>b</sup>	23
NaOH + peracetic acid	Ambient	1 d + 7 d	NaOH = 0.06; acid = 0.15	6.8 <sup>b</sup>	100 <sup>b</sup>	28

<sup>a</sup>rep., roentgen equivalent physical.

<sup>b</sup>Enzymatic digestibility.

<sup>c</sup>In vitro dry matter digestibility.

<sup>d</sup>Ethanol yield.

<sup>e</sup>In vitro digestibility.

<sup>f</sup>In vivo dry matter digestibility.

Table 2  
Pretreatment Conditions and Digestions for Newspaper

Pretreatment	Temperature (°C)	Time	Pretreatment agent loading (g/g dry biomass) or other conditions	Untreated digestibility (%) <sup>a</sup>	Treated digestibility (%) <sup>a</sup>	Referen
Physical						
Ball milling (rotary)	Ambient	7 d	36 g porcelain balls/g dry biomass	37.9	100	32
Two-roll milling	Ambient	8 min	Roll clearance = 0.05 cm	31	76	33
Ball milling (vibratory)	Ambient	2 h	Steel balls	37.1	62.5	34
Chemical						
Acid	220	22 min	0.05	57.7	80.0	35
Organosolv (EtOH)	128	3 h	1.7	27	26	36
Multiple						
AFEX (NH <sub>3</sub> + explosion)	87	3 h	2.5	27	40	36
AFEX (NH <sub>3</sub> + explosion)	65	10 min	2.8	27	40	37
H <sub>2</sub> O <sub>2</sub> + AFEX	23 + 65	6 h + 10 min	H <sub>2</sub> O <sub>2</sub> = 16; NH <sub>3</sub> = 2.8; NaOH = 7.2	27	61	37
Peracetic acid + AFEX	25 + 65	24 h + 10 min	Acid = 0.2; NH <sub>3</sub> = 2.8	27	70	37
H <sub>2</sub> O <sub>2</sub> + NH <sub>3</sub>	80 + 170	1 h + 1.5 h	H <sub>2</sub> O <sub>2</sub> = 0.05; NH <sub>3</sub> = 0.17	40	75	38

<sup>a</sup>Enzymatic digestibility.

Table 3  
Comparison of Three Common Alkali Pretreatment Agents

Alkali	Price (\$/kg) <sup>a</sup>	Operating pressure	Health hazard	Recoverability
NaOH	0.68	Low	Moderate	Hard
NH <sub>3</sub>	0.13	High	High	Easy
Ca(OH) <sub>2</sub>	0.06	Low	Low	Moderate

<sup>a</sup>Ref. 40.

CL enzyme (lot no. 17-92262-09; Environmental BioTechnologies, Santa Rosa, CA) was used in the poplar wood studies and had an activity of 89.7 FPU/mL, whereas Cytolase 300P enzyme (lot no. 14-89340-1; Genecor, Rolling Meadows, IL) was used in the newspaper studies and had an activity of 215 FPU/g of dry powder. The activity of cellobiase (Novozym 188, batch no. DCN00024; Novo Nordisk, Franklinton, NC) was 250 CBU/mL, determined by the company.

To investigate lime pretreatment conditions and enzyme loadings, enzymatic hydrolysis samples (about 4 mL) were withdrawn after 3 d and then boiled for 15 min in sealed tubes to denature the enzymes and thus prevent further hydrolysis. When the hydrolysis profiles were performed, samples were taken as a function of time (i.e., 0, 1, 3, 6, 10, 16, 24, 36, 48, and 72 h).

The sugars of the hydrolysis samples were measured and the sugar yields were used to indicate biomass digestibility. Throughout this article, the sugar yields of raw (untreated) and treated biomass are expressed per unit of raw biomass. Similarly, the sugar conversions are expressed as the percentages of raw carbohydrates converted to soluble sugars.

### Measurement of Sugar

Reducing sugars were measured using the dinitrosalicylic acid assay (47). A 200 mg/dL glucose standard solution (Yellow Springs Instruments) was used for the calibration; thus, the reducing sugars were measured as *equivalent glucose*. The sugar content in the enzymes (0.8 and 9 mg eq. of glucose/FPU for Cytolase CL and Cytolase 300P, respectively) was subtracted from the original reducing sugar yields to determine the actual amounts of reducing sugars produced from the biomass. After subtracting the enzyme sugars, the yields were multiplied by a correction factor to account for calcium acetate inhibition (from the lime neutralization) and were called *corrected* reducing sugar yields. The correction factors depend on lime loadings and were determined previously for both cellulase enzymes. At a standard lime loading of 0.1 g of Ca(OH)<sub>2</sub>/g of dry biomass, the correction factors for Cytolase CL and Cytolase 300P are 1.015 and 1.11, respectively (41,43).

Glucose and xylose were measured using high-performance liquid chromatography as previously described (41,43). Because no other carbo-

hydrates were detected, *total sugar* denotes the summation of glucose and xylose in this article.

### *Material Balances and Biomass Composition*

Untreated and pretreated poplar wood were repeatedly washed with fresh distilled water until the decanted water became colorless. The total dry weight of the sample was measured before and after the pretreatment and wash. The method of dry weight measurements for determination of sugar yield and material balances was described previously (43).

The compositions (ash, lignin, xylan, and glucan) of raw, washed only, and pretreated-and-washed poplar wood were determined using the standard analysis procedures provided by NREL (46). The crude protein and minerals were determined using a modified micro-Kjeldahl method by the Extension Soil, Water and Forage Testing Laboratory of Texas A&M University (College Station, TX).

### *Lime Recovery*

The pretreated poplar wood was mixed with distilled water and stirred for 10 min. The mixture was filtered and the wet biomass was squeezed. The squeezed liquid was combined with the filtrate, and then the mixed liquid was bubbled with CO<sub>2</sub> to precipitate CaCO<sub>3</sub> until the pH reached the minimum (about 5.1). The biomass was repeatedly washed with fresh distilled water 10 times. Ten milliliters of sample was withdrawn, and the calcium content was measured by atomic adsorption before and after carbonation.

## **Results and Discussion**

### *Effects of Pretreatment Conditions*

Our previous studies showed that pretreatment time, temperature, and lime loading have greater impacts on digestibility than water loading and biomass particle size (41,43); therefore, the pretreatment condition studies were conducted to hold the low-impact variables constant while systematically varying the high-impact variables. After the high-impact variables were determined, the low-impact variables were then investigated. Because the addition of oxygen will increase the capital and operating costs, these studies started with the nonoxidative lime pretreatment. Tables 4 and 5 show the ranges of conditions explored for poplar wood and newspaper, respectively.

#### *Poplar Wood*

The effects of pretreatment time (1, 3, 6, 10, 16, and 24 h) and temperature (60–210°C with an increment of 10°C) were systematically investigated and selectively plotted in Fig. 1A. Figure 1A shows that although nonoxidative lime pretreatment significantly enhanced the poplar wood digestibility by as much as a factor of 5.6, the best reducing sugar yield

Table 4  
Lime Pretreatment Conditions Explored for Poplar Wood

Study no.	Time (h)	Temperature (°C)	Lime loading (g Ca[OH] <sub>2</sub> /g dry biomass)	Added oxygen pressure (bar absolute) <sup>a</sup>	Water loading (mL/g dry biomass)	Particle size (mesh)
1	1-24	60-210	0.1	0	9	-40
2	1	200	0-0.3	0	9	-40
3	0.25-1	210-250	0.1	0	9	-40
4	1-24	120-180	0.1	6.9	9	-40
5	3-7.5	150	0-0.3	6.9	9	-40
6	7.5	150	0-0.3	0-13.8	9	-40
7	1-10	150	0.1	0-34.5	9	-40
8	6	150	0.1	13.8	5-15	-40
9	6	150	0.1	13.8	9	10-80

<sup>a</sup>Oxygen pressure was measured at room temperature just prior to pretreatment.

Table 5  
Lime Pretreatment Conditions Explored for Newspaper

Study no.	Time (h)	Temperature (°C)	Lime loading (g Ca[OH] <sub>2</sub> /g dry biomass)	Added oxygen pressure (bar absolute) <sup>a</sup>	Water loading (mL/g dry biomass)	Particle size (mesh)
1	1-24	120	0.3	0	6-16	-40
2	1-24	60	0.05-0.3	0	10	-40
3	24	100	0.1-0.3	0	10-15	-40
4	3	150	0.05-0.3	0	10	-40
5	24	120	0.3	0-6.9	9	-40
6	1-24	140	0.3	6.9	9	-40

<sup>a</sup>Oxygen pressure was measured at room temperature just prior to pretreatment.

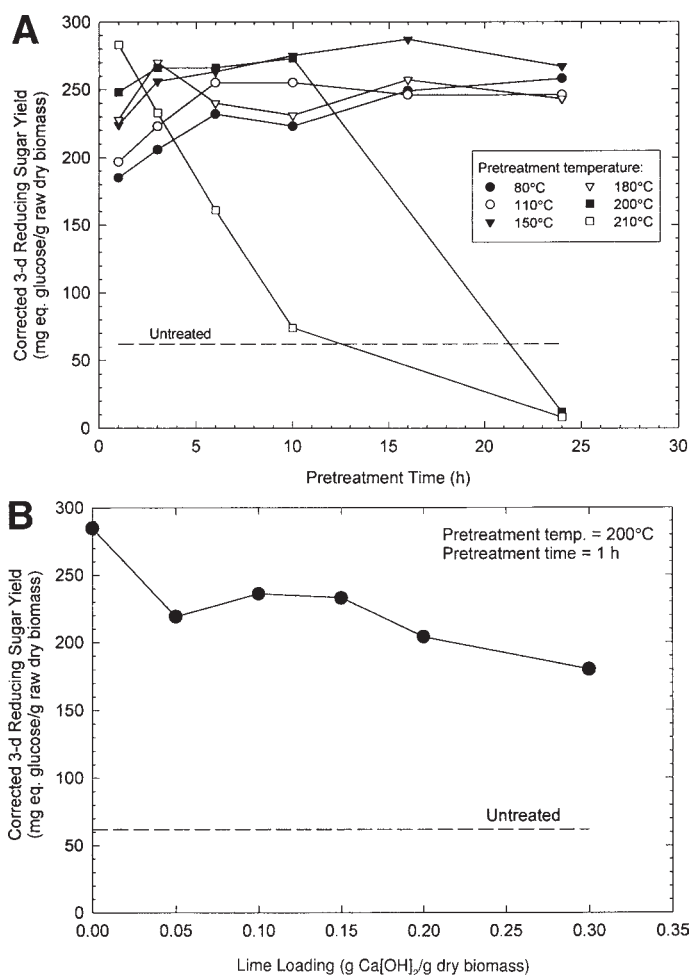


Fig. 1. Effects of nonoxidative lime pretreatment conditions for poplar wood: (A) time and temperature; (B) lime loading. Pretreatment conditions: (A) 0.1 g of  $\text{Ca}(\text{OH})_2$ /g of dry biomass, 9 mL of water/g of dry biomass; (B) 200°C, 1 h, 9 mL of water/g of dry biomass.

(283 mg of eq. glucose/g of raw dry biomass) was substantially lower than those of the lime-treated switchgrass, bagasse, and wheat straw (540, 660, and 650 mg of eq. glucose/g of raw dry biomass, respectively) (41,43).

Figure 1A also shows that at high temperatures (above 180°C), the sugar yields decreased with increasing pretreatment times. Likely this resulted from high-temperature biomass degradation that formed organic acids that neutralized lime (48). At longer pretreatment times, more acids formed and less lime remained; thus, the pretreatment became less effective. This hypothesis is supported by the laboratory observation that the volume of acetic acid needed to adjust the pH to 4.8 decreased at longer pretreatment times and higher temperatures (45). At short pretreatment times (e.g., 1 h), the sugar yields decreased as lime loadings increased

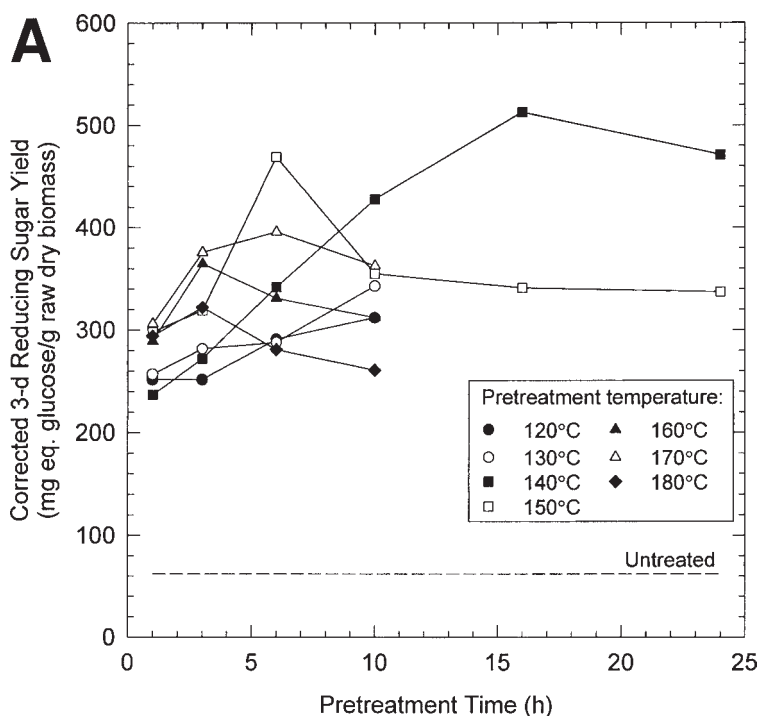


Fig. 2. Effects of oxidative lime pretreatment conditions for poplar wood: (A) time and temperature; (B) oxygen pressure and time; (C) lime loading and oxygen pressure; (D) water loading; (E) biomass particle size. Pretreatment conditions: (A) 0.1 g of  $\text{Ca}(\text{OH})_2$ /g of dry biomass, 9 mL of water/g of dry biomass, oxygen pressure = 7.1 bar absolute; (B) 150°C, 0.1 g of  $\text{Ca}(\text{OH})_2$ /g of dry biomass, 9 mL of water/g of dry biomass; (C) 150°C, 7.5 h, 9 mL of water/g of dry biomass; (D,E) 150°C, 6 h, 0.1 g of  $\text{Ca}(\text{OH})_2$ /g of dry biomass, 9 mL of water/g of dry biomass, oxygen pressure = 14.0 bar absolute.

(Fig. 1B) because the organic acids formed during biomass degradation actually served as pretreatment agents that were neutralized by lime. At higher lime loadings, there were less organic acids, and, thus, the “acidic pretreatment” was less effective.

To reduce biomass degradation, the effects of shorter pretreatment times (e.g., 15 and 30 min) were investigated at high temperatures (e.g., 210–250°C). The results show that the sugar yields increased only slightly at 240°C and 30 min (45). It appeared that nonoxidative lime pretreatment was not sufficient, and, therefore, oxygen was added to improve the digestibility of poplar wood.

Figure 2A shows the 3-d reducing sugar yields as a function of pretreatment time at various temperatures using an oxygen pressure of 7.1 bar absolute. The oxidative lime pretreatment tremendously enhanced the reducing sugar yields from 62 (untreated) to as much as 513 mg of eq. glucose/g of raw dry biomass (treated at 140°C and 16 h). However, such

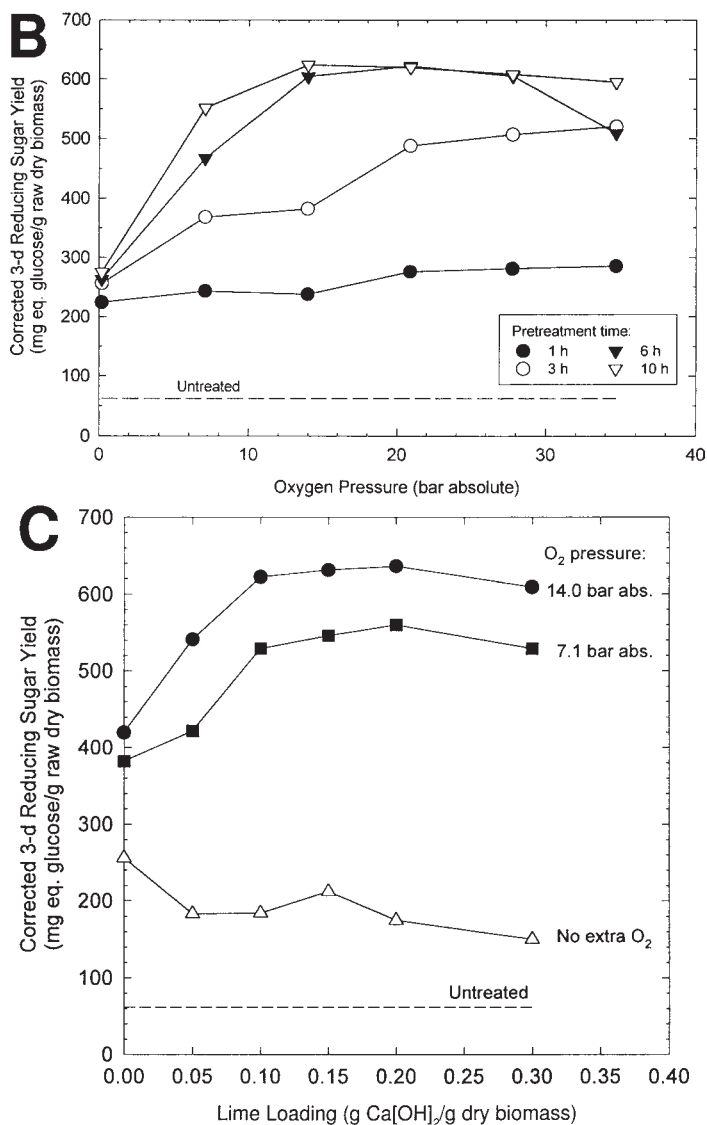


Fig. 2. (continued)

a long reaction time is not justified from an industrial viewpoint; thus, 150°C and 6 h appear to be sufficiently effective.

Figure 2B illustrates the effects of oxygen pressures at various pretreatment times. It shows that a significant pretreatment time was needed for the oxidation reactions to occur because at short pretreatment times (e.g., 1 h), the digestibility was low even with high oxygen pressures. However, pretreatment times above 6 h increased the sugar yields only slightly; thus, a pretreatment time of 6 h is sufficient. Figure 2B also shows that for a 6-h pretreatment, an oxygen pressure of 14.0 bar absolute is recommended

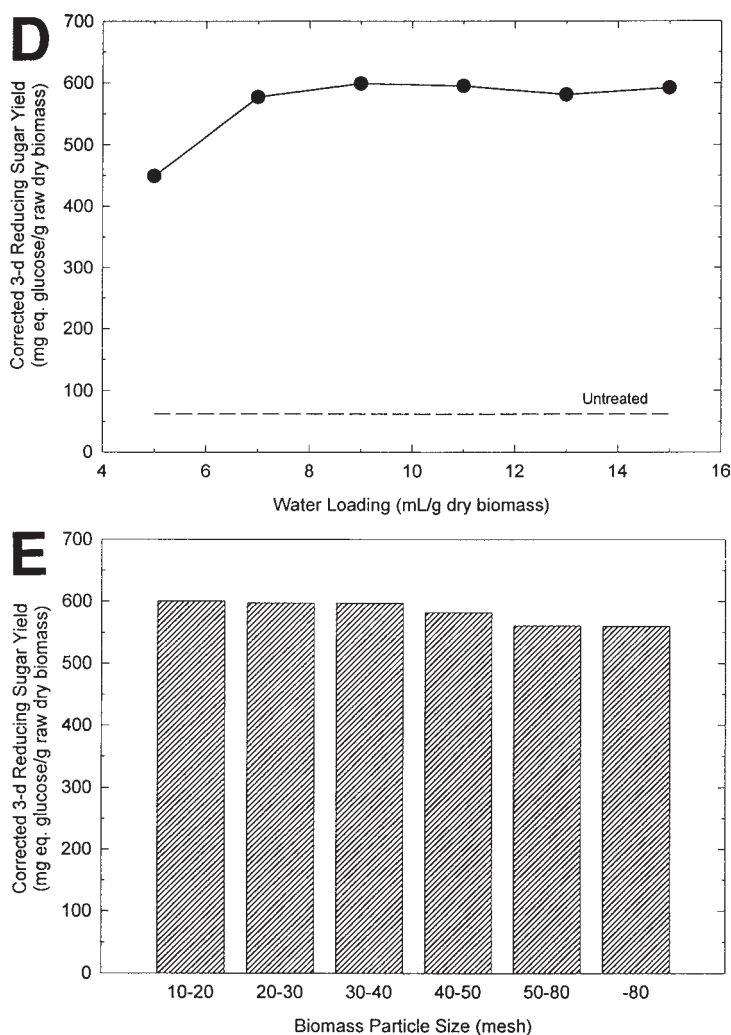


Fig. 2. (continued)

because the sugar yields declined rapidly below that pressure and increased only slightly above it.

Figure 2C shows that the most effective lime loading was 0.1 g of  $\text{Ca}(\text{OH})_2/\text{g}$  of dry biomass, similar to our previous nonoxidative studies on switchgrass, bagasse, and wheat straw (41,43). At this lime loading, the oxidative lime pretreatment increased the reducing sugar yields by a factor of 10 (from 62 to 622 mg of eq. glucose/g of raw dry biomass), whereas the nonoxidative pretreatment increased the yields only by a factor of 3 (from 62 to 184 mg of eq. glucose/g of raw dry biomass). This further demonstrates the effects of adding oxygen to lime pretreatment.

Figure 2D shows the effects of water loadings. Although water loadings as low as 7 mL/g of dry biomass are effective, there is little economic incentive to reduce the water loadings to a bare minimum.

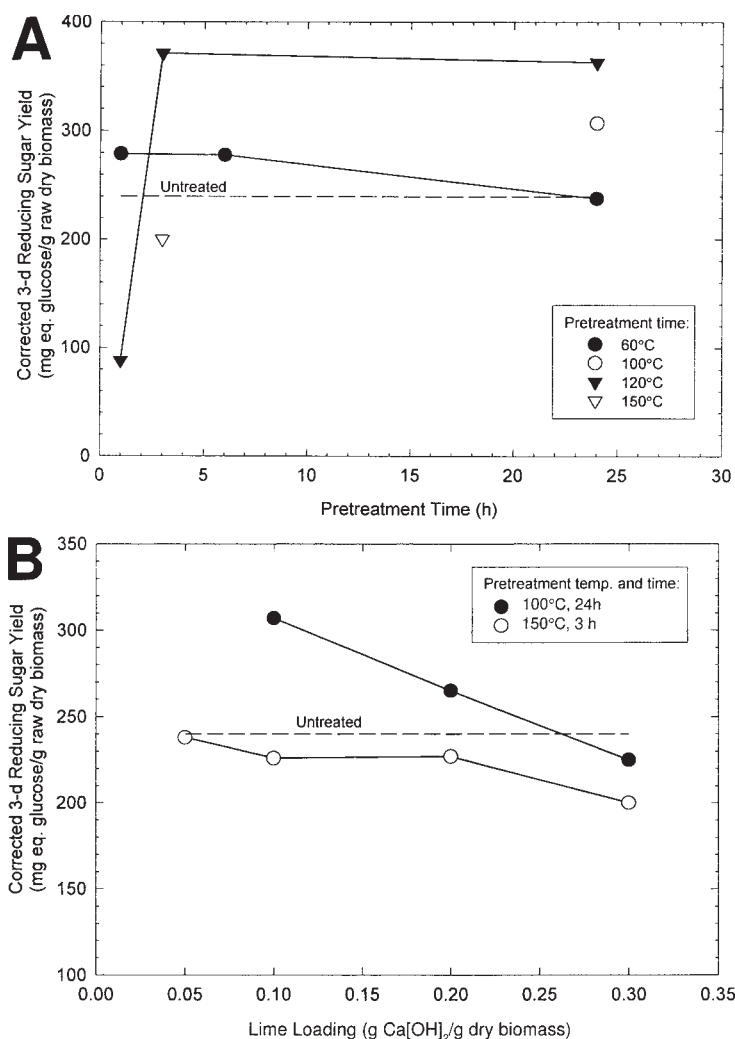


Fig. 3. Effects of nonoxidative lime pretreatment conditions for newspaper: (A) time and temperature; (B) lime loading. Pretreatment conditions: (A) 0.3 g of  $\text{Ca}(\text{OH})_2$ /g of dry biomass, 10 mL of water/g of dry biomass; (B) 10 mL of water/g of dry biomass.

Therefore, a water loading of 9 mL/g of dry biomass can be used for easier processing.

Figure 2E shows that grinding to less than 10 mesh is sufficient for lime pretreatment. Even though it was not necessary to grind biomass below 40 mesh, subsequent experiments were performed using a particle size of  $-40$  mesh because it is of a more uniform particle size, which reduces variability among experiments.

#### Newspaper

The effects of time and temperature conditions were studied for the nonoxidative lime pretreatment of newspaper. Figure 3A shows that

although the nonoxidative lime pretreatment increased the sugar yields by a factor of 1.5 (at 120°C, 3 h), the best yield (371 mg of eq. glucose/g of raw dry biomass) was low. Because newspaper contains 65–85% carbohydrates (35,38,44,49,50), the yield could be as high as 720–940 mg of eq. glucose/g of raw dry biomass. The sugar yield of untreated newspaper was substantially higher than those of other untreated lignocelluloses (240 vs 62 mg of eq. glucose/g of raw dry biomass for newspaper and poplar wood, respectively) because newspaper contains some Kraft pulp that was “pretreated” during the pulping processes.

Similar to the case of poplar wood, Fig. 3B shows that the sugar yields decreased as the lime loading increased. At high temperatures (e.g., 150°C), the sugar yields were even lower than that of the untreated sample, which might result from carbohydrate degradation.

The effects of oxidative lime pretreatment were briefly investigated for newspaper. Note that this was a short, exploratory study to determine quickly the feasibility of oxidative lime pretreatment of newspaper, not to find the best pretreatment conditions. Figure 4A shows that newspaper digestibility was significantly enhanced by the presence of oxygen. With an oxygen pressure of 7.1 bar absolute, oxidative lime pretreatment increased the sugar yields by a factor of 2.2 (from 240 to 520 mg of eq. glucose/g of raw dry biomass), whereas nonoxidative lime pretreatment increased yields only by a factor of 1.7 (from 240 to 407 mg of eq. glucose/g of raw dry biomass). Figure 4A also shows that newspaper digestibility increased with oxygen pressures. Further study is needed to determine the best oxygen pressure for newspaper.

Figure 4B illustrates the effects of pretreatment time on newspaper digestibility at 140°C with an oxygen pressure of 7.1 bar absolute. The data show that 3 h was sufficient to achieve high sugar yields (e.g., 565 mg of eq. glucose/g of raw dry biomass), but further studies at different temperatures are needed to determine the best pretreatment time for each temperature.

### *Enzyme Loading Studies*

A cellulase loading of 5 FPU/g of raw dry biomass was used when evaluating pretreatment conditions. Because enzymes can comprise as much as 44% of sugar production costs (39), it is important to determine whether there are significant yield benefits from loadings higher than 5 FPU/g of raw dry biomass or whether cellulase loadings <5 FPU/g of raw dry biomass are sufficient.

Approximately 90 g of poplar wood (–40 mesh) was pretreated with an oxygen pressure of 14.0 bar absolute for 6 h at 150°C, 0.1 g of  $\text{Ca}(\text{OH})_2$ /g of dry biomass, and 9 mL of water/g of dry biomass. The pretreated and untreated poplar wood were hydrolyzed using a cellobiase loading of 28.4 CBU/g of raw dry biomass and various cellulase loadings (0, 1, 3, 5, 10, 25, 50, and 75 FPU/g of raw dry biomass).

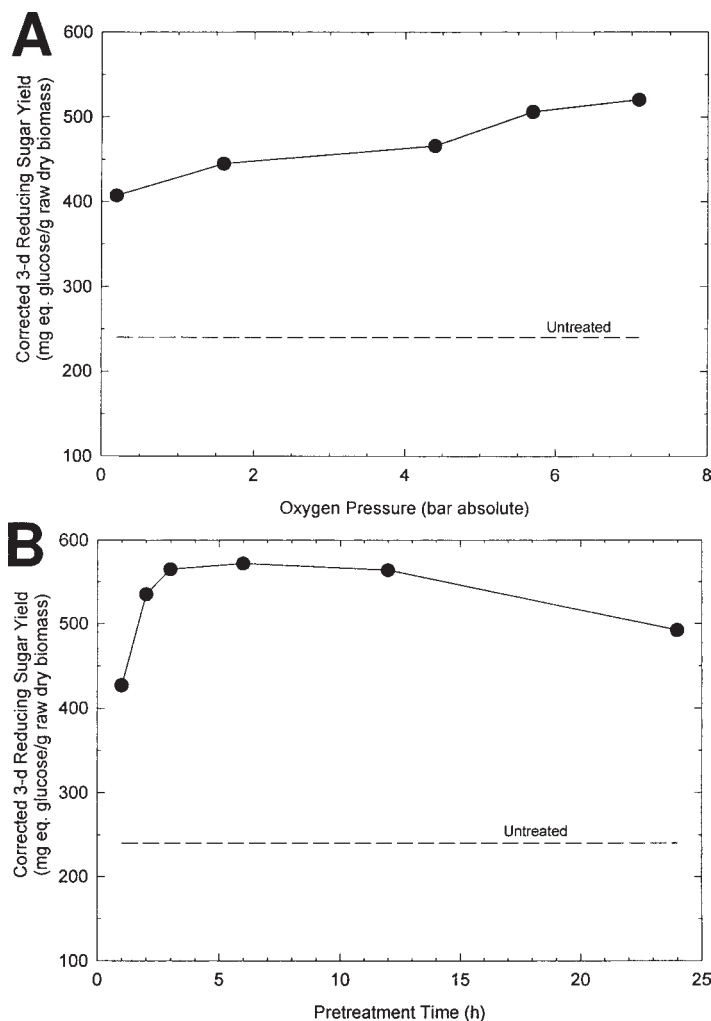


Fig. 4. Effects of oxidative lime pretreatment conditions for newspaper: **(A)** oxygen pressure; **(B)** time. Pretreatment conditions: (A) 120°C, 24 h, 0.3 g of  $\text{Ca}(\text{OH})_2$ /g of dry biomass, 16 mL of water/g of dry biomass; (B) 140°C, 0.3 g of  $\text{Ca}(\text{OH})_2$ /g of dry biomass, 16 mL of water/g of dry biomass, oxygen pressure = 7.1 bar absolute.

Figure 5 shows the effects of cellulase loadings on 3-d reducing sugar, glucose, xylose, and total sugar yields of untreated and pretreated poplar wood. The data indicate that at a cellulase loading of 25 FPU/g of raw dry biomass, the cellulose sites are saturated by the enzyme. However, a cellulase loading of 5 FPU/g of raw dry biomass is sufficient from a practical viewpoint because it represents the “shoulder” of the curve. At high cellulase loadings (e.g., 75 FPU/g of raw dry biomass), oxidative lime pretreatment increased the 3-d reducing sugar yields from 101 to 625 mg of eq. glucose/g of raw dry biomass, the 3-d glucan conversion from 16 to

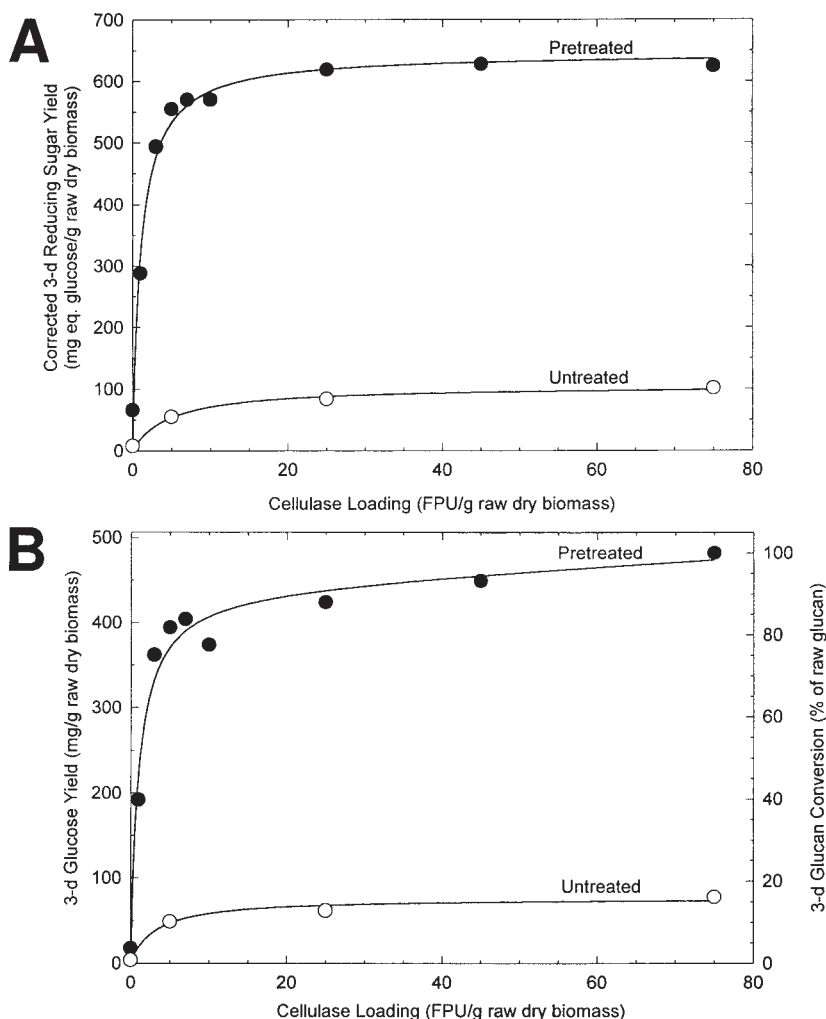


Fig. 5. Enzyme loading studies for poplar wood: **(A)** 3-d reducing sugar yields; **(B)** 3-d glucose yields; **(C)** 3-d xylose yields; **(D)** 3-d total sugar yields. Pretreatment conditions: 150°C, 6 h, 0.1 g of  $\text{Ca}(\text{OH})_2$ /g of dry biomass, 9 mL of water/g of dry biomass, oxygen pressure = 14.0 bar absolute.

100% of raw glucan, the 3-d xylan conversion from 21 to 88% of raw xylan, and the 3-d total sugar conversion from 18 to 97% of raw total sugars.

The reducing sugar yields were slightly higher than the total sugar yields (see Fig. 5A,D). The discrepancy between the reducing sugar and the total sugar measurements results from inaccuracies associated with expressing xylose as equivalent glucose.

### Enzymatic Hydrolysis Profiles

To determine the sugar yields as a function of time, enzymatic hydrolysis profiles were performed on pretreated and untreated poplar

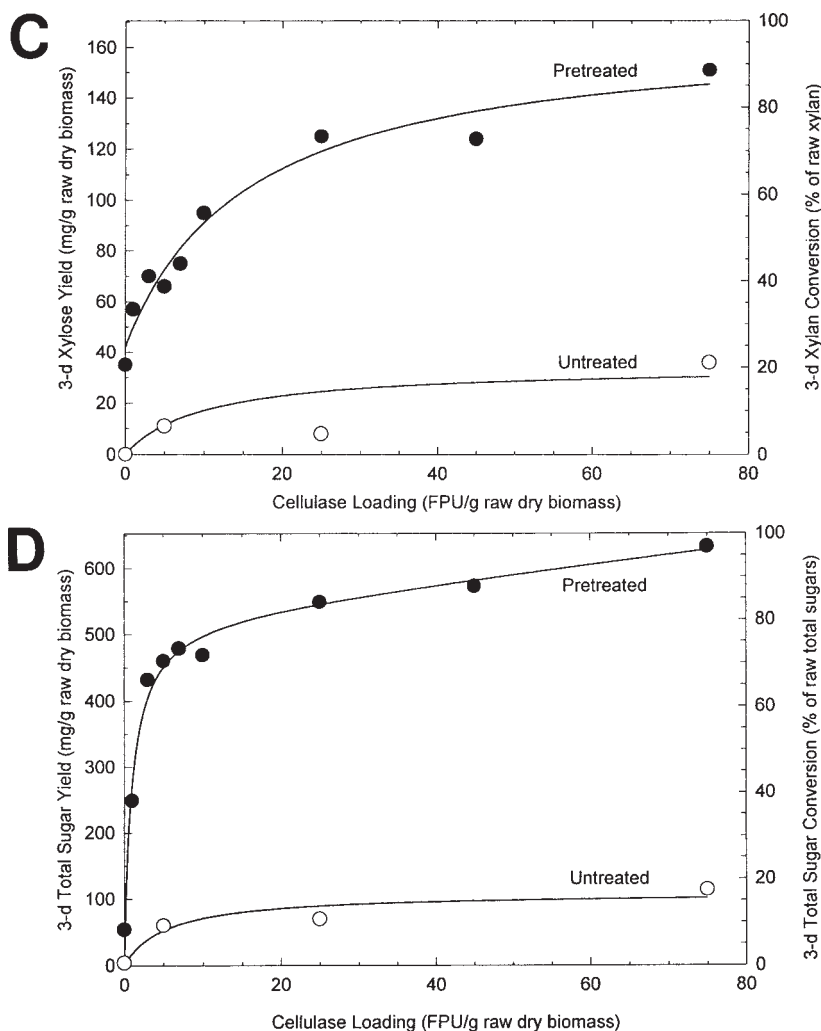


Fig. 5. (continued)

wood. Poplar wood (–40 mesh) was pretreated at 150°C for 6 h in the presence of 0.1 g of  $\text{Ca}(\text{OH})_2$ /g of dry biomass, 9 mL of water/g of dry biomass, and 14.0 bar absolute oxygen. Figure 6 shows that oxidative lime pretreatment tremendously enhanced the poplar wood digestibility. The 3-d reducing sugar yields increased by a factor of 9 (from 62 to 565 mg of eq. glucose/g of raw dry biomass), the 3-d glucan conversion increased by a factor of 11 (from 7 to 77% of raw glucan), the 3-d xylan conversion increased by a factor of 37 (from 2 to 74% of raw xylan), and the 3-d total sugar conversion increased by a factor of 13 (from 6 to 77% of raw total sugars). Unlike switchgrass and bagasse in which the hydrolysis does not proceed significantly after 24 h (41,43), the hydrolysis of pretreated poplar wood continued to proceed at 72 h. Perhaps hydrolysis times longer than 3 d may increase sugar yields significantly.

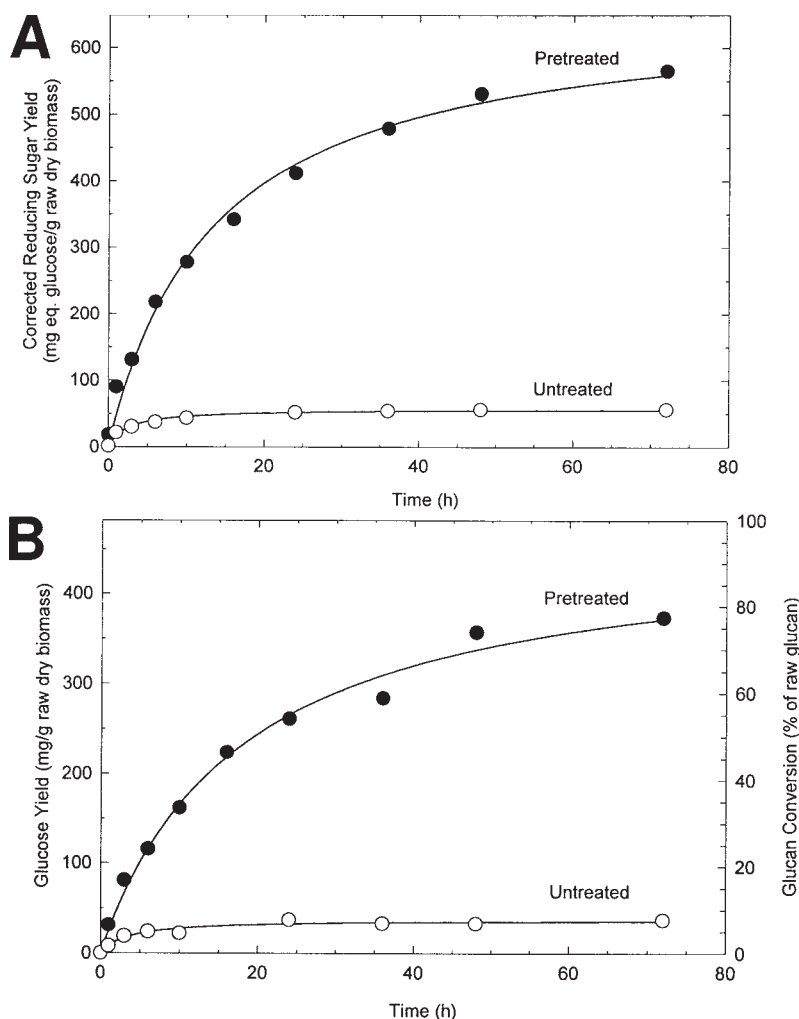


Fig. 6. Enzymatic hydrolysis profiles for poplar wood: **(A)** reducing sugar yields; **(B)** glucose yields; **(C)** xylose yields; **(D)** total sugar yields. Pretreatment conditions: 150°C, 6 h, 0.1 g of  $\text{Ca}(\text{OH})_2$ /g of dry biomass, 9 mL of water/g of dry biomass, oxygen pressure = 14.0 bar absolute.

### Material Balances

Material balances were performed on poplar wood to determine how much biomass was solubilized by oxidative lime pretreatment using the recommended conditions (150°C, 6 h, 0.1 g of  $\text{Ca}[\text{OH}]_2$ /g of dry biomass, 9 mL of water/g of dry biomass, and 14.0 bar absolute oxygen). Table 6 summarizes the water solubility of each component before and after pretreatment. Figure 7 shows the material balances for raw, washed only, and pretreated-and-washed poplar wood. All the components became more water-soluble owing to oxidative lime pretreatment, except ash and glucan. About 78% of lignin, 49% of xylan, 62% of crude protein, and 83% of other

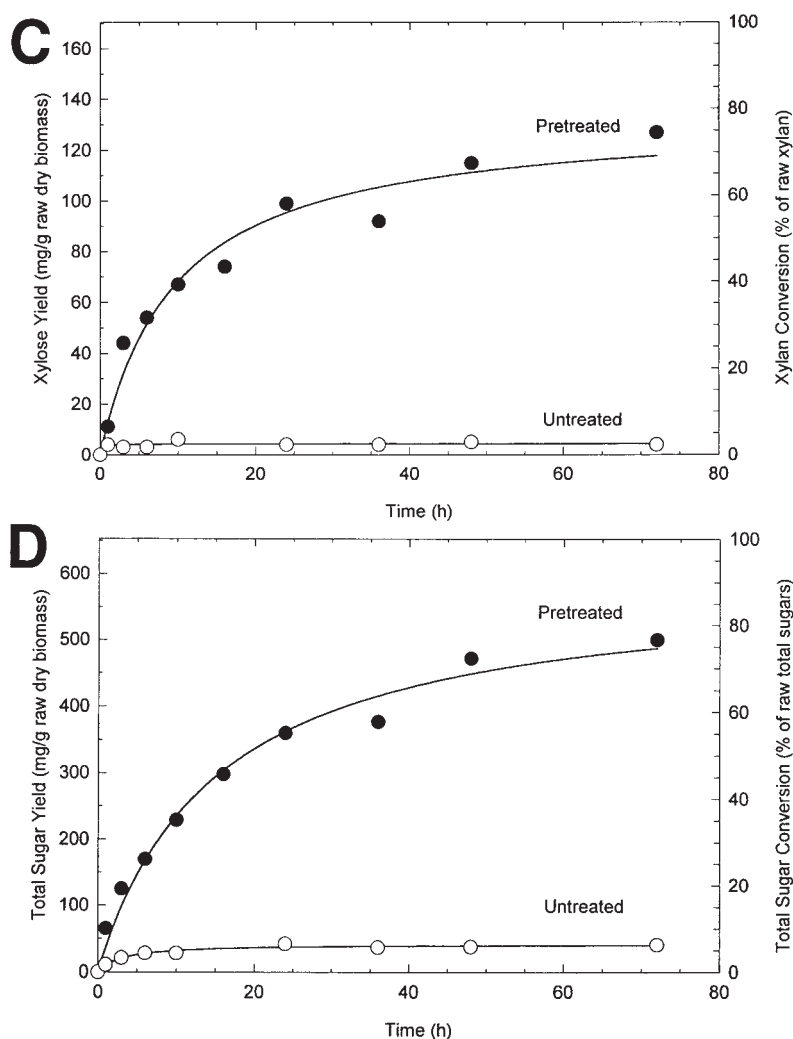


Fig. 6. (continued)

components (e.g., extractives) were removed by the pretreatment. Lignin removal is the major reason oxidative lime pretreatment enhances the digestibility (6). The data also show that glucan was barely removed. Figure 8A shows that the compositions of raw and washed only poplar wood are similar, whereas pretreated-and-washed biomass is enriched in glucan (i.e., 76%).

Similar to switchgrass and bagasse (41,43), the ash content of poplar wood increased after pretreatment. Figure 8B indicates that the ash increased because the calcium penetrated into the biomass during pretreatment. The calcium content of poplar wood increased substantially after oxidative lime pretreatment (from 0.3 to 1.2%), causing the total mineral content to increase from 0.8 to 1.3%. About 76% of sulfur and 84% of mag-

Table 6  
Water Solubility of Poplar Wood Components Before and After Oxidative Lime Pretreatment<sup>a</sup>

Component	Raw composition (g component/g total)	Weight loss percentage <sup>b</sup>		Amount removed by oxidative lime pretreatment (%) <sup>b</sup>
		Washed only (%)	Pretreated-and-washed (%)	
Ash	0.01	32.6	13.4	-19.2
Lignin	0.28	4.6	82.1	77.5
Xylan	0.15	5.3	54.1	48.8
Glucan	0.43	1.9	0.1	-1.8
Crude protein	0.02	22.2	84.2	62.0
Others	0.11	10.8	93.7	82.9
Total	1.00	4.9	42.7	37.8

<sup>a</sup>Pretreatment conditions: 150°C, 6 h, 0.1 g of Ca(OH)<sub>2</sub>/g of dry biomass, 9 mL of water/g of dry biomass, oxygen pressure = 14.0 bar absolute, and particle size = -40 mesh.

<sup>b</sup>Weight percentage is based on the initial weight of each component.

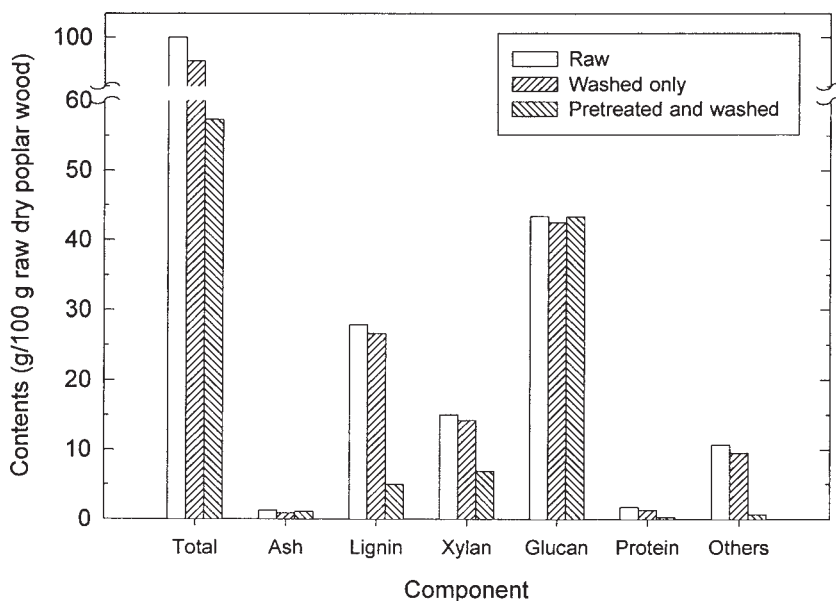


Fig. 7. Material balances for raw, washed only, and pretreated-and-washed poplar wood. Pretreatment conditions: 150°C, 6 h, 0.1 g of  $\text{Ca}(\text{OH})_2$ /g of dry biomass, 9 mL of water/g of dry biomass, oxygen pressure = 14.0 bar absolute.

nesium were removed after lime pretreatment, whereas phosphorus and potassium were fairly soluble even before the pretreatment. Other minerals (i.e., boron, iron, manganese, sodium, copper, zinc, and aluminum) constituted only a minor part of ash.

Table 7 summarizes the results of material balances from various pretreatments of poplar wood. Because the solubilization of raw biomass components was not described in most of the literature, it is difficult to compare the weight loss resulting from pretreatment alone. Thus, only the material balances between raw and pretreated-and-washed biomass are discussed for major components (i.e., lignin, glucan, and xylan).

Dilute-acid pretreatments have relatively high total solid recoveries (68–91%) (23,25–27), whereas acetic acid +  $\text{H}_2\text{O}_2$  pretreatment has a low recovery of 40% (23). The total solid recovery of oxidative lime pretreatment is moderate (57%), similar to steam and organosolv pretreatments (57–58%) (29,51). Regarding lignin removal, organosolv,  $\text{NH}_3$  +  $\text{H}_2\text{O}_2$ , peroxymonosulfate + NaOH, acetic acid +  $\text{H}_2\text{O}_2$ , peracetic acid, and oxidative lime pretreatments remove more than 80% of lignin (23,28,29,31), whereas dilute-acid, NaOH, and NaOH +  $\text{H}_2\text{O}_2$  pretreatments remove lignin only slightly (0–5%) (23,26). Regarding carbohydrate recovery, peracetic acid and oxidative lime pretreatments have the highest glucan recoveries (>99%), whereas steam pretreatment solubilizes 19% of glucan (51). Organosolv and oxidative lime pretreatments have moderate xylan recoveries (54–56%) (29), whereas steam and liquid hot water pretreatments

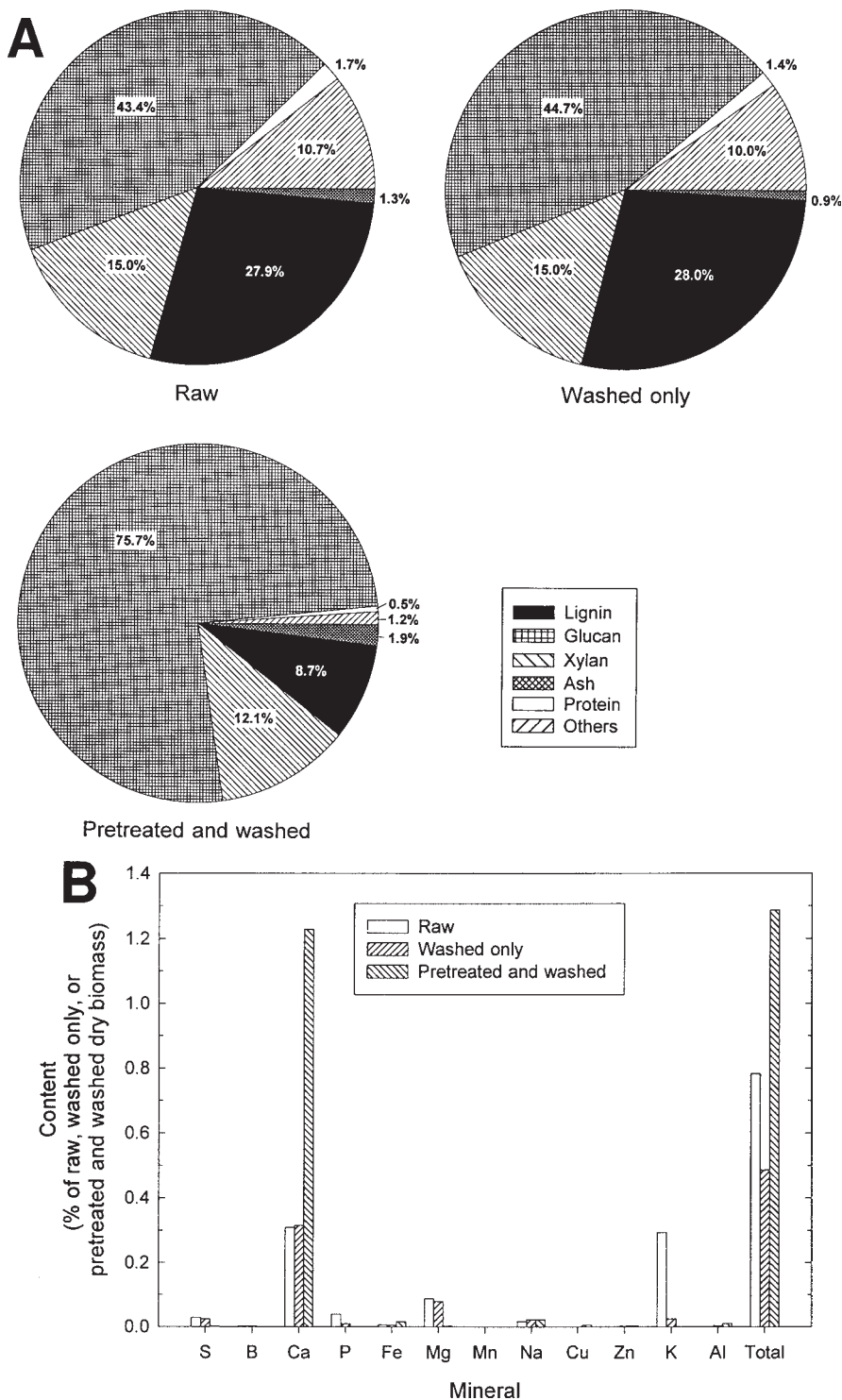


Fig. 8. Compositions of raw, washed only, and pretreated-and-washed poplar wood: **(A)** major components; **(B)** minerals. Pretreatment conditions: 150°C, 6 h, 0.1 g of  $\text{Ca}(\text{OH})_2$ /g of dry biomass, 9 mL of water/g of dry biomass, oxygen pressure = 14.0 bar absolute.

Table 7  
Summary of Material Balances from Various Pretreatments of Poplar Wood

Pretreatment <sup>a</sup>	Total solid recovered (%)	Total weight loss (%)	Composition (%)										Reference
			Lignin			Glucan			Xylan				
			Raw <sup>b</sup>	Pret. <sup>c</sup>	Loss <sup>d</sup>	Raw <sup>b</sup>	Pret. <sup>c</sup>	Loss <sup>d</sup>	Raw <sup>b</sup>	Pret. <sup>c</sup>	Loss <sup>d</sup>	Raw <sup>b</sup>	
Steam <sup>e</sup>	57	43	22	30	21	48	68	19	20	1	98	51	
Liquid hot water	68	32	—	—	37	—	—	5	—	—	100	16	
NaOH	83	17	22	26	2	—	55	—	—	10	—	23	
Dilute acid (H <sub>2</sub> SO <sub>4</sub> )	77	23	—	—	—	46	58	2	17	15	32	25	
Dilute acid (H <sub>2</sub> SO <sub>4</sub> )	68–74	26–32	18 <sup>h</sup>	26–29 <sup>h</sup>	0–5 <sup>h</sup>	50	60–66	—	18	0–3	—	26	
Dilute acid (H <sub>2</sub> SO <sub>4</sub> )	—	—	28	34	—	44	65	—	16	3	—	27	
Dilute acid (H <sub>2</sub> SO <sub>4</sub> )	91	9	22	24	0.7	—	55	—	—	10	—	23	
Peracetic acid	71	29	28	5	88	40	56	<1	13	19	1	28	
Organosolv (MeOH)	58	42	18	5	83	44	70	8	18	14	56	29	
NH <sub>3</sub> + H <sub>2</sub> O <sub>2</sub>	—	—	—	—	80	—	—	2	—	—	50	31	
NaOH + H <sub>2</sub> O <sub>2</sub>	77	23	22	28	2	—	45	—	—	<2	—	23	
PMS <sup>f</sup> + NaOH	49	51	22	3	93	—	75	—	—	10	—	23	
Acetic acid + H <sub>2</sub> O <sub>2</sub>	40	60	22	2	96	—	90	—	—	5	—	23	
NaOH + peracetic acid	78	22	28	19	48	40	50	2	13	17	1	28	
Oxidative lime <sup>g</sup>	57	43	28	9	82	43	76	<1	15	12	54	This work	

<sup>a</sup>Pretreatment conditions are listed in Table 1.

<sup>b</sup>Untreated biomass. Compositions are determined based on the dry weight of untreated biomass.

<sup>c</sup>Pretreated-and-washed biomass. Compositions are determined based on the dry weight of pretreated-and-washed biomass.

<sup>d</sup>Based on the initial dry weight of each component before pretreatment and washing.

<sup>e</sup>Pretreatment conditions: 210°C, 100 s, [SO<sub>2</sub>] = 1.6% (as a catalyst).

<sup>f</sup>Peroxymonosulfate.

<sup>g</sup>Pretreatment conditions: 150°C, 6 h, 0.1 g of Ca(OH)<sub>2</sub> / g of dry biomass, 9 mL of water / g of dry biomass, and 14.0 bar absolute oxygen.

<sup>h</sup>Klason lignin alone.

remove almost all xylan (>98%) (16,51). Peracetic acid pretreatments have the highest xylan recoveries (>98%) among these pretreatments (28).

### *Lime Recovery*

Acetic acid was used to adjust the pH in this laboratory study. However, in industry, lime can be neutralized by contacting wash water with  $\text{CO}_2$  to form insoluble  $\text{CaCO}_3$ . This eliminates the acid cost and allows lime to be recycled using a lime kiln. A lime recovery study was performed to determine how much added lime can be recovered from pretreated poplar wood. The results showed that after three washings, no more lime was removed from the pretreated biomass and about 21% of the added calcium was recovered as  $\text{CaCO}_3$ . Therefore, three washings are sufficient.

## **Conclusion**

For low-lignin biomass, such as switchgrass, bagasse, and wheat straw, enzymatic digestibility can be significantly enhanced by nonoxidative lime pretreatment (41,43). For high-lignin biomass, such as poplar wood and newspaper, oxidative lime pretreatment is necessary. The recommended conditions for treating poplar wood are 150°C, 6 h, 0.1 g of  $\text{Ca}(\text{OH})_2$ /g of dry biomass, 9 mL of water/g of dry biomass, 14.0 bar absolute oxygen, and a particle size of -10 mesh. Using these conditions, the 3-d reducing sugar yield of poplar wood increased from 62 to 565 mg of eq. glucose/g of raw dry biomass, the 3-d glucan conversion increased from 7 to 77% of raw glucan, the 3-d xylan conversion increased from 2 to 74% of raw xylan, and the 3-d total sugar (glucose + xylose) conversion increased from 6 to 77% of raw total sugars, using 5 FPU of cellulase/g of raw dry biomass. At high cellulase loadings (e.g., 75 FPU/g of raw dry biomass), the 3-d total sugar conversion was 97% of raw total sugars.

Oxidative lime pretreatment also increased newspaper digestibility substantially. Using conditions of 140°C, 3 h, 0.3 g of  $\text{Ca}(\text{OH})_2$ /g of dry biomass, 16 mL of water/g of dry biomass, and 7.1 bar absolute oxygen, the 3-d reducing sugar yields of newspaper increased from 240 to 565 mg of eq. glucose/g of raw dry biomass using 5 FPU of cellulase/g of raw dry biomass. Further study is required to improve newspaper digestibility.

A material balance study on poplar wood showed that oxidative lime pretreatment removed 38% of total biomass, including 78% of lignin, 49% of xylan, 62% of crude protein, and 83% of other components (e.g., extractives); no glucan was removed. Ash increased because calcium was incorporated during the pretreatment. The enhanced digestibility of treated biomass results mainly from lignin removal (6). After oxidative lime pretreatment, about 21% of added lime could be recovered by  $\text{CO}_2$  carbonation.

## **Acknowledgment**

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