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Lime Pretreatment of Switchgrass

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ABSTRACT

Lime (calcium hydroxide) was used as a pretreatment agent to enhance the enzymatic digestibility of switchgrass. After studying many conditions, the recommended pretreatment conditions are: time = 2 h, temperature = 100°C and 120°C, lime loading = 0.1 g $Ca(OH)_2$/g dry biomass, water loading = 9 mL/g dry biomass. Studies on the effect of particle size indicate that there was little benefit of grinding below 20 mesh; even coarse particles (4–10 mesh) digested well. Using the recommended pretreatment conditions, the 3-d reducing sugar yield was five times that of untreated switchgrass, the 3-d total sugar (glucose + xylose) yield was seven times, the 3-d glucose yield was five times, and the 3-d xylose yield was 21 times. A material balance study showed that little glucan (approx 10%) was solubilized as a result of the lime pretreatment, whereas about 26% of xylan and 29% of lignin became solubilized.

Index Entries: Lignocellulose; pretreatment; lime; cellulase; sugar.

INTRODUCTION

Lignocellulose is a valuable alternative energy source because it is widely available and can be converted to various organic compounds such as sugars and alcohols *(1)*. The susceptibility of lignocellulosic biomass to enzymatic hydrolysis is constrained because of structural characteristics such as cellulose crystallinity, hemicellulose acetylation, inaccessible surface area, and lignin content *(2,3)*. To enhance enzymatic hydrolysis, pretreatment is essential. In general, pretreatment methods are of four types: physical, chemical, multiple (i.e., physical + chemical), and biological. Excellent reviews on pretreatments have been published by Lin et al. *(4)*, Fan et al. *(5)*, Chang et al. *(6)*, and Weil et al. *(7)*.

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Of the chemicals used as pretreatment agents, alkalis have received much attention *(8)*. According to fundamental studies by Kong et al. *(3)*, alkalis remove acetate groups from hemicellulose, thereby reducing the steric hindrance of hydrolytic enzymes and greatly enhancing carbohydrate digestibility. Sodium hydroxide effectively enhances lignocellulose digestibility *(9–11)*, but it has several disadvantages; it is expensive ($0.62/kg) *(12)*, dangerous to handle, and difficult to recover. Ammonia pretreatment has also received much attention *(13,14)* because it is easy to recover; unfortunately, it is moderately expensive ($0.12/kg) *(12)* and requires careful handling. In contrast, lime (calcium hydroxide) has many advantages; it is very inexpensive ($0.04/kg) *(12)*, is safe, and can be recovered by carbonating wash water *(15)*. Table 1 summarizes the results of previous lime pretreatment studies *(16–29)*. Although these results showed that lignocellulose digestibility improved by lime pretreatment, authors usually concluded that calcium hydroxide was less effective than other alkalis such as sodium hydroxide *(16–18,22,23,25,29)*, ammonia *(20,25,29)*, and potassium hydroxide *(22,23)*. However, many of these comparisons employed the same pretreatment conditions, i.e., regardless of the alkali studied, equal amounts of water and alkali were used. Because calcium hydroxide is a weak alkali and poorly soluble in water, these studies put lime at a disadvantage. By modifying the pretreatment conditions to be compatible with lime, it is as effective as other alkalis in enhancing lignocellulose digestibility *(28)*.

The bioconversion of crop residues (e.g., wheat straw, barley straw, corn cob, etc.) is widely studied; however, relatively few studies have been performed on forage grasses. Switchgrass (*Panicum virgatum*) is a good forage species that tolerates a wide variety of environmental conditions *(30)*. It grows in all regions of the United States, except the Northwest and California *(31)*. Because of its excellent growth, switchgrass is a potential renewable energy source that deserves further investigation. In this research, the effects of lime pretreatment conditions were studied, the effect of cellulase loading was determined, hydrolysis profiles were performed, and material balances were made to determine how much biomass is solubilized as a result of lime pretreatment.

MATERIALS AND METHODS

Lime Pretreatment

Switchgrass was pretreated with lime (calcium hydroxide) in the presence of water. Six 3.8-cm I.D. × 12.7-cm long, 304 stainless steel-capped pipe nipples were used as the pretreatment reactors. To ensure thorough mixing of contents inside, the pipe nipples were mounted on a rotating shaft inside an oven providing the desired pretreatment temperature. To perform the pretreatment, the oven was heated in advance to reach the desired temperature. A measured amount of switchgrass (7.5 g dry weight) and calcium

Table 1
Summary of Lime Pretreatment Conditions and Results

Biomass	Temp. (°C)	Time (h)	Lime Loading ($g\ Ca(OH)_2$/g dry biomass)	Water Loading (mL/g dry biomass)	Particle Size	Effect on Digestibility (%)	Reference
				in vivo			
Corn stalks	amb.	24	0.12	8	2-4 cm	51.55 to 85.59[a]	*(16)*
Sorghum stalks	amb.	24	0.12	8	2-4 cm	49.93 to 83.64[a]	*(16)*
Sweet potato vines	amb.	24	0.12	8	2-4 cm	38.59 to 61.28[a]	*(16)*
Corn cobs	amb.	336	0.04	1.5	ground	improved digestibility	*(17)*
Barley straw	amb.	2160	0.056	0.9	chopped	54.8 to 56.9[b]	*(18)*
Wheat straw	amb.	24	0.09	11	3 cm	54.1 to 61.9[b]	*(19)*
Corn stover	21	336	0.04	0.54	0.95 cm	53.2 to 54.5[b]	*(20)*
				in vitro			
Poplar bark	amb.	24, **3600**	5.3, 10.6, 15.9, **21.1**	1.5	0.95 cm	38.6 to 52.0[c]	*(21)*
Corn Cobs	amb.	24	0.074, 0.0925	1	20 mesh	-----	*(22)*
Barley straw	amb.	2160	0.025, 0.05, **0.1**	1.2	5 cm	47.6 to 64.5[b]	*(23)*
Corn cobs	amb.	24 to 360	0.05	0.25, **0.67**, 1.5	-----	52 to 70[d]	*(24)*
Barley straw	amb.	24	0.05, **0.1**, 0.15	1, **20**	1.5 cm	37.9 to 52.5[e]	*(25)*
Pea straw	amb.	24	**0.05**, 0.1, 0.15	**1**, 20	1.5 cm	41.7 to 37.6[e]	*(25)*
Bagasse	amb.	24	0.05, **0.1**, 0.15	1, **20**	particulate	32.8 to 43.0[e]	*(25)*
Sunflower hulls	amb.	24	0.05, **0.1**, 0.15	1, **20**	particulate	16.6 to 20.3[e]	*(25)*
Bagasse	20	192	0.06, 0.09, 0.12, 0.15, 0.18, 0.24, **0.30**	1.74	0.225 cm	19.7 to 72.4[b]	*(26)*
Rice straw	amb.	24	0.05, 0.1, **0.2**	**4, 8, 12**	-----	40 to 59[e]	*(27)*
Corn cobs	45	>6	0.05	1.5	-----	43 to 54[f]	*(28)*
Soya-bean straw	amb.	720	0.02, 0.03, **0.04**, 0.05	0.65	chopped	35.8 to 41.3[e]	*(29)*

amb. = ambient temperature
Bold conditions correspond to the best lime pretreatment results.
[a]Crude fibre digestion coefficient
[b]Organic matter digestibility (OMD)
[c]*in vitro* true digestibility (IVTD)
[d]*in vitro* dry matter digestibility (IVDMD)
[e]*in vitro* organic matter digestibility (IVOMD)
[f]*in vitro* digestibility (IVD)

hydroxide (according to the desired lime loading) were placed in each reactor and thoroughly mixed using a spatula. Distilled water (according to the desired water loading) was then added to the dry mixture. After tightly capping the reactors, they were placed in boiling water for 10 min to quickly reach a high temperature and then put into the oven. The motor was turned on to start the rotating shaft and the reactors were left for the desired pretreatment time. After the pretreatment time elapsed, the reactors were moved out of the oven and immersed in cold water to cool them to ambient temperature. Samples were then removed for enzymatic hydrolysis.

Enzymatic Hydrolysis

Lime-pretreated switchgrass was transferred from the reactors to 500-mL Erlenmeyer flasks with distilled water. Citrate buffer (7.5 mL, 1.0*M*, pH 4.8) and sodium azide (5 mL, 0.01 g/mL) were added to the slurry to keep a constant pH and prevent microbial growth, respectively. Glacial acetic acid was added to reduce the pH from about 11.5 to 4.8. Then, the total volume of the slurry was adjusted to 150 mL by adding distilled water. The flasks were placed in a 100-rpm shaking air bath. When the temperature reached 50°C, cellulase (5 FPU/g dry biomass) and cellobiase (28.4 CbU/g dry biomass) were added to the flasks. The activity of the cellulase (Cytolase CL enzyme, lot no. 17-92262-09, Environmental BioTechnologies, Santa Rosa, CA) was 91 FPU/mL, as determined using the filter paper assay *(32)*. The activity of cellobiase (Novozym 188, batch no. DCN0015, Novo Nordisk Bioindustrials, Franklinton, NC) was 250 CbU/g.

To investigate lime treatment conditions, enzyme hydrolysis samples (approx 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; then reducing sugars were measured. When the hydrolysis profiles were performed, samples were withdrawn as a function of time (i.e., 0, 1, 3, 6, 10, 16, 24, 36, 48, and 72 h) and boiled for 15 min in sealed tubes; then glucose, xylose, and reducing sugars were measured at each time point. The same procedure was also applied to untreated switchgrass.

Sugar Measurement

Reducing sugars were measured using the dinitrosalicylic acid (DNS) assay *(33)*. A 200-mg/dL glucose standard solution (Yellow Springs Instruments, Yellow Springs, OH) was used for the calibration, thus the reducing sugars were measured as "equivalent glucose." The sugar content in the enzymes (approx 4.2 mg eq. glucose/g dry biomass) was subtracted from the original reducing sugar yields to determine the actual amounts of reducing sugar produced from the biomass. After subtracting the enzyme sugars, the yields were multiplied by a correction factor to account for calcium acetate inhibition and were called "corrected" reducing sugar yields. The correction factor depends upon lime loading (*see* Table 2).

Table 2
Effects of Calcium Acetate Inhibition and Correction Factors

Lime Addition ($g\ Ca(OH)_2/g$ dry biomass)	3-d Reducing Sugar Yield (mg eq. glucose/g dry biomass)	Correction Factor for Calcium Acetate Inhibition
0	465	1.000
0.02	491	0.948
0 05	464	1.002
0.1	458	1.015
0.2	442	1.051
0.3	419	1.110

Glucose and xylose were measured using high performance liquid chromatography (HPLC). A Bio-Rad (Cambridge, MA) Aminex HPX-87P column was used for carbohydrate separation; a refractive index detector (LDC/Milton Roy, refractoMonitor III, Riviera Beach, FL) was used to detect sugars; a Spectra-Physics (San Jose, CA) integrator (SP4270) was used for integration. Throughout the paper, total sugar denotes the summation of glucose and xylose because no other carbohydrates were detected.

There are discrepancies between the reducing sugar measurements and the total sugar measurements because of inaccuracies associated with expressing xylose as equivalent glucose; nonetheless, the DNS assay was accurate enough to rapidly screen the pretreatment conditions in the early stages of this study.

Dry Weight and Composition

Throughout the paper, the sugar yields were calculated based on the dry weight of biomass that was determined by drying the biomass at 105°C for 8 h. However, when performing material balances, the biomass samples (raw, washed only, and pretreated and washed) were dried at 45°C to prevent carbohydrate destruction. A part of the 45°C-dried samples was then dried at 105°C to determine its dry weight, whereas the rest was used to determine its composition (i.e., lignin, glucan, xylan, crude protein, and ash) *(32)*.

RESULTS AND DISCUSSION

Effects of Calcium Acetate Inhibition

After biomass is treated with lime, the pH is as high as 11.5, which is incompatible with cellulase. For convenience in a laboratory setting, the lime was neutralized with acetic acid. (Industrially, the lime would be removed by washing the lime-treated biomass with water that is subsequently carbonated to precipitate calcium carbonate. To regenerate the lime from calcium carbonate, a lime kiln would be employed.) However,

Table 3
Lime Pretreatment Conditions Explored for Switchgrass

	Time (h)	Temperature (°C)	Lime Loading (g $Ca(OH)_2$/g dry biomass)	Water Loading (mL/g dry biomass)	Particle Size (Mesh)
Study 1	1 to 24	60 to 130	0.1	10	-40
Study 2	3	100 and 120	0.01 to 0.30	10	-40
Study 3	3	100 and 120	0.1	5 to 15	-40
Study 4	3	100	0.1	9	5 to -80
Study 5	1 to 3	100	0.1	9	-40

the resulting calcium acetate may inhibit cellulase. To measure the effects of calcium acetate inhibition, the following experiment was conducted:

A large sample (approx 200 g) of switchgrass (–40 mesh) was treated with lime. The pretreatment was performed using recommended conditions by Nagwani *(15)* (temperature = 100°C, time = 1 h, lime loading = 0.1 g $Ca(OH)_2$/g dry biomass, and water loading = 10 mL/g dry biomass). The pretreated switchgrass was washed with fresh distilled water 10 times to remove the lime. Then, the pretreated-and-washed switchgrass was air-dried and divided into 12 flasks that contained citrate buffer and various lime additions (0, 0.02, 0.05, 0.1, 0.2, and 0.3 g $Ca(OH)_2$/g dry biomass, each in duplicate). The lime added to each flask was neutralized by adding various amounts of acetic acid such that the pH of each flask was 4.8. Then enzymatic hydrolysis was performed at 50°C for 3 d. The reducing sugar yields at each lime loading were measured as a function of time using the DNS assay. The reducing sugar yields of the time-zero samples determined the sugar content of the enzymes and were subtracted from the sugar yields at other time points.

Table 2 summarizes the 3-d reducing sugar yields. As anticipated, calcium acetate inhibited the enzyme which caused about 1.5% loss of sugar yields at recommended lime loadings. The correction factors reported in Table 2 were used in the subsequent experiments to correct the 3-d reducing sugar yields.

Effects of Pretreatment Conditions

The pretreatment conditions were systematically varied to explore the effects of process variables (i.e., time, temperature, lime loading, water loading, and biomass particle size) on digestibility. Although some researchers have explored the effects of lime loading *(21–23,25–27,29)* and water loading *(24,25,27)*, little has been done on the effects of pretreatment time *(21)* or temperature. Recently, Nagwani *(15)* determined that time and temperature had the greatest impact on biomass digestibility. Lime load-

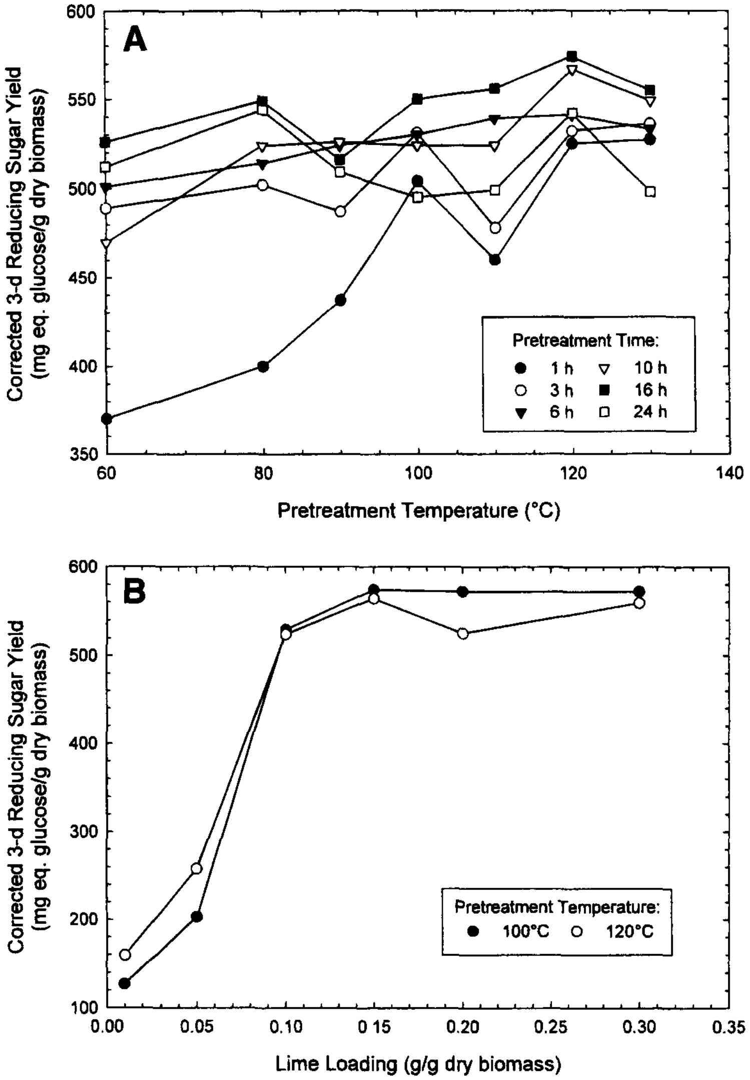

Fig. 1. Investigation of lime pretreatment conditions: **(A)** temperature and time, **(B)** lime loading, **(C)** water loading, **(D)** particle size.

ing generally had a critical value (approx 0.1 g $Ca(OH)_2$/g dry biomass) below which the digestibility greatly declined, and above which the digestibility only increased slightly. Water loading had little effect on the digestibility. Therefore, this study was conducted to hold the low-impact variables (e.g., lime loading, water loading, and particle size) constant while systematically varying the high-impact variables (e.g., time and temperature). Table 3 shows the range of conditions explored.

Figure 1A shows the 3-d reducing sugar yields as a function of pretreatment temperature at various reaction times. The best temperature lies between 100 and 120°C. The best pretreatment resulted after 16 h, but this

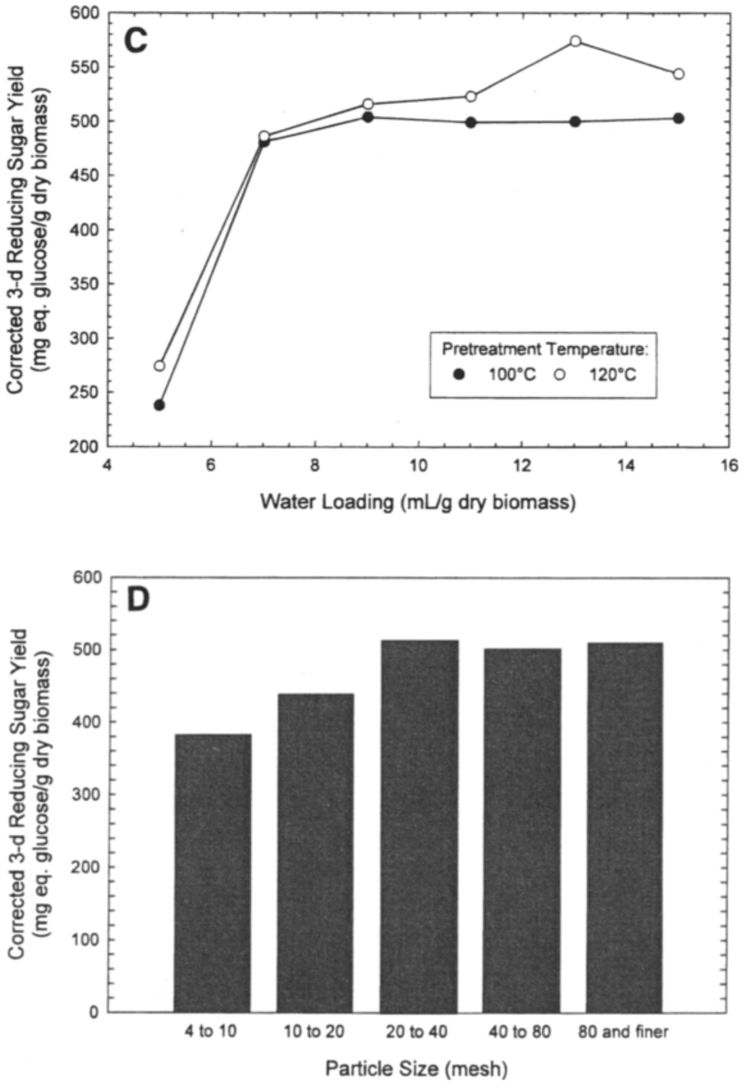

Fig. 1. *(continued)*

is excessively long from an economic viewpoint. One hour of reaction time was obviously insufficient to achieve good sugar yields, whereas pretreatment times longer than 3 h had little additional benefit. Therefore, 3 h was temporarily selected as a standard pretreatment time with which other investigations were conducted.

Figure 1B shows the effect of lime loading at two pretreatment temperatures (100 and 120°C). The most effective lime loading was 0.1 g $Ca(OH)_2$/g dry biomass. Although slightly greater sugar yields were obtained at a lime loading of 0.15 g $Ca(OH)_2$/g dry biomass, the 50% increase in lime consumption cannot be justified from an economic viewpoint. The results at 100 and 120°C were very similar.

Table 4
Further Investigation of Pretreatment Time

Pretreatment Time (h)	3-d Corrected Reducing Sugar Yield (mg eq glucose/g dry biomass)
1	447
1.5	469
2[a]	483
3	486

[a]Recommended pretreatment time.

Figure 1C shows the effect of water loading at two pretreatment temperatures (100 and 120°C). Although water loadings as low as 7 mL/g dry biomass are effective, there is little economic incentive to reduce the water loadings to a bare minimum. Therefore, a water loading of 9 mL/g dry biomass, which has a slight extra benefit (approx 5% increase in sugar yields), can be used.

Figure 1D shows the effect of biomass particle size on the digestibility. Five different particle sizes were studied. Grinding to less than 20 mesh is sufficient for lime pretreatment. Even though it was not necessary to grind biomass below 40 mesh, subsequent experiments were performed using particle sizes less than 40 mesh because there was a large quantity of this material available. Also, it is of a more uniform particle size that reduces variability between experiments.

A higher resolution study of pretreatment times was performed to focus in the range of 1 to 3 h. The reducing sugar yields were measured for biomass samples that had been pretreated for 1, 1.5, 2, and 3 h. Table 4 shows that full pretreatment likely occurs after 2 h; therefore, it was selected as the standard pretreatment time.

Enzyme Loading Studies

A cellulase loading of 5 FPU/g dry biomass was used in the studies presented in Fig. 1 and Table 2. Here, cellulase loading is studied to determine if there are yield benefits from loadings higher than 5 FPU/g dry biomass or if cellulase loadings less than 5 FPU/g dry biomass are acceptable.

Approximately 90 g of switchgrass (–40 mesh) was pretreated using the recommended conditions (i.e., time = 2 h, temperature = 120°C, lime loading = 0.1 g $Ca(OH)_2$/g dry biomass, water loading = 9 mL/g dry biomass). The pretreated and untreated switchgrass (concentration = 50 g/L) were hydrolyzed at 50°C, pH 4.8 for 3 d in a 100-rpm air shaker, using an excess cellobiase loading (i.e., 28.4 CbU/g dry biomass) and various cellulase loadings (i.e., 0, 1, 3, 5, 10, 25, 50, 75, 100 FPU/g dry biomass).

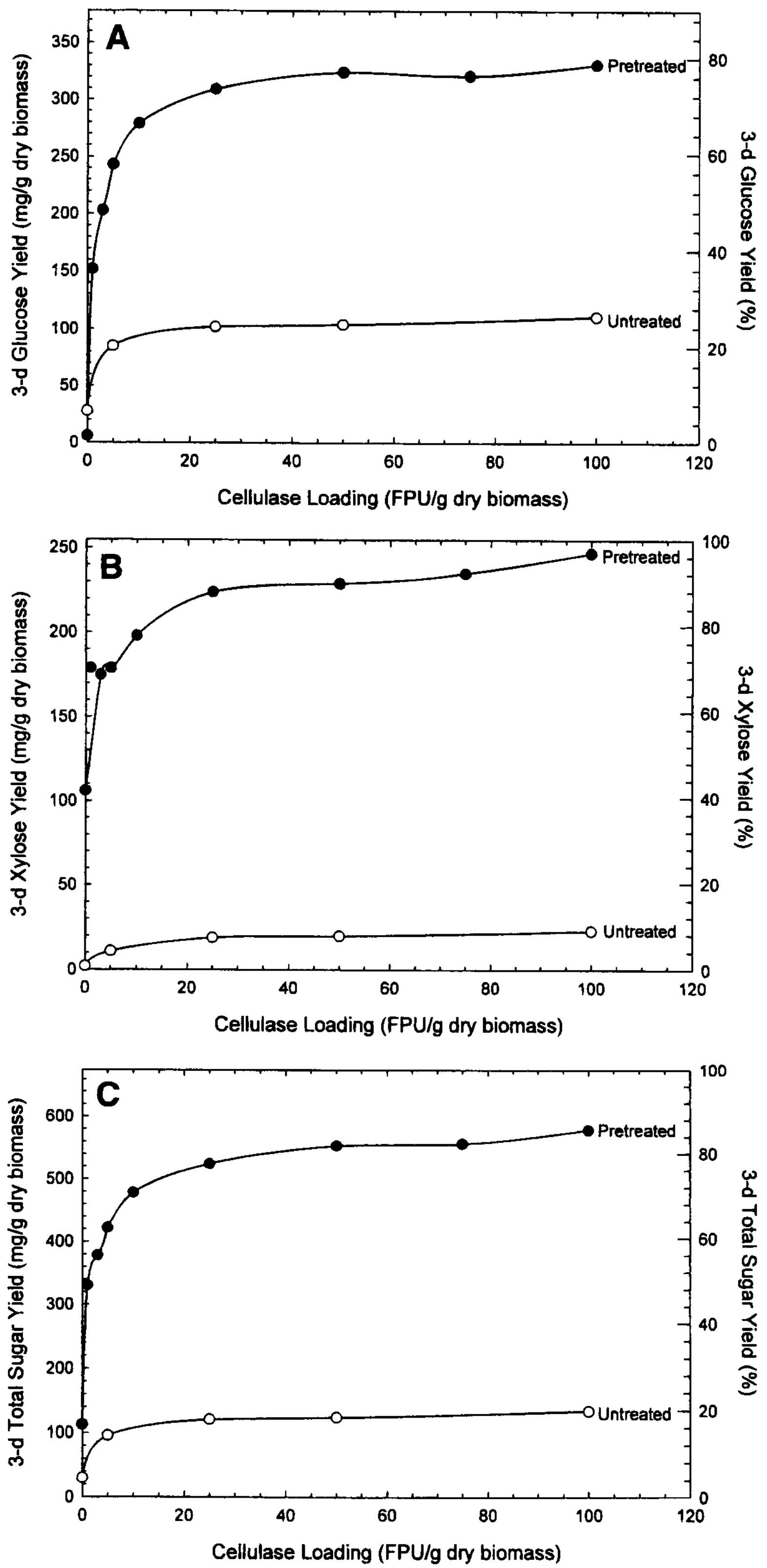

Fig. 2. Enzyme loading studies: **(A)** glucose yields, **(B)** xylose yields, **(C)** total sugar yields. (Pretreatment conditions: 120°C, 2 h, 0.1 g $Ca(OH)_2$/g dry biomass, 9 mL water/g dry biomass. Hydrolysis conditions: 50°C, pH 4.8, 28.4 CbU cellobiase/g dry biomass.)

The 3-d glucose, xylose, and "total sugar" (glucose + xylose) yields of untreated and pretreated switchgrass at various cellulase loadings are shown in Fig. 2. For cellulase loadings larger than 25 FPU/g dry biomass, the sugar yields remain essentially constant because the cellulose sites are likely saturated by the enzyme. The maximum total sugar yield was 85%. A cellulase loading of 5 FPU/g dry biomass is the "shoulder" of the curve. Although a cellulase loading of 10 FPU/g dry biomass increases total sugar yield by about 13%, doubling enzyme usage cannot be justified economically. (For example, assuming an enzyme cost of $9.4/million FPU *(34)* and an ethanol yield of 100 gal/t dry biomass, an enzyme loading of 5 FPU/g dry biomass corresponds to $0.47/gal ethanol, which is about 43% of its sales price; doubling the enzyme loading is impossible unless enzyme cost is reduced significantly.)

Hydrolysis Profiles

Instead of measuring just 3-d reducing sugar yields, a complete hydrolysis profile was measured so the sugar yields were determined as a function of time. Switchgrass (–40 mesh) was pretreated at 120°C for 2 h in the presence of 0.1 g $Ca(OH)_2$/g dry biomass and 9 mL water/g dry biomass. The pretreated switchgrass was then transferred from the reactors to Erlenmeyer flasks for enzymatic hydrolysis. This experiment was performed in triplicate.

Using cellulase loadings of 5 FPU/g dry biomass, Fig. 3 shows the yields of reducing sugars, glucose, xylose, and "total sugar" (glucose + xylose). (Note: Because of high cellobiase activity, cellobiose concentrations were negligible.) The sugar yields from pretreated switchgrass are significantly higher than from untreated switchgrass. The 3-d reducing sugar yield of pretreated switchgrass increased about five times (i.e., from 102 to 538 mg eq. glucose/g dry biomass), the 3-d total sugar yield increased about seven times (i.e., from 8.7% to 58.1%), the 3-d glucose yield increased about five times (i.e., from 12.3% to 58.0%), and the 3-d xylose yield increased about 21 times (i.e., from 2.8% to 58.1%). This dramatic increase in xylose yield indicates that lime has a selective effect on hemicellulose. The likely mechanism is that lime removes acetate groups from hemicellulose rendering it more accessible to hydrolytic enzymes *(3)*.

Material Balances

To remove solubles, switchgrass (either untreated or pretreated with the recommended conditions) was 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 compositions (i.e., glucan, xylan, lignin, crude protein, and ash) of raw, washed only, and pretreated and washed switchgrass were then determined (*see* Fig. 4).

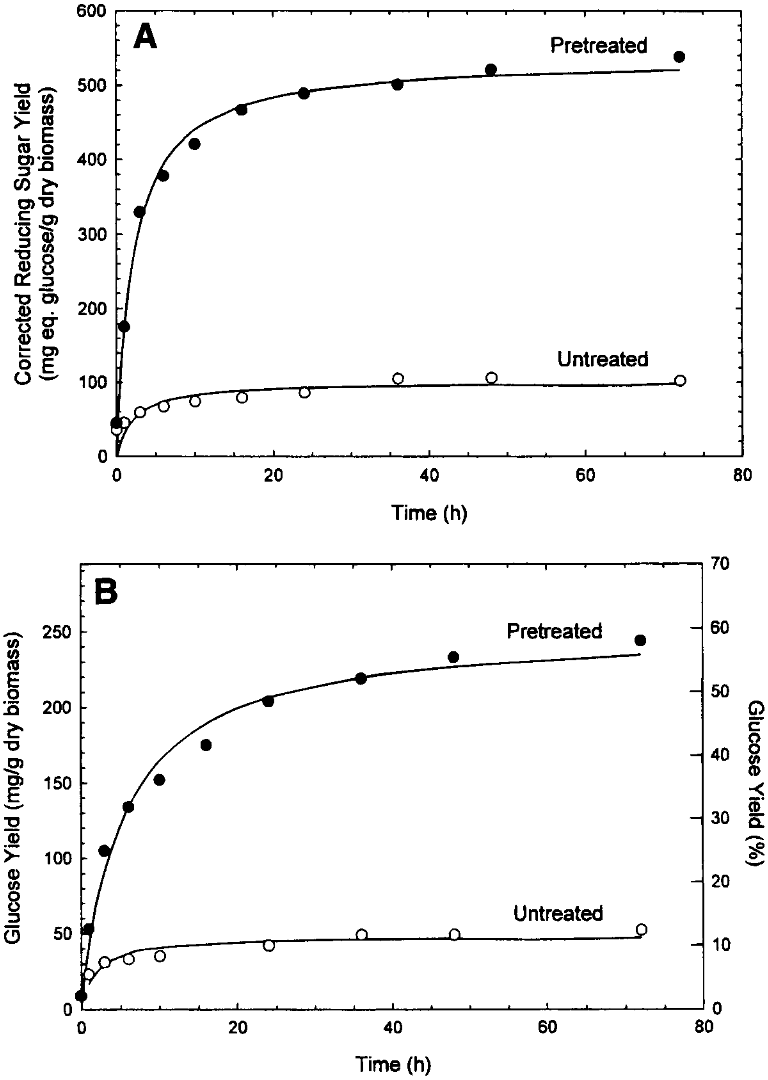

Fig. 3. 3-d hydrolysis profiles: **(A)** corrected reducing sugar, **(B)** glucose, **(C)** xylose, **(D)** total sugar. (Pretreatment conditions: 120°C, 2 h, 0.1 g $Ca(OH)_2$/g dry biomass, 9 mL water/g dry biomass. Hydrolysis conditions: 50°C, pH 4.8, 5 FPU cellulase/g dry biomass, 28.4 CbU cellobiase/g dry biomass.)

Table 5 summarizes the losses of each component before and after pretreatment. All the components became more water soluble because of the lime pretreatment, except ash. Fairly large quantities of lignin, xylan (hemicellulose), crude protein, and other components (e.g., extractives) were removed by the lime pretreatment, whereas little glucan (cellulose) was removed; hence, the pretreated and washed biomass was slightly enriched in cellulose. The results suggest that the removal of lignin and hemicellulose both contribute to the increase of the biomass digestibility.

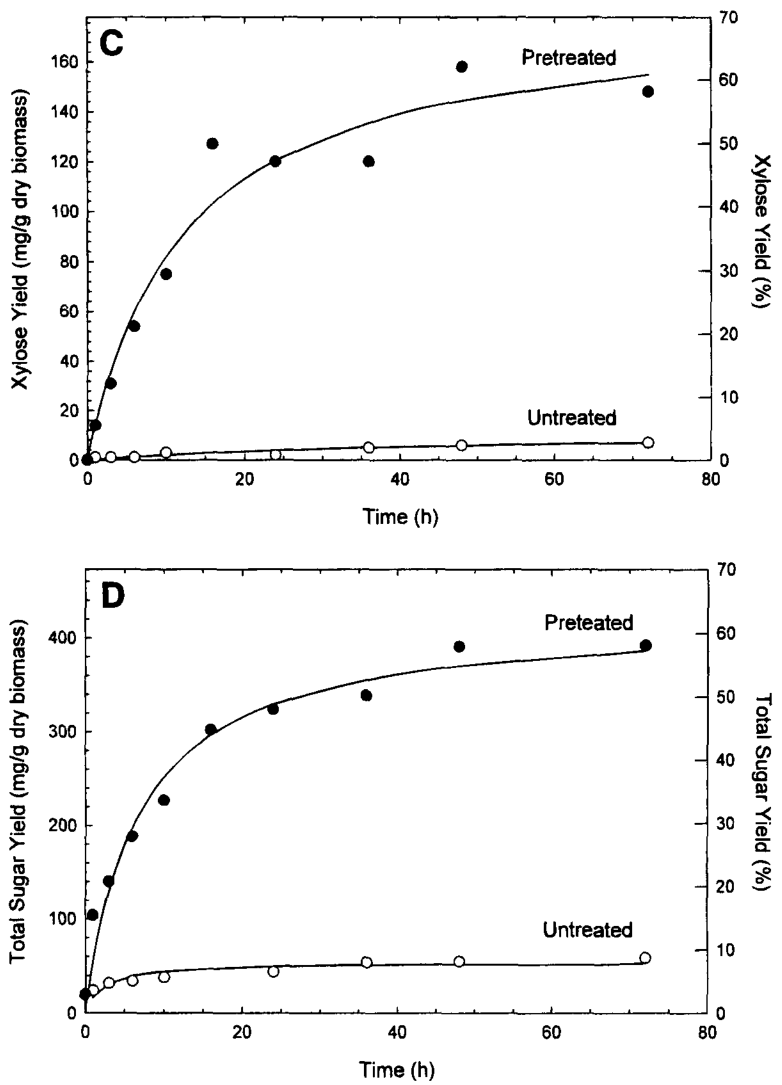

Fig. 3. *(continued)*

Lime Recovery and Recycle

Lime, although inexpensive, still requires recovery and recycle for it to be an economically viable pretreatment agent. The costs of various recovery alternatives have been evaluated *(35)*. For example, the pretreated biomass can be washed with water to remove the lime. The wash water can be contacted with carbon dioxide to precipitate calcium carbonate, which subsequently may be separated from the liquid and converted to lime using a lime kiln. The lime kiln operates at a high temperature (900°C), so the exhaust gases are hot enough to generate steam for motive power or process heat; thus, there is not a significant energy penalty asso-

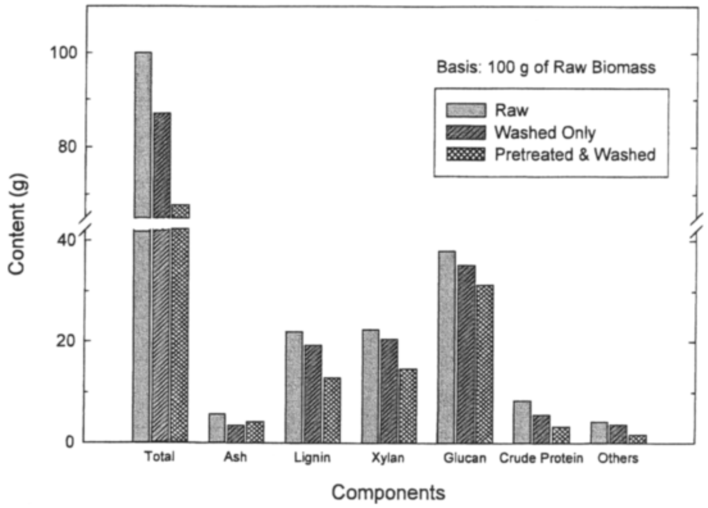

Fig. 4. Material balances for raw, washed only, and pretreated and washed switchgrass. (Pretreatment conditions: 120°C, 2 h, 0.1 g $Ca(OH)_2$/g dry biomass, 9 mL water/g dry biomass.)

Table 5
Summary of Water-Solubility of Switchgrass Components Before and After Lime Pretreatment

Components	Raw Composition (g component/g total)	Weight Loss Percentage[a] Washed Only	Weight Loss Percentage[a] Pretreated & Washed	Amount Removed by Lime Pretreatment
Total	-----	13.0%	32.4%	19.4%
Ash	0.06	40.6%	27.6%	-13.0%
Lignin	0.22	11.4%	40.8%	29.4%
Xylan	0.22	8.5%	35.0%	26 5%
Glucan	0.38	7.7%	17.3%	9.7%
Crude Protein	0 08	31.8%	59.7%	27.9%
Others	0.04	19.2%	63.3%	44.1%

[a]Weight percentage based on the initial weight of each component.

ciated with operating the lime kiln. This process allows lime to be recycled so that the cost of lime consumption is effectively minimized.

CONCLUSIONS

The recommended conditions for lime pretreatment of switchgrass are: temperature = 100–120°C, time = 2 h, lime loading = 0.1 g $Ca(OH)_2$/g dry biomass, water loading = 9 mL/g dry biomass, and particle size = 20 mesh and finer. Under these conditions, the 3-d total sugar yield of pre-

Table 6
Summary of Effects of Various Alkaline Pretreatment Agents

Pretreatment Agent	Biomass	Untreated Digestibility (%)	Treated Digestibility[a] (%)	Ratio of Digestibility	Reference
NaOH	Corn stalks	51.55[b]	91.84[b]	1.78	*(16)*
	Sorghum stalks	49.93[b]	93.64[b]	1.88	*(16)*
	Sweet potato vine	38.59[b]	78.05[b]	2.02	*(16)*
	Barley straw	54.8[c]	67.4[c]	1.23	*(18)*
	Barley straw	47.6[c]	75.7[c]	1.59	*(23)*
	Barley straw	37.9[d]	59.7[d]	1.58	*(25)*
	Pea straw	41 7[d]	54 6[d]	1.31	*(25)*
	Bagasse	32.8[d]	51.7[d]	1.58	*(25)*
	Sunflower hulls	16.6[d]	23.1[d]	1.39	*(25)*
	Soya-bean straw	35.8[d]	44.2[d]	1.23	*(29)*
NH_3 or NH_4OH	Corn stover	53.2[c]	62 8[c]	1.18	*(20)*
	Barley straw	37.9[d]	59.7[d]	1.58	*(25)*
	Pea straw	41.7[d]	54 6[d]	1.31	*(25)*
	Bagasse	32.8[d]	51.7[d]	1.58	*(25)*
	Sunflower hulls	16.6[d]	23 1[d]	1 39	*(25)*
	Soya-bean straw	35.8[d]	43.3[d]	1.21	*(29)*
KOH	Barley straw	47.6[c]	69.7[c]	1.46	*(23)*
$Ca(OH)_2$	Switchgrass	19.8[e]	85.6[e]	4.32	This work

[a]Best result reported in the literature.
[b]Crude fibre digestion coefficient.
[c]Organic matter digestibility (OMD).
[d]*in vitro* organic matter digestibility (IVOMD).
[e]3-d total sugar yield at 100 FPU cellulase/g dry biomass.

treated switchgrass increases from 19.8 to 85.6% at an excess enzyme loading. Table 6 summarizes the effects of pretreatments using various alkalis as pretreatment agents. In most cases, the authors concluded that calcium hydroxide is not as effective as other alkalis such as sodium hydroxide, ammonia, and potassium hydroxide. However, when proper conditions are employed, the digestibility improvement resulting from lime pretreatment is significant.

For industrial applications, a pretreatment agent must be effective, economical, safe, environmentally friendly, easy-to-use, and easy-to-recover. Lime meets these objectives and warrants further research.

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