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Lime Pretreatment of Crop Residues Bagasse and Wheat Straw

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ABSTRACT

Lime (calcium hydroxide) was used as a pretreatment agent to enhance the enzymatic digestibility of two common crop residues: bagasse and wheat straw. A systematic study of pretreatment conditions suggested that for short pretreatment times (1–3 h), high temperatures (85–135°C) were required to achieve high sugar yields, whereas for long pretreatment times (e.g., 24 h), low temperatures (50–65°C) were effective. The recommended lime loading is 0.1 g $Ca(OH)_2$/g dry biomass. Water loading had little effect on the digestibility. Under the recommended conditions, the 3-d reducing sugar yield of the pretreated bagasse increased from 153 to 659 mg Eq glucose/g dry biomass, and that of the pretreated wheat straw increased from 65 to 650 mg Eq glucose/g dry biomass. A material balance study on bagasse showed that the biomass yield after lime pretreatment is 93.6%. No glucan or xylan was removed from bagasse by the pretreatment, whereas 14% of lignin became solubilized. A lime recovery study showed that 86% of added calcium was removed from the pretreated bagasse by ten washings and could be recovered by carbonating the wash water with CO_2 at pH 9.5.

Index Entries: Crop residues; pretreatment; lime; cellulase; sugar.

INTRODUCTION

Crop residues are the nonedible portions of agricultural plants that remain in the field after harvest (e.g., wheat straw) or fractions discarded during crop processing (e.g., sugarcane bagasse). The US Department of

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Agriculture estimates that 333 million dry metric tons of crop residues will be produced during the year 2000 *(1)*. Because of the large quantity available and their richness in holocellulose (cellulose + hemicellulose), crop residues are a promising lignocellulosic biomass resource that can be converted into liquid fuels *(2)*.

Owing to lignocellulose structural characteristics, such as its lignin barrier, cellulose crystallinity, and hemicellulose acetylation *(3,4)*, crop residues require pretreatment to enhance their digestibility for biomass conversion processes. Pretreatments are usually grouped into three categories: physical, chemical, and multiple (physical + chemical). Tables 1 and 2 summarize the pretreatment conditions and results from previous studies of bagasse and wheat straw, respectively. It is obvious that chemical pretreatments have received the most attention, because although simple and clean, physical pretreatments are relatively ineffective in enhancing biomass digestibility *(14)*, and multiple pretreatments rarely result in higher biomass digestibility than that achieved by single pretreatments *(13)*.

Many chemicals have been studied as pretreatment agents for chemical pretreatments, such as acids *(6,13,14,20)*, alkalis *(7,9,10,13,16–20)*, gases *(9)*, cellulose solvents *(11,20)*, alcohols *(13,14)*, oxidizing agents *(9,14)*, and reducing agents *(14)*. Among these chemicals, alkali is the most popular pretreatment agent, because it is relatively inexpensive and results in less cellulose degradation *(22)*. Sodium hydroxide *(7,9,13,16)*, lime (calcium hydroxide) *(9,10,17,18)*, ammonia *(9,10,12,20)*, and urea *(9,10,17)* are the common alkalis used as pretreatment agents. Although sodium hydroxide increases biomass digestibility significantly, it is difficult to recycle *(10)*, is relatively expensive ($0.68/kg) *(23)*, and is dangerous to handle. Because it is volatile, ammonia can be recycled easily, but it is moderately expensive ($0.25/kg) *(23)* and needs careful handling. Urea is safe to handle, but it is also modestly expensive ($0.17/kg) *(23)* and is less effective in enhancing biomass digestibility. In contrast, lime has many advantages. It is safe and very inexpensive ($0.06/kg) *(23)*. In addition, this article will show that lime can be recovered by carbonating wash water with CO_2.

In fact, because of its low cost and safety, other researchers have observed that lime has a great potential as a pretreatment agent *(10,17,18)*. Because lime is less soluble than other alkalis, Ibrahim and Pearce *(9)* first applied a soaking method, where a high water loading (20 mL/g dry biomass) was used to overcome its low solubility. They concluded that the soaking method only increased biomass digestibility slightly (41% for spray method vs 43% for soaking method) and lime was not as effective as sodium hydroxide in enhancing bagasse digestibility (43% for lime vs 57% for NaOH). Please note that the same pretreatment conditions (ambient temperature and 24 h) were employed for both pretreatments, regardless

of the type of alkali used. This put lime pretreatment at a disadvantage, because lime is a weaker alkali and has a lower solubility than sodium hydroxide. Playne *(10)* achieved a significant improvement in bagasse digestibility (20% untreated vs 72% pretreated) by increasing the lime loading to 0.3 g $Ca(OH)_2$/g dry biomass and the pretreatment time to 192 h. Verma *(17)* studied the effects of various lime loadings and pretreatment times for wheat straw and increased the digestibility from 48 to 74%. Although these authors enhanced the effectiveness of lime pretreatment by varying one or two process variables, none of them studied systematically the effects of different combinations of all process variables. Furthermore, the effect of pretreatment temperature was rarely studied; ambient temperature was used in most literature. This study systematically explores the effects of various lime pretreatment conditions for two common crop residues: bagasse and wheat straw. In addition, enzymatic hydrolysis profiles, material balances, and lime recovery were performed on bagasse.

MATERIALS AND METHODS

Sample Preparation

Bagasse and wheat straw were ground using a Thomas-Wiley laboratory mill and then passed through a 40-mesh screen.

Lime Pretreatment

Bagasse and wheat straw were pretreated with lime (calcium hydroxide) in the presence of water under various conditions (i.e., temperature, time, lime loading, water loading, and biomass particle size). For laboratory purposes, after pretreatment, the pH was adjusted to 4.8 by adding acetic acid. The detailed procedure of lime pretreatment was described previously *(24)*.

Enzymatic Hydrolysis

The pretreated and untreated biomass were hydrolyzed at 50°C, pH 4.8, for 3 d in a 100-rpm air-bath shaker, with a cellulase loading of 5 FPU/g dry biomass and an excess cellobiase loading of 28.4 CBU/g dry biomass. The cellobiase (Novozym 188, Novo Nordisk Bioindustrials, Inc., Franklinton, NC) was employed to relieve the cellobiose inhibition on cellulase *(22)* and had an activity of 250 CBU/mL. Two batches of cellulase were used in this study. The cellulase used in the pretreatment condition studies was Cytolase 300P (Genecor, Inc., Rolling Meadows, IL) and had an activity of 215 FPU/g dry powder. The cellulase used in the hydrolysis pro-

Table 1
Pretreatment Conditions[a] and Digestions for Bagasse

Pretreatment	Temperature °C	Time	Water loading mL/g dry biomass	Pretreatment agent loading, g/g dry biomass or other conditions	Particle size	Untreated digestibility %	Best-treated digestibility %	Reference
Physical								
γ-Ray irradiation	—	1, 3, **20** h	—	Radiation dosage = 10^6, 10^7, 5×10^7, **10^8** rad	N/A[b]	10[c]	30[c]	*(5)*
Solvolysis	185, 197, **208**	20, 25, **29** min	N/A[b]	Pressure = 10, 13.5, **17** atm	N/A[b]	35[d]	64[d]	*(6)*
Steam explosion	195	15 min	—	—	N/A[b]	34[e]	62[e]	*(7)*
Liquid hot water	220	2 min	N/A[b]	Pressure = 5 MPa	+14 mesh	11[f]	107[f]	*(8)*
Chemical								
NaOH	Ambient	24 h	1	0.03, 0.06, **0.09,** 0.12	Particulate	33[h]	57[h]	*(9)*
NaOH	Ambient	2 h	30	2.4	2 mm	34[e]	99[e]	*(7)*
$Ca(OH)_2$	Ambient	24 h	1, **20**	0.05, **0.1,** 0.15	Particulate	33[h]	43[h]	*(9)*
$Ca(OH)_2$	Ambient	192 h	1.74	0.06–**0.3**	2.25 mm	20[h]	72[h]	*(10)*
$Ca(OH)_2$ + other chemicals	Ambient	192 h	1.74	$Ca(OH)_2$ = 0.18, NH_3 = 0.025, NaOH = 0.024, **$NaCO_3$ = 0.04**	2.25 mm	20[h]	73[h]	*(10)*
NH_3	Ambient	120, **240** h	0	0.035, **0.058**	Particulate	33[h]	44[h]	*(9)*
NH_4OH	Ambient	720 h	0.3	0.026, 0.052, **0.077**	Particulate	33[h]	37[h]	*(9)*
Urea	Ambient	24, 168, 336, **672** h	1	0.02, 0.04, 0.06, **0.08**	Particulate	33[h]	47[h]	*(9)*
$NaClO_2$	Ambient	168 h	2	**0.03,** 0.06, 0.09, 0.12	Particulate	33[h]	44[h]	*(9)*

$NaClO_2$ + NH_3	Ambient	168 h	2	**0.03,** 0.06, 0.09, 0.12	Particulate	33[h]	47[h]	*(9)*
SO_2	20, 60, **120**	1, 2, **3** h	3	N/A[b]	Particulate	33[h]	44[h]	*(9)*
Cl_2	Ambient	N/A[b]	—	0.03, **0.06,** 0.09	Particulate	33[h]	20[h]	*(9)*
H_3PO_4	185, 197, **208**	20, 25, **29** min	4	0.015, 0.022, **0.029,** 0.036, 0.044	N/A[b]	35[d]	70[d]	*(6)*
Cadoxen solvent	Ambient	12 h	5	N/A[b]	0.5–2 mm	20[g]	98[g]	*(11)*
Multiple								
Steam explosion + alkalis (NH_3 + urea)	steam exp. = 200, alkali = ambient	steam exp. = 5 min, alkali = 504 h	Alkali = 1.048	NH_3 = 0.247, urea = 0.094, pressure = 6.9 MPa	2.25 mm	20[h]	74[h]	*(10)*
Steam explosion + NaOH washing	steam exp. = 190, washing = 100	steam exp. = 15 min, washing = 1 h	0.05	NaOH = 1%	N/A[b]	34[c]	91[c]	*(7)*
AFEX	93	50 min	0.25	NH_3 = 1.5, pressure = 32.2 atm	1 × 15 mm	11[c]	68[c]	*(12)*

[a]Bold conditions correspond to the best pretreatment results.
[b]Not available.
[c]Enzymatic digestibility.
[d]*In situ* dry matter digestibility.
[e]*in situ* degradability potential of insoluble fraction.
[f]Ethanol yield during SSF.
[g]Glucan conversion.
[h]*In vitro* organic matter digestibility.

Table 2
Pretreatment Conditions[a] and Digestions for Wheat Straw

Pretreatment	Temperature, °C	Time	Water loading, mL/g dry biomass	Pretreatment agent loading, g/g dry biomass or other conditions	Particle size	Untreated digestibility, %	Best-treated digestibility, %	Reference
Physical								
Ball milling	—	4 h	—	Rotation speed = 52 rpm	−10 mesh	5.5[c]	24[c]	*(13)*
Fitz milling	—	N/A[b]	—	—	−10 mesh	5.5[c]	9[c]	*(13)*
Roller milling	—	0.25, **0.5** h	—	—	−10 mesh	5.5[c]	15[c]	*(13)*
Extrusion	N/A[b]	N/A[b]	—	—	−10 mesh	5.5[c]	5.8[c]	*(14)*
γ-Ray irradiation	—	N/A[b]	—	Radiation dosage = 10^7, 3×10^7, **5×10^7** rad	−10 mesh	5.5[c]	19[c]	*(14)*
Steam explosion	230	1 min	—	—	5 cm	12[c]	47[c]	*(15)*
Chemical								
NaOH	N/A[b]	N/A[b]	0.3	0.01–0.15	0.3175 cm	34[d]	82[d]	*(16)*
NaOH	Ambient, **129**	2 h	10	0.1	−10 mesh	6[c]	62[c]	*(13)*
$Ca(OH)_2$	Ambient	24, 48, **72,** 96, 120 h	N/A[b]	**1%,** 2%, 3%, 4%	N/A[b]	48[h]	74[h]	*(17)*
$Ca(OH)_2$	Ambient	24 h	11	0.09	3 cm	54[i]	62[i]	*(18)*
NaOH + $Ca(OH)_2$	Ambient	120 h	1.5	1% + 3%, 3% + 1%, 3% + 2%, **4% + 1%,** 4% + 0%	2.54 cm	41[d]	76[d]	*(19)*
NH_4OH	25°C for 8 h +	55°C for 72 h	N/A[b]	5%, **25%,** 50%	2.5–7.5 cm	11[c]	43[c]	*(20)*
Urea	Ambient	5, 6, 7, 8, 10, 12 weeks	1.2	3%, 4%, **5%**	N/A[b]	46[h]	59[h]	*(17)*

EDTA[f]	25°C for 8 h + 55°C for 72 h		N/A[b]	5% (v/w)	2.5–7.5 cm	11[c]	14[c]	*(20)*
H_2SO_4	98	1 h	16	0.73	−10 mesh	6[c]	25[c]	*(14)*
H_2SO_4	95, 120, **140,** 160	0–8.3 h	N/A[b]	5%	2 mm	23[c]	87[c]	*(21)*
Peracetic acid	100	0.5 h	—	10 mL/g dry biomass	−10 mesh	6[c]	55[c]	*(13)*
Ethylene glycol + HCl	ambient, **129**	1 h	—	Ethylene glycol = 12.5 mL/g dry biomass, HCl = 0.27 mL/g dry biomass	−10 mesh	6[c]	55[c]	*(13)*
DMSO[e]	25°C for 8 h + 55°C for 72 h		N/A[b]	5% (v/w)	2.5–7.5 cm	11[c]	16[c]	*(20)*
EDA[g]	25°C for 8 h + 55°C for 72 h		N/A[b]	28% (v/w)	2.5–7.5 cm	11[c]	60[c]	*(20)*
Na_2SO_3	Ambient, **129**	2 h	6	0.96	−10 mesh	6[c]	38[c]	*(14)*
NaOCl	Ambient, **129**	2 h	9.5	0.5	−10 mesh	6[c]	56[c]	*(14)*
Butanol	175	1.5 h	5	5 mL/g dry biomass	−10 mesh	6[c]	11[c]	*(14)*
Multiple								
Fitz milling + NaOH	Ambient, **129**	2 h	10	0.1	−10 mesh	6[c]	49[c]	*(13)*

[a]Bold conditions correspond to the best pretreatment results.
[b]Not available.
[c]Glucan conversion.
[d]In vitro dry matter digestibility.
[e]Dimethylsulfoxide.
[f]Ethylenediaminetetraacetic acid.
[g]Ethylenediamine.
[h]In vivo dry matter digestibility.
[i]In vivo organic matter digestibility.

file studies was Cytolase CL (Environmental BioTechnologies, Inc., Santa Rosa, CA) and had an activity of 91 FPU/mL. The activities of the cellulase were determined using the filter paper assay *(25)*.

To investigate lime pretreatment conditions, enzymatic hydrolysis samples (ca. 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.

Sugar Measurement

Reducing sugars were measured using the dinitrosalicylic acid (DNS) assay *(26)*. A 200 mg/dL glucose standard solution (Yellow Springs Instruments Co., Inc., Yellow Springs, OH) was used for the calibration. Thus, the reducing sugars were measured as "equivalent glucose." The sugar content in the enzymes (ca. 45 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 (from the lime neutralization) and were called "corrected" reducing sugar yields. The correction factor depends lime loading (*see* Effects of Calcium Acetate Inhibition).

Glucose and xylose were measured using high-performance liquid chromatography (HPLC). A Bio-Rad Aminex HPX-87P column was used for carbohydrate separation. Degassed reverse osmosis deionized (RODI) water, which was filtered through a 45-μm nylon membrane filter, was used as the mobile-phase liquid. The column operating temperature was 85°C and the eluant flow rate was 0.6 mL/min. Because of high cellobiase loading, cellobiose concentrations were negligible. Therefore, throughout the article, "total sugar" denotes the summation of glucose and xylose, because no other carbohydrates were detected.

Although HPLC produces more accurate analytical results, it was too time-consuming for a broad survey of lime pretreatment conditions. The DNS assay was accurate enough to screen the pretreatment conditions rapidly in the early stages of this study. The most promising conditions then were verified using HPLC.

Material Balances

To remove solubles, untreated and pretreated bagasse 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 sugar yield determination and material balances was described previously (*24*).

The composition of bagasse was determined using the standard analysis procedures provided by the National Renewable Energy Laboratory (NREL) (*25*). Two-stage sulfuric acid hydrolysis was used to determine carbohydrates (glucan and xylan). Klason lignin was the insoluble residue remaining after first swelling bagasse in 72% sulfuric acid at 20°C for 2 h and then boiling the sample in 3% sulfuric acid for 4 h. The soluble fraction was used to determine the acid-soluble lignin using a spectrophotometric method. The lignin content was determined as the summation of Klason lignin and acid-soluble lignin. The ash content was determined as the weight of the bagasse residue remaining after ignition at 575°C for at least 8 h. The crude protein was determined using a modified micro-Kjeldahl method by the Forage Testing Laboratory of Texas A&M University.

Lime Recovery

The method for recovering lime is to wash pretreated biomass with water and then to contact the wash water with CO_2 to form insoluble $CaCO_3$, which can be separated from liquid and converted to lime. The lime recovery study was conducted using two approaches: continuous recovery and batch recovery.

Continuous Recovery

An apparatus for continuous recovery is employed to simulate the actual industrial process (Fig. 1). The pretreated bagasse was packed in a 2.5 cm id × 21.6 cm high glass column. A peristaltic pump was used to maintain the flow rate at 20 mL/min. Carbon dioxide was bubbled through the wash water in a 300-mL flask to precipitate $CaCO_3$ until the pH of the wash water reached 9.5 from about 12.0. A glass fiber filter was used to remove $CaCO_3$ present in the overflow from the flask. To measure the calcium concentration in the water during washing, 1-mL samples were periodically withdrawn from the column inlet and outlet. The calcium concentration of the liquid was measured using an atomic adsorption apparatus with potassium chloride (1 mg/mL) as an internal standard. Depending on the calcium concentration, the samples were diluted from 11 to 1331 times to reduce the calcium concentration to the calibration range of 1–5 ppm. The washing process took about 110 min. The washed bagasse was removed from the column and air-dried. About 0.5 g of the air-dried sample was dissolved in nitric acid to determine its calcium content by atomic adsorption.

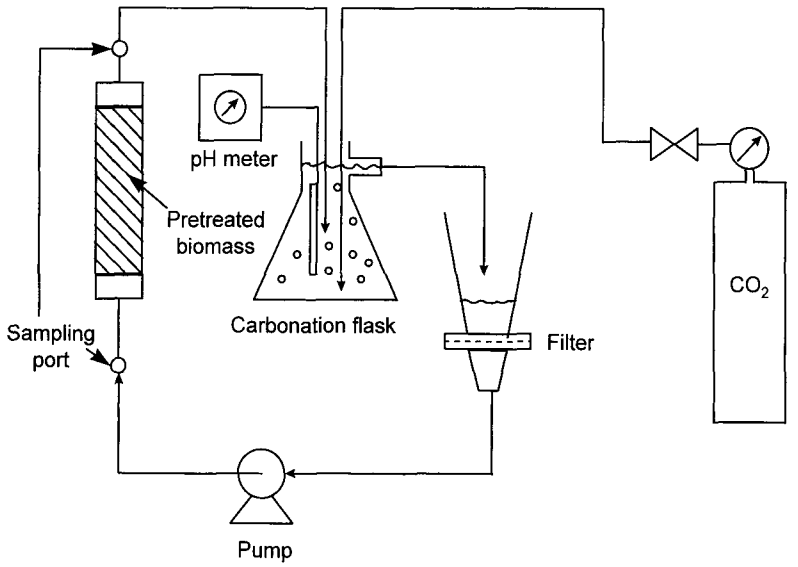

Fig. 1. Apparatus for continuous lime recovery.

Batch Recovery

The principle of batch recovery is the same as that of continuous recovery. The pretreated bagasse was transferred to a 250-mL beaker and mixed with $CaCO_3$-saturated water, which was used as wash water to imitate the real recovery process. The mixture was stirred for 10 min and then filtered through a double-layer filter consisting of a polyester cloth as the upper layer and a Rapid-Flow Milk Filter (Johnson and Johnson, New Brunswick, NJ) as the lower layer. The filtrate was bubbled with CO_2 until the pH dropped from about 12.0 to 7.0. Ammonium hydroxide was added to increase the pH to 9.5. The carbonated liquid was left for 24 h to settle. One milliliter of sample was taken before carbonation and after precipitation to measure the calcium concentration by atomic adsorption. Some experiments used 6 washings, whereas some used 10 washings.

RESULTS AND DISCUSSION

Effects of Calcium Acetate Inhibition

The pH of lime-pretreated biomass is between 11.0 and 12.0, which is much higher than the pH optimum of cellulase (i.e., 4.8). Therefore, either biomass washing or pH adjustment with acids is necessary. Industrially, the pretreated biomass will be washed with water to remove the lime. The wash water will then be carbonated with CO_2 and the resulting $CaCO_3$ will be converted to lime in a lime kiln. The feasibility of lime recovery will be discussed later. For convenience in a laboratory setting, the lime was neu-

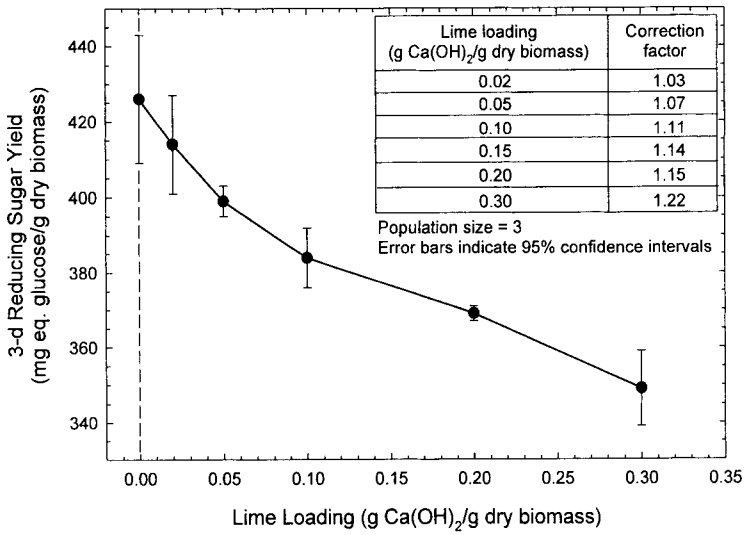

Fig. 2. Effects of calcium acetate inhibition. Pretreatment conditions: 120°C, 1 h, 0.1 g $Ca(OH)_2$/g dry biomass, 10 mL/g dry biomass.

tralized with acetic acid. However, the resulting calcium acetate may inhibit cellulase *(24)*. The following experiment was conducted to measure the effects of calcium acetate inhibition.

A large sample (ca. 300 g) of bagasse (−40 mesh) was treated with lime under the following conditions: 120°C, 1 h, 0.1 g $Ca(OH)_2$/g dry biomass, 10 mL water/g dry biomass. The pretreated bagasse was washed with fresh distilled water 11 times to remove the lime. Then, the pretreated and washed bagasse was squeezed to remove liquid, and the solids were divided into 21 flasks containing citrate buffer (0.05 *M*, pH 4.8) and various lime additions—0, 0.02, 0.05, 0.1, 0.15, 0.2, and 0.3 g $Ca(OH)_2$/g dry biomass, each in triplicate. 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 were measured using the DNS assay at time zero and after 3 d. The reducing sugar yields of the time-zero samples determined the sugar content of the enzymes and were subtracted from the 3-d sugar yields.

Figure 2 shows the 3-d reducing sugar yields and the correction factors at various lime loadings for Cytolase 300P. Calcium acetate inhibition caused about 3–18% loss of sugar yields. The correction factors reported in Fig. 2 were used in the pretreatment condition studies to correct the 3-d reducing sugar yields. The calcium acetate inhibition on Cytolase CL was previously determined *(24)* and a correction factor of 1.015 was used to correct the reducing sugar yields in the hydrolysis profile studies where the lime loading was 0.1 g $Ca(OH)_2$/g dry biomass.

Table 3
Lime Pretreatment Conditions Explored for Bagasse

	Time, h	Temperature, °C	Lime loading, g $Ca(OH)_2$/g dry biomass	Water loading, mL/g dry biomass	Particle size
Study 1	6, 12	65, 125	0.3	6–16	−40 mesh
Study 2	1–24	135	0.1–0.3	10, 15	−40 mesh
Study 3	1–24	100	0.1–0.3	10, 15	−40 mesh
Study 4	1–24	50	0.02–0.3	10	−40 mesh
Study 5	3, 24	85	0.05–0.2	10	−40 mesh
Study 6	3–24	65	0.05–0.2	10	−40 mesh
Study 7	24	65	0.1, 0.15	10	−1 × 1 mm +40 mesh, −40 mesh

Because calcium sulfate is insoluble and might not inhibit cellulase, we studied the feasibility of using sulfuric acid to replace acetic acid for neutralization. The resulting sugar yields were extremely low (about zero) at lime loadings > 0.1 g $Ca(OH)_2$/g dry biomass. This resulted because the insoluble calcium sulfate blocked sulfuric acid from reaching lime at the interior of the biomass particle; that is, the lime was coated by a layer of calcium sulfate. Over a brief period of time, the lime appeared to be neutralized; however, after a long contact, the lime slowly leaked out and raised the pH such that the enzyme activity was significantly destroyed.

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. Tables 3 and 4 show the ranges of conditions explored for bagasse and wheat straw, respectively.

Bagasse

Figure 3A shows that there was not much effect from water loading. Although water loadings as low as 6 mL/g dry biomass are effective, and decreasing water loading would decrease equipment size, low water loading results in a very thick paste that may cause handling and transportation problems. Therefore, a water loading of 10–14 mL/g dry biomass, which gave slightly better sugar yields (ca. 5–9% increase in sugar yields), can be used.

Figure 3B shows that the effect of lime loading had a similar pattern for four pretreatment times (1, 3, 6, and 24 h). The reducing sugar yields

Table 4
Lime Pretreatment Conditions Explored for Wheat Straw

	Time, h	Temperature, °C	Lime loading, g $Ca(OH)_2$/g dry biomass	Water loading, mL/g dry biomass	Particle size
Study 1	3, 24	65	0.1	6–19	−40 mesh
Study 2	1, 3	65	0.05–0.2	10	−40 mesh
Study 3	3, 24	50	0.05–0.2	10, 15	−40 mesh
Study 4	1–24	85	0.05–0.2	10	−40 mesh
Study 5	1–24	125	0.05–0.2	10	−40 mesh

increased when the lime loading increased from 0.02 to 0.15 g $Ca(OH)_2$/g dry biomass and decreased thereafter. It indicates that a lime loading of at least 0.1 g $Ca(OH)_2$/g dry biomass is required for an effective pretreatment. Figure 3B also shows that the sugar yields increased with increasing pretreatment times.

Figure 3C shows the 3-d reducing sugar yields as a function of pretreatment temperature at various pretreatment times. For short pretreatment times (i.e., 1–3 h), high temperatures (i.e., 85–135°C) are required to achieve good sugar yields, whereas for long pretreatment times (e.g., 24 h), temperatures as low as 65°C are effective.

Figure 3D shows the effect of biomass particle size for two lime loadings (0.1 and 0.15 $Ca[OH]_2$/g dry biomass). Two different particle sizes were studied. It shows that the lime pretreatment is as effective for coarser biomass (between 1 × 1 mm and 40 mesh) as for fine biomass (−40 mesh). However, this comparatively coarse biomass is still rather fine. A study with larger particles is needed.

Wheat Straw

Similar to the case of bagasse, water loading had little effect on the digestibility (Fig. 4A), so a water loading of 10 mL/g dry biomass was used for the subsequent experiments.

Figure 4B shows the effect of pretreatment time and temperature. For pretreatment times as short as 1 h, high temperatures (e.g., 125°C) are necessary for effective pretreatments. For pretreatment times of 3 and 24 h, the reducing sugar yields were high for 50–85°C and decreased substantially when the temperature rose to 125°C. These results were similar to those obtained from bagasse.

Four combinations of pretreatment times and temperatures that resulted in high sugar yields were employed to explore the effect of lime loadings. High temperatures (85–125°C) were expected to increase the

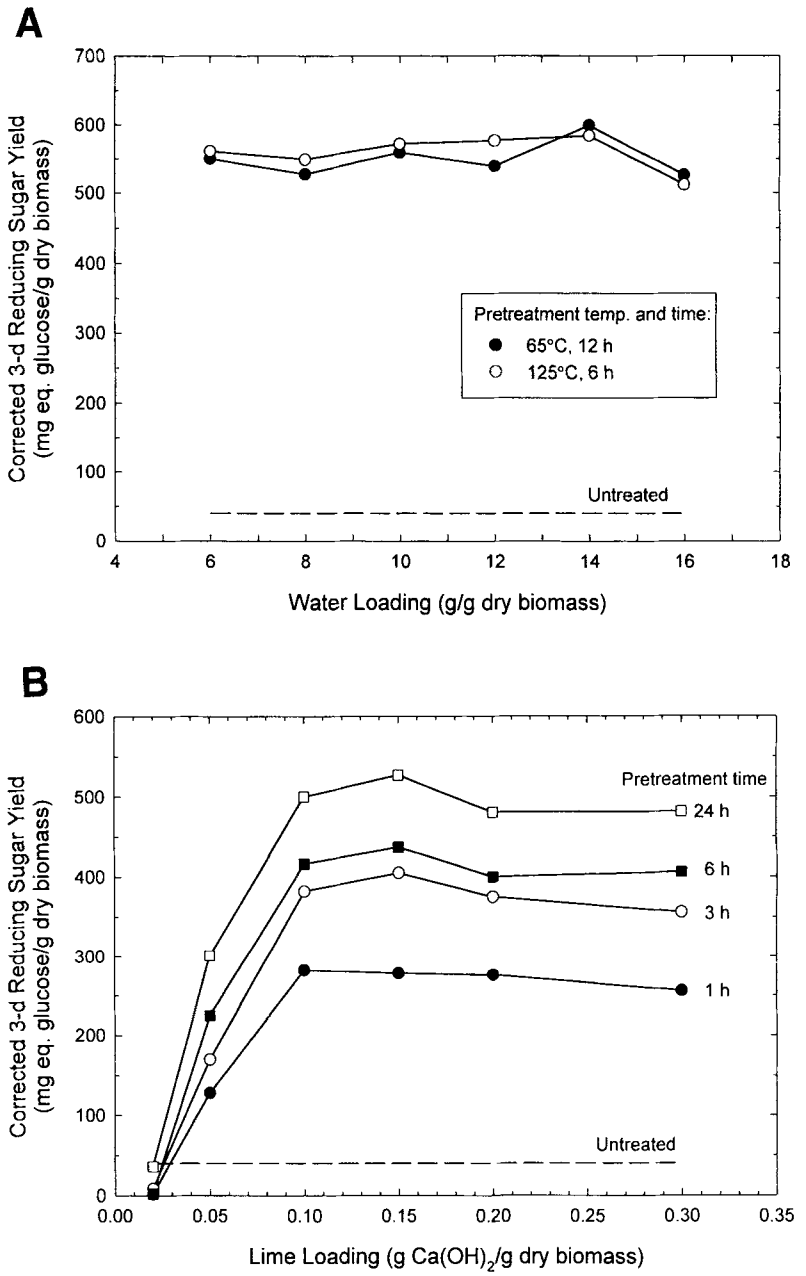

Fig. 3. Effects of lime pretreatment conditions for bagasse: (**A**) water loading, (**B**) lime loading, (**C**) temperature and time, (**D**) particle size. Pretreatment conditions: (**A**) 0.3 g $Ca(OH)_2$/g dry biomass, (**B**) 50°C, 10 mL water/g dry biomass, (**C**) 0.1 g $Ca(OH)_2$/g dry biomass, 10 mL water/g dry biomass, (**D**) 65°C, 24 h, 10 mL water/g dry biomass; enzymatic hydrolysis condition: 50°C, 3 d, pH 4.8, 5 FPU cellulase/g dry biomass, 28.4 CBU cellobiase/g dry biomass.

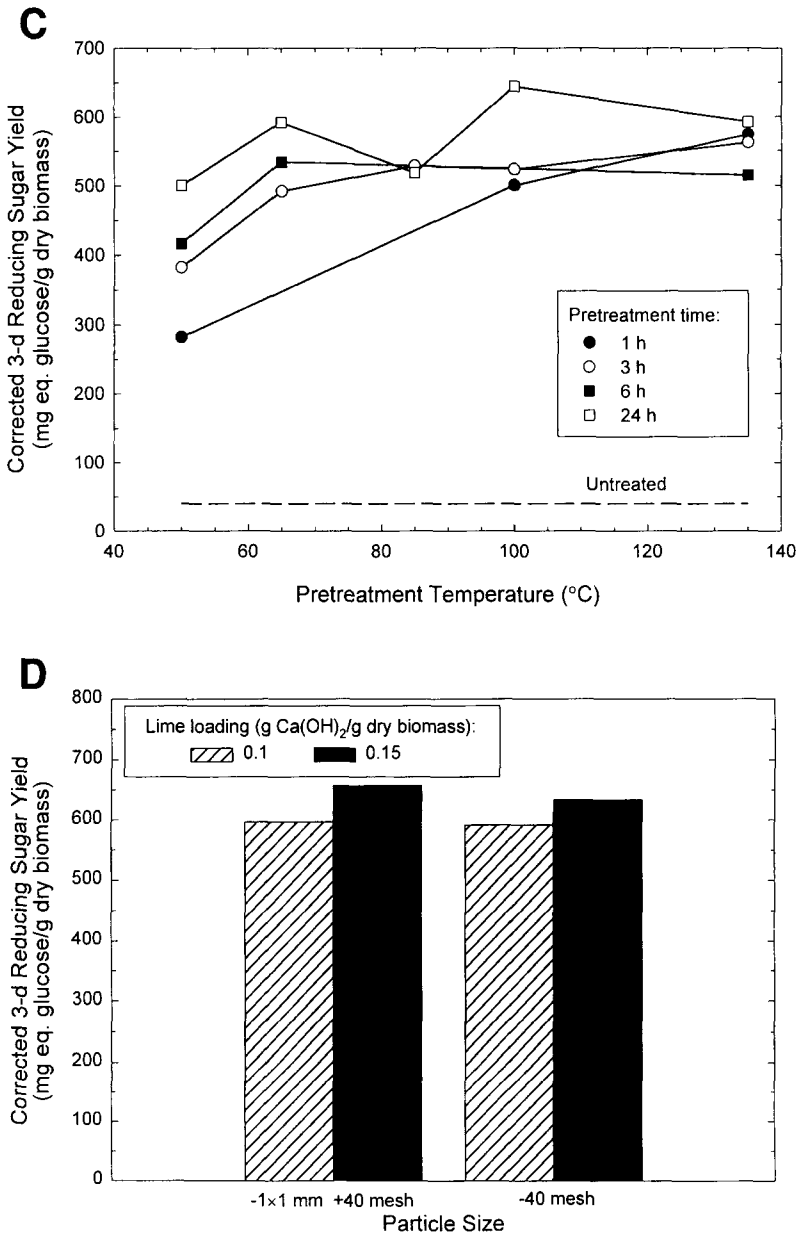

Fig. 3. *(continued)*

reaction rate, so that the digestibility might increase significantly at high lime loadings. However, Fig. 4C suggests that the digestibility increased only slightly at lime loadings > 0.1 $Ca(OH)_2$/g dry biomass, as similarly shown in Fig. 3B. Therefore, there appears to be a "critical" lime loading above which further lime addition is ineffective. A "critical" lime loading is consistent with the hypothesis that the biomass digestibility is sig-

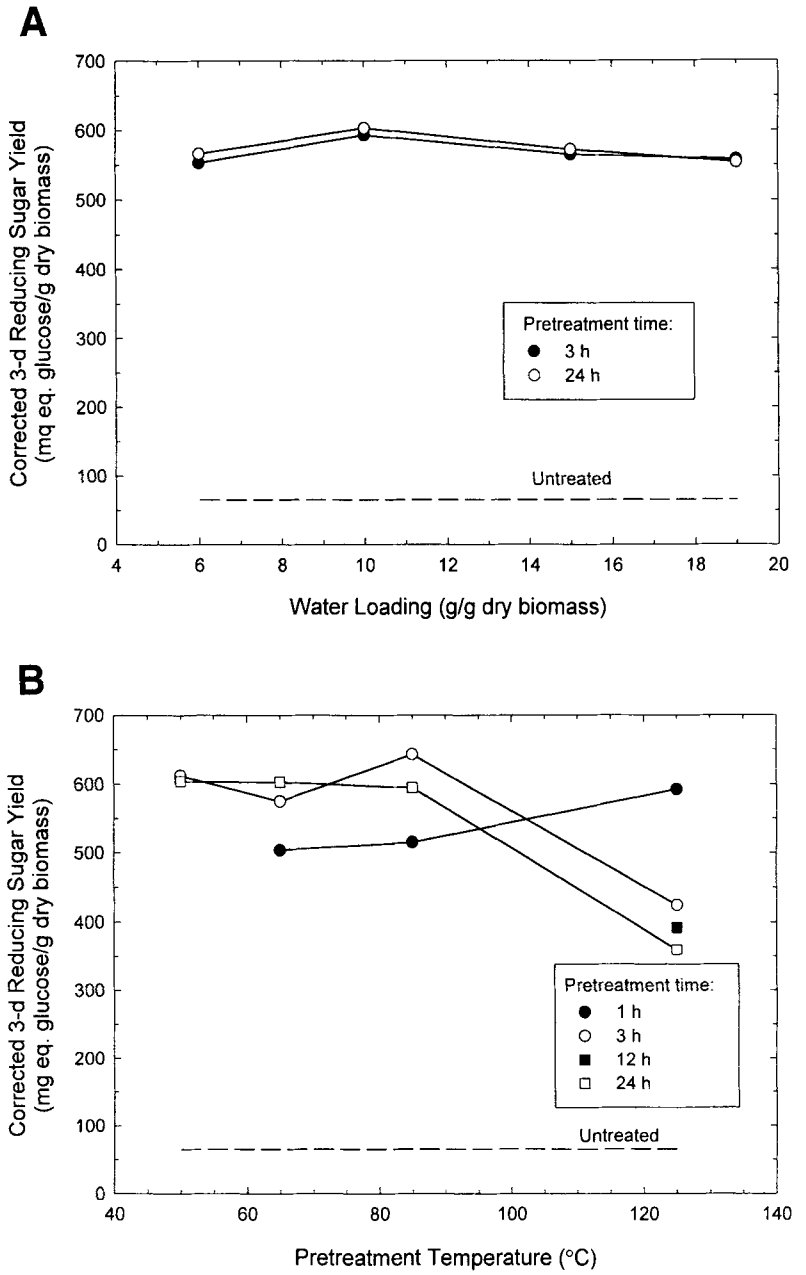

Fig. 4. Effects of pretreatment conditions for wheat straw: (**A**) water loading, (**B**) temperature and time, (**C**) lime loading. Pretreatment conditions: (A) 65°C, 0.1 g $Ca(OH)_2$/g dry biomass, (B) 0.1 g $Ca(OH)_2$/g dry biomass, 10 mL water/g dry biomass, (C) 10 mL water/g dry biomass; enzymatic hydrolysis condition: 50°C, 3 d, pH 4.8, 5 FPU cellulase/g dry biomass, 28.4 CBU cellobiase/g dry biomass.

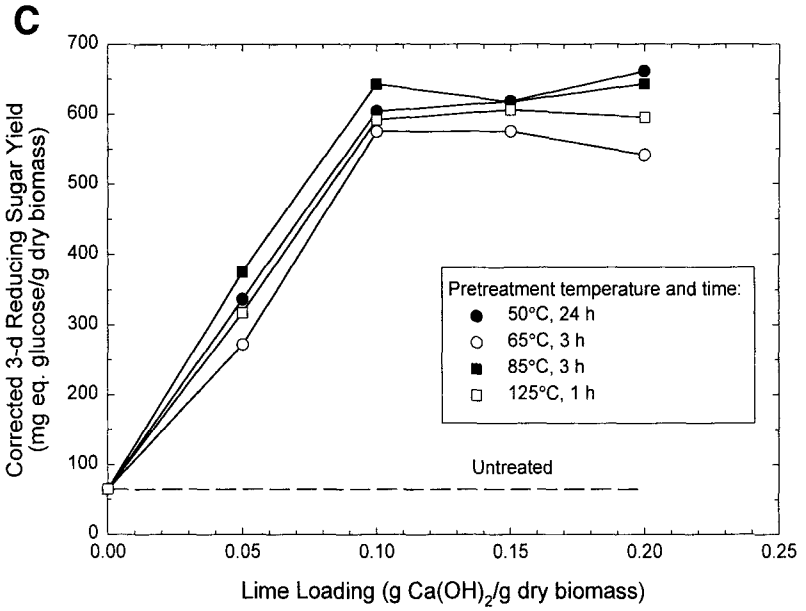

Fig. 4. *(continued)*

nificantly enhanced by removing acetate groups from hemicellulose *(4)*. Apparently, when enough lime has been added to remove acetate, further lime addition is not beneficial.

Hydrolysis Profiles

Three-day hydrolysis profiles were performed on pretreated and untreated bagasse to determine the reducing sugar yields as a function of time, as shown in Fig. 5. The bagasse was pretreated at 120°C for 1 h in the presence of 0.1 g $Ca(OH)_2$/g dry biomass and 10 mL water/g dry biomass. Figure 5 shows that lime pretreatment enhanced the digestibility significantly. Compared with the untreated bagasse, the 3-d reducing sugar yield and the 3-d total sugar yield of pretreated bagasse increased about 4 times (153 vs 659 mg Eq glucose/g dry biomass and 119 vs 499 mg/g dry biomass, respectively), the 3-d glucose yield increased about 3 times (99 vs 301 mg/g dry biomass), and the 3-d xylose yield increased about ten times (20 vs 197 mg/g dry biomass). This drastic increase in xylose yield was similar to the results obtained from our previous studies on switchgrass and poplar wood *(24,27)*, indicating 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 *(4)*. The hydrolysis profiles also show that 85% of the sugars were released in 24 h (Fig. 5D).

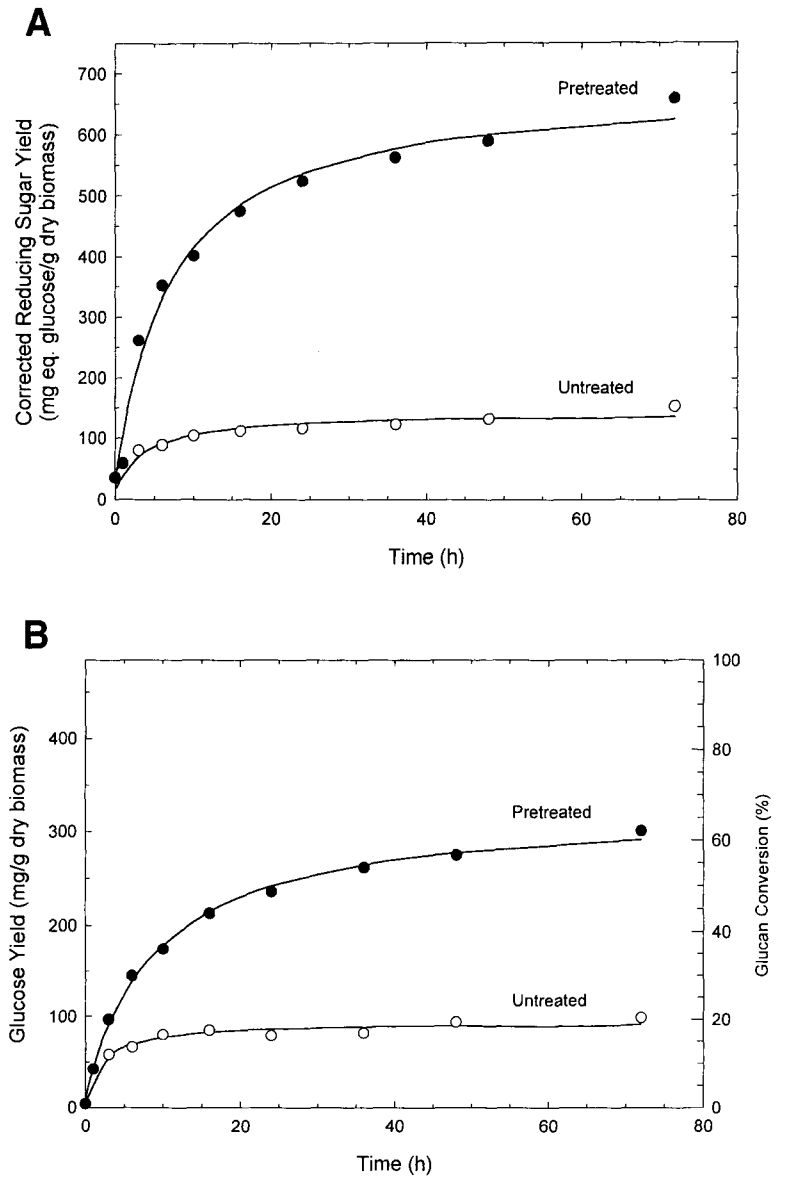

Fig. 5. Hydrolysis profiles for bagasse: (**A**) corrected reducing sugar, (**B**) glucose, (**C**) xylose, (**D**) total sugar. Pretreatment conditions: 120°C, 1 h, 0.1 g $Ca(OH)_2$/g dry biomass, 10 mL water/g dry biomass. Hydrolysis conditions: 50°C, pH 4.8, 5 FPU cellulase/g dry biomass, 28.4 CBU cellobiase/g dry biomass.

Discrepancies were observed between the reducing sugar yields and the total sugar yields. They may have resulted from the inaccuracies associated with expressing xylose as equivalent glucose.

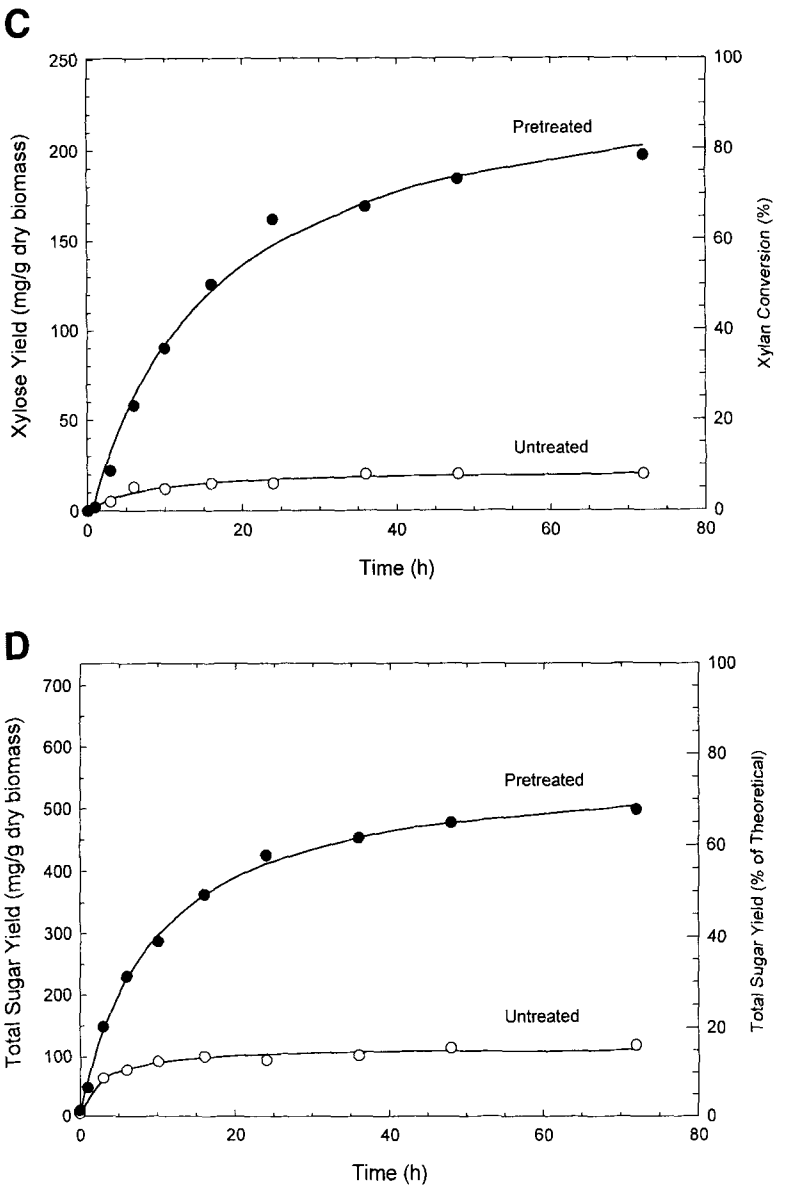

Fig. 5. *(continued)*

Material Balances

Figure 6 shows the compositions of raw, washed only, and pretreated-and-washed bagasse. Table 5 summarizes the losses of each component in bagasse before and after pretreatment, showing that the lime pretreatment does not remove much biomass (i.e., 3.64%). This result was expected,

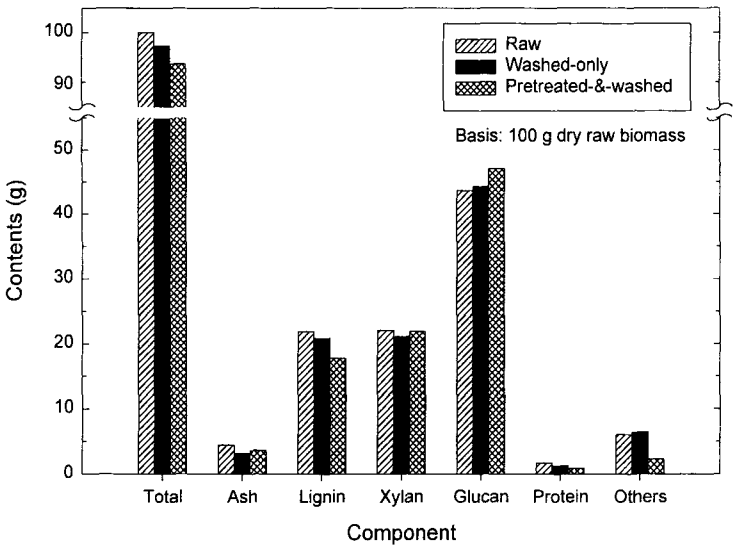

Fig. 6. Material balances for raw, washed only, and pretreated-and-washed bagasse. Pretreatment conditions: 120°C, 1 h, 0.1 g $Ca(OH)_2$/g dry biomass, 10 mL water/g dry biomass.

because bagasse is a crop residue in which the extractable biomass (mostly sucrose and other soluble components) has been removed during processing. No removal of ash, xylan, and glucan was observed, whereas 14% of lignin and 23% of crude protein were removed by the lime pretreatment.

Compared with other pretreatments, lime pretreatment is mild. Figure 7 summarizes the biomass yields (the ratios of the insoluble biomass solids after pretreatment and washing to the raw biomass) for bagasse pretreated by different methods. The biomass yield using lime pretreatment is the highest (i.e., 93.6%), which is an advantage for commercialization, because a high biomass yield is a basic prerequisite to high sugar yields in the subsequent enzymatic hydrolysis.

Lime Recovery

To minimize lime consumption, the feasibility of lime recovery was evaluated for bagasse. Figure 8A shows the calcium concentrations in the wash water for the continuous recovery process. After 60 min, the $CaCO_3$ in the wash water was allowed to settle for 24 h, so there is a break in the graph. The time-course data shown in Fig. 8A indicate that the carbonation and precipitation reduced the calcium content of the biomass considerably. The calcium analysis of the unwashed and washed bagasse shows that 75% of added calcium was removed from biomass by the continuous recovery process.

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

Components	Raw composition, g component/ g total	Weight loss percentage[a]		Amount removed by lime pretreatment
		Washed only	Pretreated-and-washed	
Total	—	2.76%	6.41%	3.64%
Ash	0.05	28.63%	19.69%	−8.95%
Lignin	0.22	4.89%	18.93%	14.03%
Xylan	0.22	4.39%	0.90%	−3.49%
Glucan	0.44	−1.42%	−7.85%	−6.42%
Crude Protein	0.02	24.56%	47.82%	23.27%
Others	0.06	−6.18%	61.89%	68.07%

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

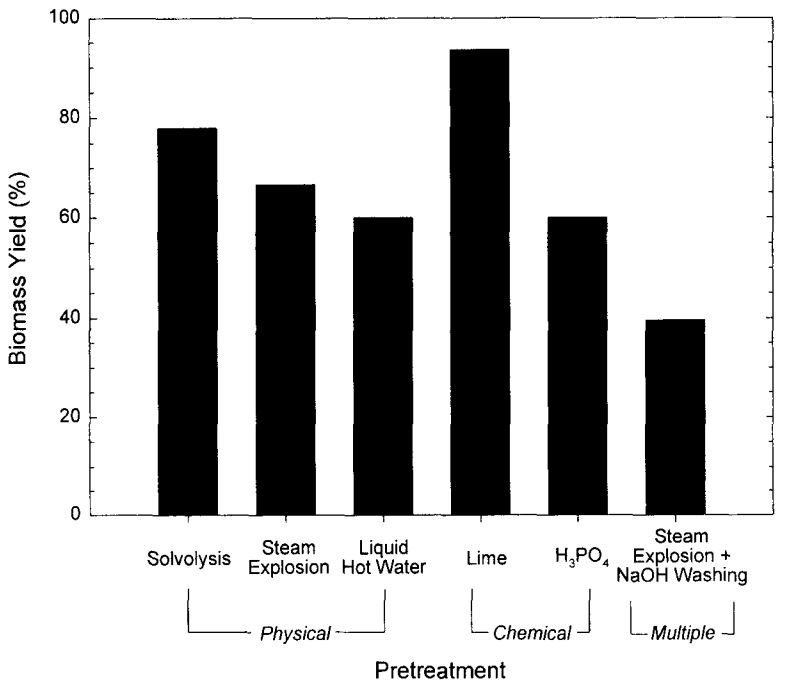

Fig. 7. Biomass yields for bagasse pretreated by several methods *(7,8)*.

Channeling inside the bagasse-filled column was observed during the continuous recovery, which might prevent the wash water from contacting some pretreated bagasse and thus lower the process efficiency. Therefore, a batch recovery—which provides good contact—was performed to overcome the difficulty. Figure 8B shows that the calcium in the

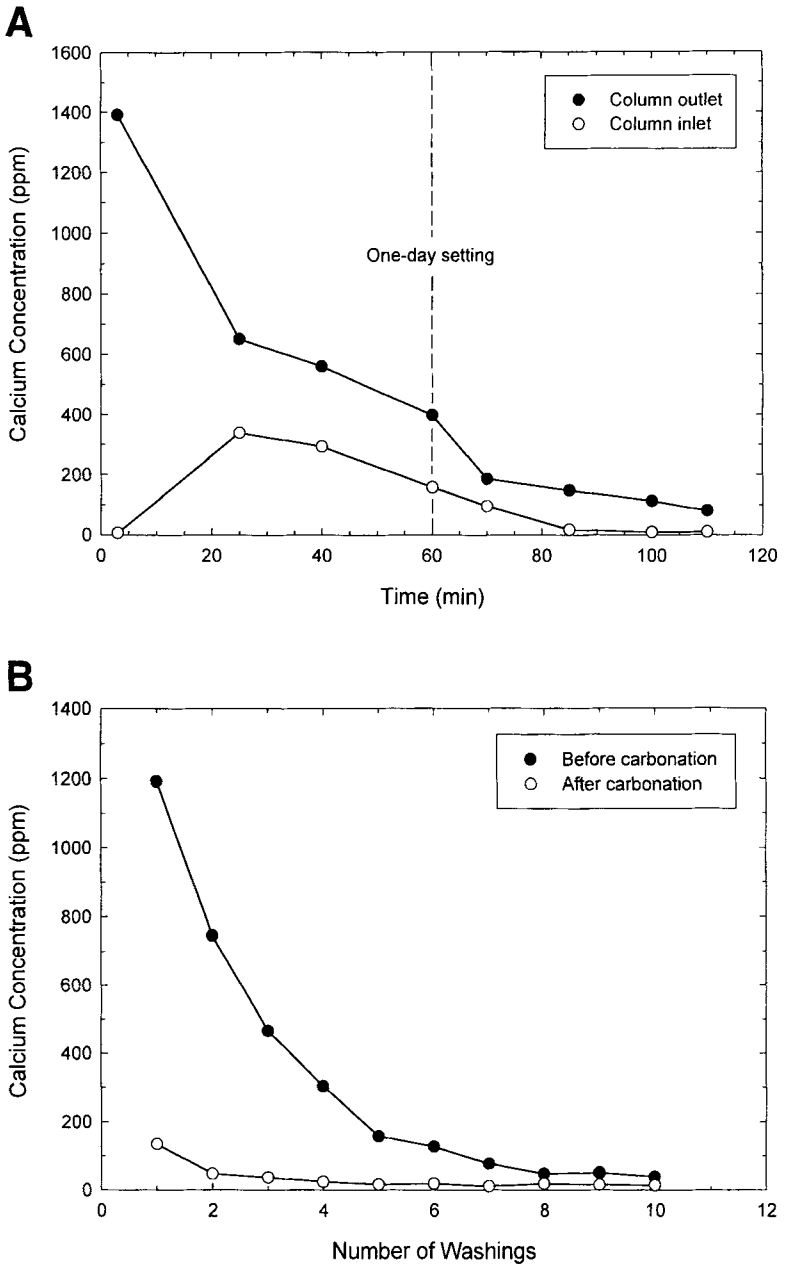

Fig. 8. Lime recovery studies for bagasse: (**A**) continuous, (**B**) batch, (**C**) effects of pH. Pretreatment conditions: (A) 85°C, 3 h, 0.15 g $Ca(OH)_2$/g dry biomass, 10 mL water/g dry biomass, (B,C) 65°C, 24 h, 0.15 g $Ca(OH)_2$/g dry biomass, 10 mL water/g dry biomass.

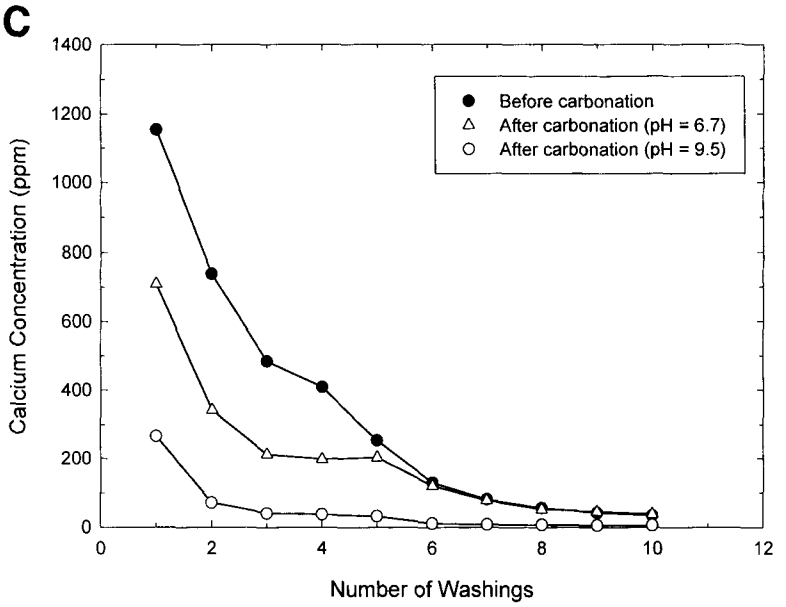

Fig. 8. *(continued)*

pretreated bagasse was effectively removed and precipitated as $CaCO_3$ after carbonation. The calcium analysis of bagasse before and after washing shows that 75% of added calcium was removed from biomass by 6 washings and 86% by 10 washings.

In the previous experiments, ammonium hydroxide was added to adjust the pH of carbonated wash water from about 7.0 to 9.5 to ensure that less-soluble carbonate ions (rather than more-soluble bicarbonate ions) dominated. This could potentially increase the operating cost of the process owing to extra chemical consumption. Therefore, it is worthwhile to investigate the possibility of avoiding ammonium hydroxide addition. Figure 8C shows the calcium concentrations of two wash waters, one of which was carbonated to pH 6.7 and the other in which the pH was raised to 9.5 by adding ammonium hydroxide. The data show that pH 9.5 is essential to recover calcium effectively from the wash water. However, industrially, this can be achieved with good control of CO_2 addition; that is, only the amount of CO_2 that reduces the pH from about 12 to 9.5 would be added.

CONCLUSIONS

The systematic studies on the effects of lime pretreatment conditions showed that time and temperature had the greatest impact on enzymatic digestibility. For short pretreatment times (1–3 h), high temperatures

(85–135°C) are required to ensure an effective pretreatment. For long pretreatment times (e.g., 24 h), low temperatures (50–65°C) are sufficient to achieve high sugar yields. To determine which combination is best for commercialization requires an economic analysis to determine the gains and losses for each combination. Lime loading had a critical value—0.1 g $Ca(OH)_2$/g dry biomass—below which the digestibility greatly declined and above which the digestibility increased only slightly, or even decreased in some cases. Water loading had little effect on the digestibility. The particle size study shows that lime pretreatment has a considerable potential for treating coarse material.

Under the conditions of 120°C, 1 h, 0.1 g $Ca(OH)_2$/g dry biomass, and 10 mL water/g dry biomass, the 3-d corrected reducing sugar yield of the pretreated bagasse was 4.3 times that of untreated bagasse (659 vs 153 mg Eq glucose/g dry biomass). The pretreated wheat straw—under 50°C, 24 h, 0.1 g $Ca(OH)_2$/g dry biomass, and 15 mL/g dry biomass—also produced much higher reducing sugar yields (e.g., 650 mg Eq glucose/g dry biomass) than the untreated wheat straw (65 mg Eq glucose/g dry biomass). The hydrolysis profiles show that 85% of the sugars can be released in 24 h. Compared with other pretreatments that were used to treat bagasse and wheat straw (Tables 1 and 2), lime pretreatment is very effective in enhancing the digestibility of biomass.

Material balances show that lime pretreatment is mild, because the biomass recovery is high (93.6% for bagasse). Lime recovery studies with bagasse show that lime can be easily recovered and recycled, making the pretreatment not only effective, but also economical and environmentally friendly.

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