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Dilute-Acid Pretreatment of Corn Residues and Short-Rotation Woody Crops

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ABSTRACT

As a prerequisite for the enzymatic saccharification or simultaneous saccharification and fermentation process for ethanol synthesis, a dilute-acid pretreatment of the biomass has been shown to be a very effective first step in the yeast-catalyzed bioprocess. Three hardwoods (silver maple, sycamore, and black locust) and two corn residues (cobs and stover) were chosen and subjected to prehydrolysis with dilute sulfuric acid at 140 and 160°C for reaction times ranging from 5 to 60 min. Although the hemicelluloses from all five samples could be completely hydrolyzed at both 140 and 160°C, hydrolysis at 160°C for the woods and stover produced a superior substrate for the cellulase enzyme from *Trichoderma reesei*, in which >90% of the cellulose was hydrolyzed by the enzyme. Corn cobs produced an excellent substrate after only 5 min at 140°C. Small amounts of lignin and glucan were also solubilized by the acid in all samples.

Index Entries: Dilute-acid pretreatment; short-rotation crops; hardwoods; herbaceous crops; cellulase digestion.

INTRODUCTION

It has been estimated that the ethanol made from underutilized lignocellulosic biomass, properly managed, could meet all annual transportation needs in the United States if ethanol was used as a neat fuel. The concept of substituting ethanol or ethanol blends for gasoline in the United

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States stems from the estimate that, by the turn of the century, the US will import up to 60% of its oil, thus opening the door to further dominance by OPEC in world energy markets (*1*).

The economics of ethanol production from lignocellulosics are yield-sensitive (*1*) and are thus directly related to feed costs, which are, in turn, a function of transportation. Thus, a proposed ethanol-from-biomass process, which could be used at a variety of locations, must be versatile enough to accept a variety of feedstocks, such as hardwoods or agricultural residues, depending on the supply and demand in each locality.

All lignocellulosic biomass resists cellulytic breakdown of its major chemical component, cellulose, which can compose up to 50% of its dry weight. To open up the biomass structure to the cellulase enzyme complex, we have chosen dilute sulfuric acid prehydrolysis (*2–4*), which breaks the lignin–hemicellulose barrier and liberates free pentose and some hexose sugars. The solid portion remaining, after prehydrolysis, is amenable to enzymatic breakdown of cellulose to glucose.

We (*2–8*) have studied dilute sulfuric acid prehydrolysis of several hardwoods, grasses, and wheat straw at moderate temperatures (120–180°C). Grethlein's group (*9–14*) has investigated prehydrolysis with similar concentrations of sulfuric acid (0–1 wt%) using oak, poplar, mixed birch, and maple at higher temperatures (180–220°C). Sudo et al. (*15*) also obtained high enzymatic digestibility of cellulose in acid-pretreated larch wood (*15*). All of the above-mentioned groups obtain biomass substrates that are highly enzymatically digestible and easily converted to ethanol.

The objective of this study is to extend investigations of dilute sulfuric acid pretreatment of the biomass currently under large-scale energy production by the DOE Biomass Production Program managed by Oak Ridge National Laboratory, Oak Ridge, Tennessee, to characterize conversion rates of the sugars to ethanol.

MATERIALS AND METHODS

The short-rotation woody crops were provided by the Biomass Production Program at Oak Ridge National Laboratory. The corn crops were obtained locally and selected for absence of rot. Hardwood stems 2–4 in. in diameter were cut into sections 2–4 ft long, air-dried, and stored in a dry room at ambient temperature. Whole aerial parts of mature corn stalks and cobs were also harvested, dried, and stored as above.

The following species were used for pretreatment investigations:

1. Hardwoods
   silver maple (*Acer saccharinum*)
   sycamore (*Platanus occidentalis*)
   black locust (*Robinia pseudoacacia*)

2. Corn residues
   corn cobs
   corn stover

The bark was loosened by brief steaming (30 min, dry cycle) in the autoclave. The bark sections were then stripped from steamed wood by hand or loosened with a knife. The wood sections were air-dried, chipped, and knife-milled to pass through a 2-mm rejection screen. Dry corn samples were also knife-milled (Thomas-Wiley laboratory mill, Arthur H. Thomas Co., Philadelphia, PA) to pass through a 2-mm rejection screen. Milled material was not separated into additional fractions.

A cellulase preparation (Celluclast, 1.5 L) produced by *T. reesei* was a gift from NOVO Industries, Ltd. (Copenhagen, Denmark). The cellulase preparation was in liquid form, stabilized by the addition of glycerol. The specific activity of the enzyme preparation was approx 72 International Filter-Paper Units (IFPU)/mL (*14*). Fungal β-glucosidase [Novozyme 188, NOVO, Ltd., specific activity (*16*) 250 IU/mL] was used to supplement the β-glucosidase activity in the cellulase preparation. The remaining chemicals were purchased from national laboratory supply houses. Cellulose powder (α-cellulose) was used as a control substrate and was obtained from Sigma Chemical Co. (St. Louis, MO).

Chemical Pretreatments

The wood and corn-residue particles were pretreated with dilute (0.45–0.50%, v/v) sulfuric acid solutions in a 1-L stainless steel reactor (Carpenter 20 Cb-3, Parr Co., Moline, IL) equipped with an impeller mixer and a pressurized injection device (*17*). In consequence of mixing limitations of impeller mixers with biomass particles, only low-solid slurries (10%) were investigated. Dilute-acid-pretreatment experiments were performed at 140 and 160°C. Reaction began when the slurries of biomass in deionized water reached the desired reaction temperature; acid was then injected. Zero time denotes biomass slurries heated in water to the reaction temperature. The acid concentrations were calculated to yield a hydrolysis pH of 1.35–1.40 after being heated at 140°C for 30 min. All pretreated biomass particles were exhaustively washed in deionized water to remove water-soluble components.

A portion of the solid wet residues was stored frozen at −20°C for subsequent enzymatic hydrolysis. The remaining material was air-dried at 45°C for subsequent chemical analyses. Volumes of combined filtrate and washes were measured and recorded. The combined liquids were neutralized with calcium carbonate, filtered, and stored at 4°C for analyses.

Enzymatic Hydrolysis

Enzymatic hydrolysis was performed in batch mode at 50°C, pH = 4.8, in gently rotated glass vials (*3,4*). Cellulase enzyme loading was approx

42 IFPU/gm of cellulose (β-glucosidase activity was approx 4.9 IU/gm of cellulose), and initial cellulose concentrations were adjusted to 1%. The reaction time for digestibility determination was usually 4 d and was defined by negligible additional release of glucose from the α-cellulose control and pretreated substrates. Glucose released by the enzymatic hydrolysis was determined with a YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH) and by ion-moderated partition (IMP) chromatography when information about other sugars was required. Enzymatic digestibility was calculated as the percentage of the total glucose (corrected for hydration) released from the total anhydroglucose in the biomass sample at the end of hydrolysis. Release of the other sugars was not considered in this study, because they are solubilized by the pretreatment.

Analytical Methods

Dry weights and ash content of all solids were determined by standard methods (*18,19*). Lignin and other acid-insoluble components were determined as Klason lignin (*18*). Carbohydrate composition of biomass solids was determined by a two-stage sulfuric acid hydrolysis (*18*) followed by determination of monomeric sugars by IMP chromatography. Monosaccharides in all neutralized hydrolysates were determined by IMP chromatography using an Aminex HPX 87-P column (Bio-Rad, Richmond, CA), deionized water as eluant, and refractive-index detection. Acetic acid and furfural in aqueous solutions were determined by gas chromatography (*2,3*).

RESULTS AND DISCUSSION

This study is a continuation of our efforts to determine the universal applicability of dilute sulfuric acid pretreatment to complete enzymatic breakdown of cellulose found in short-rotation woody and herbaceous crops (*2–8*). We have previously shown that a direct relationship exists between dilute-acid-mediated xylan removal and concomitant increased enzymatic breakdown of cellulose (*3*). It has also been reported that the economics of ethanol production using lignocellulosic biomass as a feedstock depends on yield and the cost of the feedstock (*1*). It is, therefore, advantageous to have one dilute-acid-pretreatment facility, using a variety of lignocellulosic feedstocks depending on supply, demand, and price of the feed. Our research, in collaboration with the DOE Biomass Production Program managed by Oak Ridge National Laboratory, expects to discover suitable lignocellulosic biomass feedstocks for the pretreatment and bioconversion to fuel-grade ethanol.

Hardwoods

The approximate chemical compositions of the debarked wood samples from three types of short-rotation trees are shown in Table 1. The compo-

Table 1
Chemical Composition of Three Hardwood and Two Corn Samples
(Weight %, mositure-free basis)

	Silver Maple	Sycamore	Black Locust	Corn Cobs	Corn Stover
Klason Lignin (unextracted biomass)	20.8	22.8	21.5	17.5	16.7
Ash	0.4	0.5	0.6	2.7	6.3
Glucan	45.9	44.0	49.4	39.4	40.9
Xylan	17.1	16.3	16.2	28.4	21.5
Galactan	ND	ND	ND	1.1	1.0
Arabinan	0.7	0.6	0.4	3.6	1.8
Mannan	1.2	0.9	1.0	ND	ND
Acetyl Groups	3.9	3.6	3.8	1.9	1.9
TOTALS	90.0	88.7	92.9	94.6	90.1

ND = Not Detected

sitions of all three samples were typical of woods from hardwood trees. The major component was cellulose (glucan) followed by lignin, xylan, and a variety of minor components. The very low ash content of all wood samples is also notable. The conditions (i.e., pH, acid concentration, and reaction temperature) of pretreatment and enzymatic hydrolysis were chosen to be the same as those used for the woody and herbaceous crops (*2–4,6,8*) previously investigated. By keeping the key parameters constant, these results can be compared to other short-rotation hardwoods reported previously. The key results are shown in Figs. 1–4 and Tables 1 and 2.

Dilute sulfuric acid prehydrolysis of lignocellulosics for the biosynthesis of ethanol is used to cleave several classes of covalent bonds, both in the lignin and hemicellulose fractions, producing greater pore volume and greater accessibility for cellulase enzymes (*11*). The hemicellulose fraction is nearly completely hydrolyzed to monomeric sugars, whereas, the lignin primarily recondenses, forming an altered lignin polymer. Since the hemicellulose and other minor components of wood can be completely hydrolyzed by the acid, the changes in dry weight loss as a function of time of hydrolysis is a useful parameter to determine completion of hydrolysis at a given temperature. The loss of dry weight of maple, black locust, and sycamore wood meals as a function of pretreatment time at 140 or 160°C is shown in Fig. 1; results are very similar for all three woods. Small amounts of wood (3–8%) became solubilized with hot water and much larger (34–42%) amounts were solubilized during dilute acid pretreatment. The hydrolyses of substrates pretreated at 140°C were practically complete in a 15- to 30-min interval; hydrolyses were practically complete in 5 min when pretreatment was at 160°C. This is very similar to the results previously obtained with other short-rotation woody and herbaceous crops (*2,4,6,8*).

It was mentioned earlier that the economics of ethanol production from biomass is yield-sensitive (*1*). Therefore, because the dilute-acid pretreatment is a hydrolytic reaction releasing free hemicellulosic sugars as well as some glucose from cellulose or glucans, and because the degradation of these sugars is also acid-catalyzed, hydrolytic conditions must be chosen that assure that the solid cellulosic product is enzymatically degradable and that the yield of free sugars in the supernatant is maximized. Solubilization of the major components of the three hardwoods for averaged reaction times of 5–10 min at 160°C is shown in Table 2. Results for pretreatments at 140°C with reaction times of 30 to 60 min were very similar (data not shown). The lignin was solubilized only slightly (6–16% loss) during the pretreatments at both temperatures, and a large part of solubilization (40–100%) occurred during the initial heating period in water (data not shown). Glucan (cellulose) hydrolysis was also low; only 13–17% was solubilized from all three woods. It should be emphasized at this point, however, that solubilization of sugars during dilute-acid pretreatment does not represent any large actual loss for subsequent

Table 2
Solubilization of Hardwood and Corn Components
During Pretreatment at 160°C for 5 to 10 min (% of original)

	Silver Maple	Sycamore	Black Locust	Corn Cobs	Corn Stover
Klason Lignin	13	6	16	26	9
Glucan	13	15	17	14	25
Xylan	97	98	97	100	93
Galactan	--	--	--	100	100
Arabinan	100	100	100	100	100
Mannan	100	100	100	--	--
Acetyl Groups	100	100	100	100	100

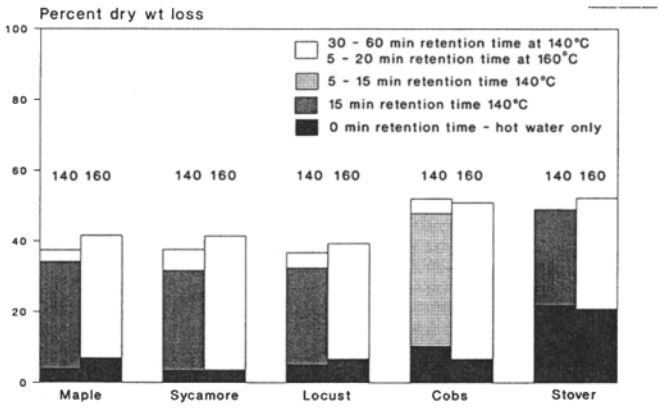

Fig. 1. Pattern of dry weight loss during acid pretreatment of hardwood and corn samples.

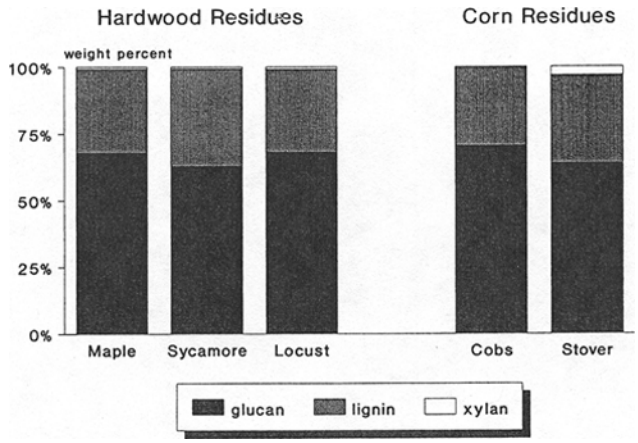

Fig. 2. Average residue compositions of hardwood and corn samples after pretreatment at 160°C for 10 min.

fermentations. All sugars can be recovered in aqueous solutions after pretreatment and eventually fermented to ethanol or other products. Actual recoveries of hemicellulosic sugars in liquids from pretreatments at 140°C (15–30 min reaction time [rt]) and 160°C (5–10 min rt) were approx 80–90% for xylose and 90–100% for galactose and arabinose. Pretreatments at 160°C for 20 min were too severe, and higher destruction of sugars was observed. Small amounts of furfural (0.04 to 0.08 wt%) and acetic acid (0.3 wt%) also accumulated in liquids from these pretreatments. Overall, material balances for glucan and xylan were similar for short reaction times, and recoveries were calculated at 94–103% from the starting biomass.

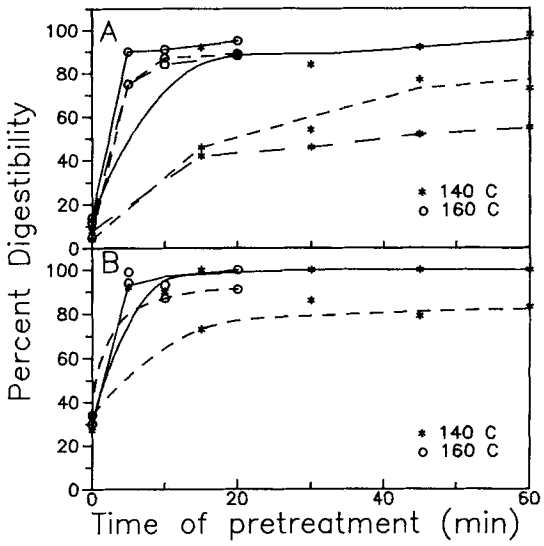

Fig. 3. Panel (A): Changes in enzymatic digestibility of cellulose in maple (——), black locust (— —), and sycamore (- - -) wood samples as a function of reaction time in dilute acid pretreatment. Panel (B): Changes in enzymatic digestibility of cellulose in corn cobs (——) and stover (- - - -) as a function of reaction time in dilute acid pretreatment.

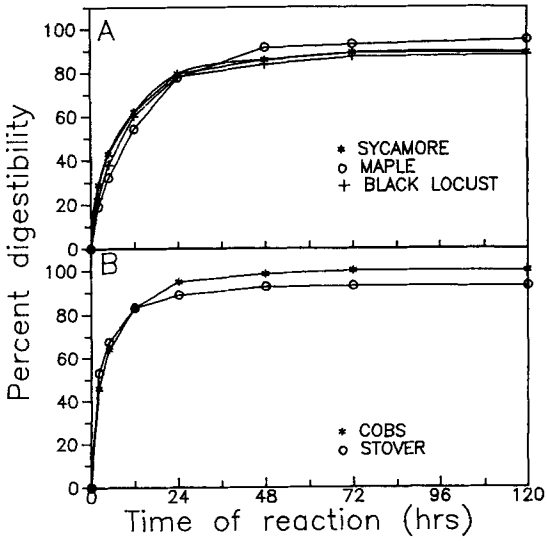

Fig. 4. Kinetics of enzymatic release of glucose from wood and corn samples pretreated at 160°C for 10 min in dilute acid: (A) Hardwood samples, (B) Corn samples.

The compositions of all three pretreated wood solids were very similar after removal of hemicelluloses. Average compositions for samples pretreated at 160°C for 10 min are shown in Fig. 2. The main component (64–70%) is cellulose, followed by lignin. Traces of xylan (2%) also remained in many pretreated samples.

The dilute-acid pretreatment dramatically increased the enzymatic digestibility of all three hardwoods when performed at 160°C (Fig. 3). Cellulose digestibility in all three woods reached 88–94% after 10 min, with maple achieving the highest level. The development of high digestibilities of cellulose in all three woods pretreated at 160°C coincided with hydrolysis and solubilization of xylan. When 140°C was used as a prehydrolysis temperature, only maple yielded a highly digestible substrate, and the degree of digestibility was again directly correlated with xylan removal (data not shown). When either the sycamore or black locust was pretreated at 140°C, lower digestibilities (Fig. 3) were observed (75% for sycamore and only 52% for black locust) even though near-complete removal of xylan was observed (data not shown). This indicates that additional reactions, such as lignin condensation, may contribute to development of porosity and enhanced cellulose accessibility during dilute acid-pretreatment (*11*).

The rates of enzymatic release of glucose from all three woods pretreated at 160°C for 10 min were nearly identical (Figure 4). The enzymatic reaction was complete after 48 h. This observation is different from those made in previous work using other hardwoods, in which it took from 3 to 5 d to completely digest the cellulose (*8*).

Corn Residues

The approximate chemical compositions of the corn samples are shown in Table 1. The composition of the stover is very similar to that of wheat straw, with a slightly lower ash content. The cobs, however, have a very high xylan content and an ash level significantly lower than that found in stover or wheat straw. Because of the lower ash content found in cobs, less sulfuric acid (0.45% v/v, compared to 0.5% v/v required for stover) is necessary to achieve the hydrolysis pH of 1.35 for the cob slurries than is needed for stover.

The patterns of dry weight loss (Fig. 1) vs time at 140 and 160°C were very similar for the two substrates, except for the initial time. The cobs behaved more like wood, with less than 10 wt% being solubilized by hot water, whereas hot water solubilized slightly more than 20 wt% of the stover, as was the case for wheat straw. More than 50% of each substrates was solubilized during dilute-acid pretreatment. The hydrolyses were practically complete in a 5-min interval at 140°C for the cobs and in 15–30 min at 140°C for the stover; at 160°C, both pretreatments were complete in 5 min. These results are very similar to the results previously obtained (*8*).

Although the hemicellulosic sugars from both substrates were almost completely solubilized during the pretreatment, lignin and glucan solubili-

zation was substrate-specific (Table 2). At either temperature, the lignin from the stover was solubilized only slightly (13–17% loss); the glucan solubilization was higher (9–28% loss). Using cobs as the substrate at either temperature, as much as 33% of the lignin was solubilized, and only 6–14% of the glucan was released. The solubilized sugars were again recovered from liquid fractions in high yields, together with minor amounts of furfural (0.3% for cobs and 0.1% for stover) and acetic acid (0.4% for both) (data not shown). The composition of the pretreated solids changes very little after removal of hemicelluloses (Fig. 2). The main component (70%) is cellulose, followed by lignin. Traces of xylan (3%) also remained in the pretreated stover, whereas all xylan was removed from the cob residues.

Cellulose digestibility in both substrates reached 90–100% after 5–10 min of pretreatment at 160°C (Fig. 3). Slightly lower (79–86%) digestibilities were observed after 30–60 min at 140°C for the stover, but, after only 5 min at 140°C, the cellulose in cobs was 90% digestible. The development of high enzymatic digestibilities of cellulose in both substrates pretreated at 160°C coincided with hydrolysis and solubilization of xylan, as we previously observed for dilute-acid pretreatment of aspen and wheat straw. The rates of enzymatically released glucose from the corn residues pretreated at 160°C for 10 min is seen in Fig. 4. The rates are identical for both substrates, and the reaction is essentially complete after only 24 h. These results are very similar to those for grasses and wheat straw (*3,8*), and are 2 to 3 times faster than those for hardwoods (*8*). Additional studies using narrower ranges of particle-size are needed to establish whether cellulose fibers in pretreated corn residues are inherently more digestible than those in pretreated hardwoods, or if the differences are caused by other factors, such as particle dimensions and enzyme penetration into the interior of pretreated particles.

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