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Dilute-Acid Pretreatment of Two Short-Rotation Herbaceous Crops

Scientific Note

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INTRODUCTION

The development of fuel ethanol from cellulosic biomass is of increasing importance to provide an alternative transportation fuel because of several factors. First is the greatly increased concern with local air pollution associated with use of gasoline. In addition, the buildup of carbon dioxide with combustion of fossil fuels could lead to global climate change owing to the "greenhouse effect." Furthermore, with the United States now importing approx 50% of its petroleum, changes in petroleum prices and availability could have profound influences on our economy (*1*).

Blends of ethanol with gasoline reduce carbon monoxide emissions, and use of neat ethanol reduces smog relative to gasoline (*2*). Because growing biomass for ethanol production recaptures the CO_2 released in combustion, ethanol production from cellulosic biomass does not contribute to the possibility for global climate change resulting from increased atmospheric concentrations of CO_2. Additionally, enough land could be made available for growing energy crops during the next 50 yr to generate potentially enough ethanol to replace all gasoline used in the United States (*2*). Thus, substitution of ethanol produced from cellulosic biomass reduces the vulnerability of the country to disruptions in the supply and price of

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transportation fuels, and decreases the trade deficit for petroleum imports.

To convert the carbohydrate fractions of lignocellulosic biomass into ethanol via an enzymatically catalyzed process requires that the resistance of biomass to carbohydrate degradation be addressed. Thus, "pretreating" the substrate to overcome this resistance is one of the key elements in the biomass-to-ethanol conversion process. The primary purpose of pretreatment is to make the cellulosic biomass amenable to the action of cellulase enzymes. In the case of dilute-acid pretreatment, which is a widely accepted method at the present time, acids hydrolyze the hemicellulose fraction of biomass to primarily xylose (and a few other minor sugars) in solution leaving a porous solid substrate that is accessible for conversion to ethanol. The key requirements are to maximize the susceptibility of cellulose to hydrolysis to glucose while minimizing the loss of sugars (primarily xylose and glucose) during the pretreatment step.

Our research, in collaboration with the DOE Biomass Production Program managed by Oak Ridge National Laboratory (ORNL), has focused on identification of suitable lignocellulosic biomass feedstocks for pretreatment and bioconversion to fuel-grade ethanol. We (*3–10*) have previously measured the performance of dilute-sulfuric acid prehydrolysis for several high-productivity energy crops (HPEC) currently undergoing large-scale production/availability studies. At moderate temperatures (120–180°C) and low-acid loading (0.8–1.1 wt% acid), a digestible pulp is produced while minimizing degradation of the soluble hemicellulosic sugars for all species tested other than legumeous species. These species seem to require harsher pretreatment conditions than were studied to yield completely digestible pulps. We have previously demonstrated that two factors seem to affect the yields of glucose from the enzymatic breakdown of cellulose: (1) dilute-acid-mediated xylan removal and (2) temperature of pretreatment for some species (i.e., even when similar amounts of xylan are released at two different temperatures, the higher pretreatment temperature produces a more digestible pulp). In this study, we are extending the previous work to include dilute-acid pretreatment of the legume, flatpea hay, and the grass, reed canary grass, to culminate the initial screening of selected HPEC from ORNL as to the efficacy of dilute-acid pretreatment for ethanol synthesis (*3–10*).

MATERIALS AND METHODS

The two HPEC were provided through the Biomass Production Program at ORNL by J. Cushman. Common reed canary grass (*Phalaris arundinacea L.*) was supplied by J. H. Cherney at Purdue University. It was harvested in September 1989 and air-dried at 60°C before being baled. The flatpea hay (*Lathyrus sylvestris L.* var *Lathco*), a gift from G. W. Fick at Cornell University, Ithaca, NY, was harvested in August 1989 and air-

dried. Both samples were knife-milled (Thomas-Wiley laboratory mill, Arthur H. Thomas Co., Philadelphia, PA) to pass through a 2-mm round hole rejection screen. Milled material was not screened into additional fractions.

A cellulase preparation (Celluclast, 1.5 L) produced by *Trichoderma 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 (*11*). Fungal β-glucosidase (Novozyme 188, NOVO, Ltd., specific activity 250 IU/mL) (*11*) 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 Company (St. Louis, MO).

Chemical Pretreatments

Particles from two herbaceous crops were pretreated with dilute (0.55% 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 (*12*). Because of the limitations of impeller mixers with biomass particles, only low-solids slurries (10%) were investigated in this study. Dilute-acid-pretreatment experiments were performed at 140, 160, and 180°C. Reactions were initiated when the slurries of biomass (in deionized water) reached the desired reaction temperature. At this time, acid was 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 and 105°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 analysis.

Enzymatic Hydrolysis

Enzymatic hydrolysis was performed in a batch mode at 50°C, pH 4.8, in gently rotated glass vials (*4,5*). The cellulase enzyme loading was approx 42 IFPU/g of cellulose content (β-glucosidase activity was approx 4.9 IU/g of cellulose content), and initial cellulose concentrations were adjusted to 1 wt%. 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

Table 1
Chemical Composition of Reed Canary Grass and Flatpea Hay, Wt %, Moisture-Free Basis

	Reed canary grass	Flatpea hay
Klason Lignin[1], unextracted biomass	15.6	24.5
Ash	13.9	10.2
Glucan	26.0	28.9
Xylan	9.8	7.4
Galactan	.08	1.5
Arabinan	2.4	2.0
Mannan	0	0.1
Acetyl groups	0.9	1.4
Protein	18.4	20.4
Totals	87.8	96.5

[1]Corrected for ash content.

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 during the pretreatment.

Analytical Methods

Dry weights and ash contents of all solids were determined by standard methods (*13,14*). Lignin and other acid-insoluble components were measured as Klason lignin (*13*). The carbohydrate composition of biomass solids was evaluated by a two-stage sulfuric acid hydrolysis (*13*) followed by measurement 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) and refractive-index detection. The acetic-acid composition in aqueous solutions was evaluated by gas chromatography (*4,5*).

RESULTS AND DISCUSSION

The approximate chemical compositions of the two herbaceous species reported in this study are shown in Table 1. Reed canary grass has only 39.0 wt% carbohydrate, whereas flatpea hay has only 40.0 wt% carbohydrate. Because the economics of the biomass-to-ethanol process benefit

greatly from high ethanol yields (*15*), both crops must benefit from significant byproduct credits, such as electricity or protein-rich animal feed sales, or be very inexpensive to supply to the plant, to be serious feedstock candidates. Thus, it would appear, strictly from a compositional standpoint, that neither species would be a good candidate for ethanol synthesis.

Another drawback for ethanol synthesis is the high ash content, which would be carried through the process and require disposal. Other feedstock components are measured by the Klason lignin assay procedure for the direct estimation of all components that are insoluble in both concentrated and hot dilute sulfuric acid. As long as it is understood that the Klason lignin content for the herbaceous plants represents not only lignin, but also aliphatic waxy compounds, acid-insoluble ash (e.g., silica), and some protein, this determination is very useful for investigation of dilute-acid pretreatment. Klason lignin components are also generally insoluble in dilute-acid pretreatment liquors and represent a significant fraction of water-insoluble plant components that are not carbohydrates. The fate of carbohydrates during pretreatment enzymatic hydrolysis can than be determined separately from the rest of the plant material.

The conditions of pretreatment (i.e., pH, acid concentration, and reaction temperature) and enzymatic hydrolysis were chosen to be the same as those used for the woody and herbaceous crops previously investigated (*3–5,7,9,10*). By keeping the key parameters constant, these results can be compared to the other HPEC 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 (*16*). The hemicellulose is nearly completely hydrolyzed to monomeric sugars, whereas the lignin and possibly some protein and aliphatic waxy compounds are thought to recondense, forming altered polymers. Because the hemicellulose and other minor components can be completely hydrolyzed by acid, the change 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 and the composition of the solid residues of the two herbaceous samples as a function of pretreatment time and temperature are shown in Fig. 1 and Table 2.

The patterns of dry weight loss during dilute-acid pretreatment of the two herbaceous crops differed slightly. The water-soluble extractives of reed canary grass were 32% with only a small increase from 140 to 160°C. However, the water-soluble fraction of flatpea hay increased from 24 wt% at 140°C to nearly 31 wt% at 180°C. Pretreatment of reed canary grass at either temperature (independent of time) caused a total of more than 60% of the biomass to be solubilized. This very high biomass solubilization is significantly greater than any other samples tested to date (*3–5,7,9,10*) and appears to be a result of low glucan content of reed canary grass.

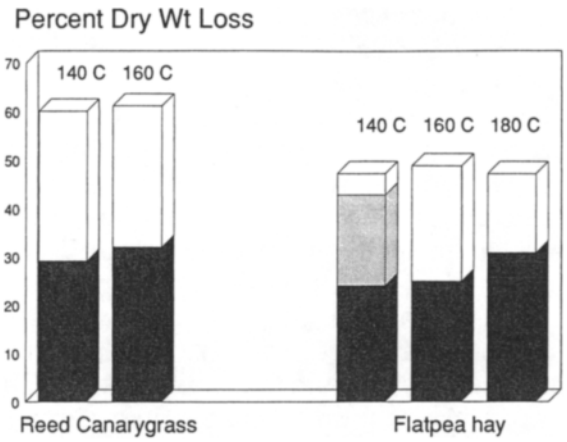

Fig. 1. Pattern of dry weight loss during acid pretreatment of reed canary grass and flatpea hay under varying temperatures and retention times. □ 5–20 min retention time at 180°C. 30–60 min retention time at 140°C (flatpea hay). 5–20 min retention time at 160°C. 5–60 min retention time at 140°C (reed canary grass). ▨ 15 min retention time at 140°C (flatpea hay). ■ 0 min retention time—hot water only.

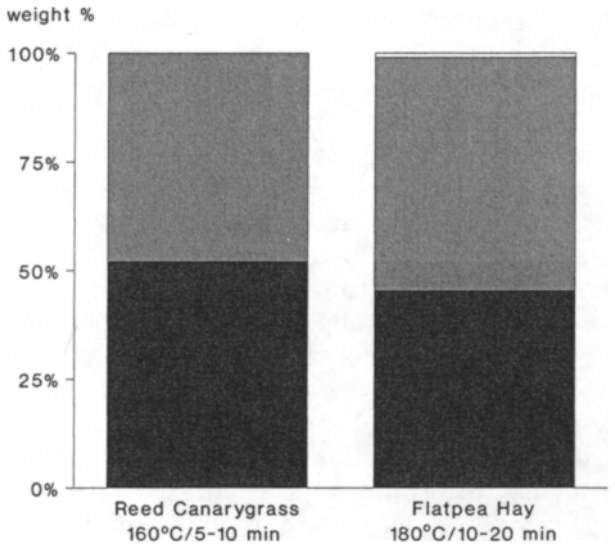

Fig. 2. Average compositions of dilute-acid-pretreatment reed canary grass and flatpea hay residues using either 160°C or 180°C, as indicated. □ xylan, ▨ lignin, ■ glucan.

For flatpea hay, an additional 17–24 wt% of the solids could be solubilized by acid depending on the temperature and times of pretreatment, which is consistent with the observations for previously tested herbaceous crops (*3–5,7,9*). As shown in Table 2, the hemicellulose fractions from both substrates were nearly completely hydrolyzed and solubilized along with minor amounts of glucan (approx 22% for reed canary grass and 19% for

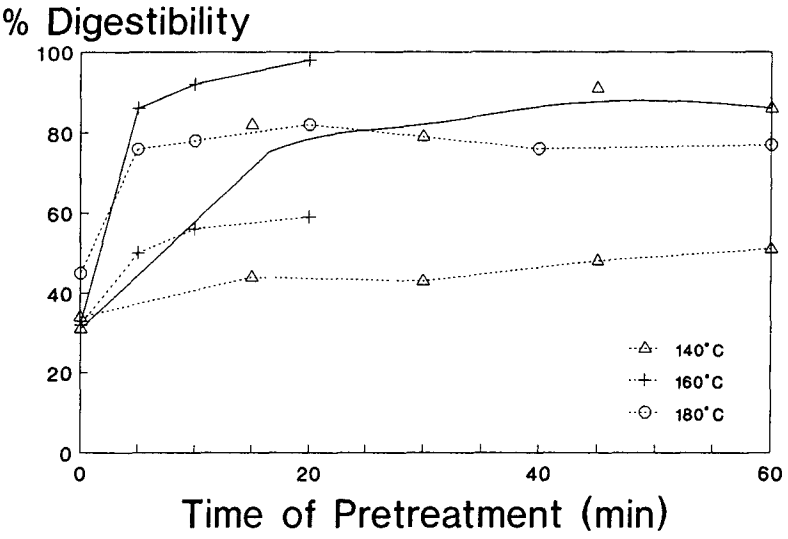

Fig. 3. Changes in enzymatic digestibilities of glucan in pretreated reed canary grass (—) and flatpea hay (- -) as a function of reaction time and temperature.

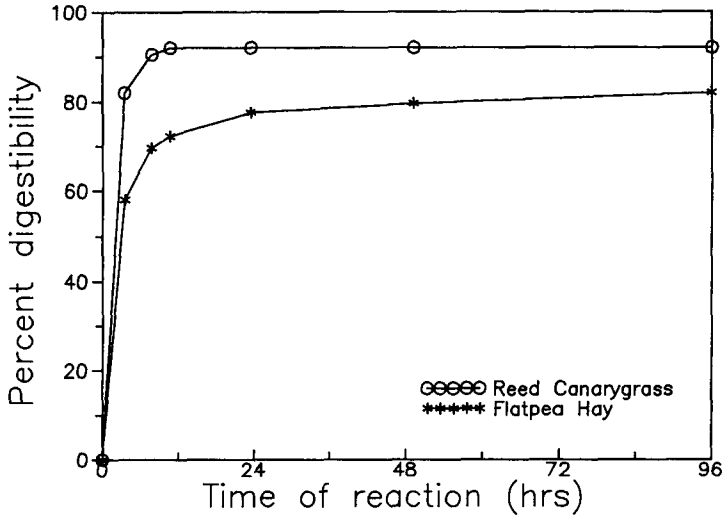

Fig. 4. Enzymatic release of glucose from reed canary grass and flatpea hay pretreated at 160°C for 10 min in dilute acid for the canary grass and 180°C for 10 min for the hay.

flatpea hay). The solubilized sugars were recovered from liquid fractions in high yields, together with minor amounts of furfural (ca. 0.02%) and acetic acid (ca. 0.3%).

The average compositions of the solids after pretreatment at 160°C for 5-10 min for the grass and 180°C for 10-20 min for the flatpea hay are shown in Fig. 2. The relatively low percentage of glucan in each substrate

Table 2
Solubilization of Reed Canary Grass and Flatpea Hay Carbohydrate During Dilute-Acid Pretreatment[1], % of Original

	Reed canary grass	Flatpea hay
Glucan	22	19
Xylan	100	87–100
Galactan	100	100
Arabinan	100	100
Mannan	N/A	100
Acetyl groups	100	100

[1]Conditions of pretreatment were 160°C for 5–10 min for reed canary grass and 180°C for 10–20 min for flatpea hay.

is directly a consequence of the low glucan content in the native plants and similar to previously reported pretreated herbaceous samples (*9*). The enzymatic digestibility of glucan in the pretreated herbaceous samples is shown in Fig. 3. The lower digestibilities observed with the legume, compared to the grass under similar pretreatment conditions, confirm previous observations that legumes are more resistant to dilute-acid prehydrolysis (*9*) and require harsher conditions to obtain a digestible pulp. The enzymatic digestibility of glucan in the grass increases from about 90% when pretreated at 140°C to 98% when pretreated at 160°C, even though identical amounts of xylan have been removed from the substrate prior to enzymatic hydrolysis (data not shown). The digestibility of glucan in the legume pretreated at 140 and 160°C increased from only approx 50% to about 60%. Significant digestibility (approx 80%) of glucan in this legume was only achieved after a 20-min pretreatment at 180°C.

High enzymatic digestibility of glucan still coincides with the complete hydrolysis of xylan bonds (data not shown) for pretreatments at 140 and 160°C. However, the strong temperature effects on digestibility of cellulose in the range of 140–180°C do not seem to be solely related to the removal of hemicelluloses. Thus, changes in cell wall structure and porosity may have a strong influence on the effectiveness of the dilute-acid pretreatment of this legume. It has been observed by other workers (*17–20*) that the cell wall structure and composition of herbaceous legumes may be signifiantly different from grasses.

Finally, we have also performed a limited comparative investigation of differences between rates of enzymatic hydrolysis of the pretreated herbaceous residues as shown in Fig. 4. The pretreated grass achieved complete saccharification in just 10 h. For the legume, most of the sugars were released in 24 h, but 4 d were required to saccharify the available glucan completely. Additional studies at narrower particle size ranges are needed to establish whether cellulose fibers in the pretreated grass are inherently more digestible than in the pretreated legume or if the differ-

ences are caused by other factors, such as particle dimensions, enzyme penetration into the interior of pretreated particles or lignin content.

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