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Dilute Acid Pretreatment of Short Rotation Woody and Herbaceous Crops

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ABSTRACT

Large-scale production of ethanol or other transportation fuels by biological conversion of lignocellulosic biomass will eventually require integration with large-scale production of biomass substrates. The most promising candidates for efficient production of biomass in the US are short rotation hardwoods and herbaceous crops. The following samples of short rotation hardwoods were provided by the Biomass Production Program managed for the Department of Energy Biofuels and Municipal Waste Technology Division by the Oak Ridge National Laboratory: Poplar hybrid NE388 (*Populus, maximowiczii* × *P. trichocarpa*), Poplar hybrid N11 (*Populus. trichocarpa* × *P. deltoides*), and Sweetgum (*Liquidambar styraciflua*). Samples of two short rotation grasses (weeping lovegrass and switchgrass) and one herbaceous legume (sericea lespedeza) were also obtained from the same source. Milled and debarked hardwoods and herbaceous samples were subjected to prehydrolysis with dilute sulfuric acid at 140 and 160°C for reaction times ranging from 5 to 60 min. The dilute sulfuric acid hydrolyzed all hemicelluloses at longer reaction times (30–60 min at 140°C and 5–10 min at 160°C), but solubilized very small amounts (< 15%) of lignin and cellulose. Cellulose in all three pretreated hardwoods became highly digestible by cellulase enzyme from *Trichoderma reesei*. The two grasses also responded to dilute acid pretreatment very well.

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

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INTRODUCTION

Prehydrolysis of selected hardwood, corn stover, and wheat straw meals with dilute sulfuric acid at moderate (120–160°C) and high (180–230°C) temperatures has been shown to be an effective pretreatment for increasing the digestibility of cellulose by a cellulase enzyme complex produced by mutants of the fungus *Trichoderma reesei* (*1–13*). We have investigated (*4–7,12,13*) prehydrolysis of aspen wood and wheat straw with dilute sulfuric acid at 95–160°C and obtained highly digestible substrates for enzymatic hydrolysis. Grethlein and coworkers investigated prehydrolysis with similar concentrations (0–1%) of sulfuric acid at 180–220°C and obtained highly digestible substrates with oak (*1,9*), poplar (*3,10,11*), mixed birch and maple (*2,3,8*), corn stover (*9*), and newsprint (*9*). They also observed only moderate (ca. 65%) enzymatic digestibility of cellulose in pretreated white pine (*2,3*), which is a softwood. Sudo and coworkers (*11*) investigated acid-catalyzed steam explosion of larchwood (*Larix leptolepis*) and observed that this softwood responded to the acid catalyzed pretreatment very well. This accumulated evidence indicates that dilute acid prehydrolysis is an effective pretreatment for hardwoods and two major agricultural residues, both of which are grasses.

Since large scale production of ethanol or other transportation fuels by biological conversion of lignocellulosic biomass will eventually require integration with biomass production, we have extended investigations of dilute acid pretreatment to short rotation woody and herbaceous crops. These crops are being developed for large scale energy production by the DOE Biomass Production Program managed by the Oak Ridge National Laboratory, Oak Ridge, TN.

MATERIALS AND METHODS

Materials

The biomass was harvested in the fall of 1987. Hardwood stems 2–4 inches in diameter were cut into sections 2–4 feet long, air dried, and stored in a dry room at ambient temperature. Whole aerial parts of mature herbaceous crops were also harvested, dried, and stored as above.

The following species and hybrids were used for pretreatment investigations: (a) hardwoods: Poplar hybrid NE388 (*Populus maximowiczii* × *P. trichocarpa*); Poplar hybrid N11 (*Populus trichocarpa* × *P. deltoides*); and Sweetgum (*Liquidambar styraciflua*); and (b) herbaceous plants: Switchgrass (*Panicum virgatum*); Weeping lovegrass (*Eragrotis curvula*); and Sericea lespedeza (*Lespedeza cuneata*). The biomass samples were provided to us by the Biomass Production Program at Oak Ridge National Laboratory. Since all hardwood trees were harvested in the dormant season (late fall), the bark adhered tightly to the wood in stem sections. The bark was loosened

by brief steaming (30 min, liquid cycle) in the autoclave. The bark sections could then be stripped from steamed wood by hand, or easily loosened with a knife. The wood sections were air dried, chipped, and knife milled to pass through a 2 mm rejection screen. Dry herbaceous crop samples were also milled in a knife mill (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 approximately 72 International Filter Paper Units (IFPU)/mL (*14*). Fungal β-glucosidase [Novozyme 188, NOVO, Ltd., specific activity (*14*) 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), used as a control substrate, was obtained from Sigma Chemical Co. (St. Louis, MO).

Chemical Pretreatments

The wood and herbaceous particles were pretreated with dilute (0.45–0.5%, v/v) sulfuric acid solutions in a 1 L stainless steel reactor (Carpenter 20 Cb-3, Parr Co., Moline, IL), equipped with impeller mixers and a pressurized injection device (*15*). Because of mixing limitations of an impeller mixer with biomass particles, only low (10%) solids slurries were investigated. Dilute acid pretreatment experiments were performed at 140 and 160°C. Reaction times are specified in the text and start when the slurries of biomass in deionized water reach the desired reaction temperature, and acid is 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 1.35–1.45 after 140°C for 30 min. All pretreated biomass particles were exhaustively washed with deionized water to allow removal of 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 (*5,6*). Cellulase enzyme loading was approximately 42 IFPU/g cellulose (β-glucosidase activity was approximately 4.9 IU/g cellulose), and initial cellulose concentrations were adjusted to approximately 1%. The reaction time for digestibility determination was usually 4 d and 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 ion moderated partition chromatography (IMP) 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 the present study because they are solubilized by the pretreatment.

Analytical Methods

Dry weights and ash content of all solids were determined by standard methods (*16,17*). Lignin and other acid insoluble components were determined as Klason lignin (*16*). Carbohydrate composition of biomass solids was determined by a modified two-stage sulfuric acid hydrolysis (*4,16*), followed by determination of monomeric sugars by IMP chromatography. Monosaccharides in all neutralized hydrolyzates were determined by IMP chromatography, using 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 (*4,5*).

Uronic acid content of starting biomass samples was determined by the colorimetric dimethyl-phenol procedure (*19*). Acetyl group content was determined by dilute sulfuric acid hydrolysis, followed by gas chromatography (*20*).

RESULTS AND DISCUSSION

Hardwoods

The approximate chemical compositions of the wood samples from three short rotation trees are shown in Table 1. The composition of all three samples was typical for 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 investigated previously with aspen wood and wheat straw (*4–6,12*). By keeping the key parameters constant, we can compare the results obtained with short rotation hardwoods to those previously obtained with aspen wood. The key results are shown in Figs. 1–3 and Tables 1 and 2.

Since dilute acid pretreatment is primarily a hydrolytic process leading to solubilization of hemicellulosic sugars and other components, the changes in dry weight loss are a useful overall parameter for monitoring

Table 1
Chemical Composition of Three Hardwood and Three Herbaceous Samples

	Poplar hybrid NE388	Poplar hybrid N11	Sweetgum	Switchgrass (SG)	Weeping lovegrass (WLG)	Sericea lespedeza (SL)
Klason lignin (unextracted biomass)	21.8	22.5	21.8	21.9	21.2	31.6
Ash	0.7	0.6	0.4	5.6	4.8	4.8
Glucan	48.6	51.8	49.5	36.6	36.7	31.5
Xylan	14.6	11.3	17.5	16.1	17.6	14.5
Galactan	0.3	0.7	0.3	1.2	1.7	0.9
Arabinan	0.3	0.3	0.4	2.2	2.6	1.6
Mannan	0.5	0.3	0.4	0	0	0
Protein	ND[a]	ND[a]	ND[a]	6.3	7.6	8.4
Uronic acids	2.3	2.2	2.6	1.5	1.4	3.2
Acetyl groups	2.2	1.9	2.3	1.1	1.1	1.3
	91.3%	91.6%	95.2%	92.5%	94.7%	97.8%

[a]ND = Not Determined

Table 2
Solubilization of Hardwood and Herbaceous Components During Pretreatment at 160°C

	NE388	N11	Sweetgum	SG[a]	WLG[a]	SL[a]
Lignin	18	17	17	4	12	3
Glucan	10	9	16	18	18	8
Xylan	96	98	98	93	92	85
Galactan	100	100	100	100	100	100
Arabinan	100	100	100	100	100	100
Mannan	100	100	100	–	–	–
Acetyl	100	100	100	100	100	100

[a]*See* Table 1 for abbreviations.

the progress of pretreatment. The pattern of dry weight loss vs time at 140 and 160°C was very similar for all woods (Fig. 1). Small amounts of wood (5–10%) became solubilized with hot water, and much larger (34–40%) amounts were solubilized during dilute acid pretreatment. The hydrolyses of substrates pretreated at 140°C were practically complete in a 15–30 min interval and in five minutes when pretreatment was at 160°C. This is very similar to results previously obtained with aspen wood and wheat straw (*4–6,12*).

The major part of the weight loss during dilute acid pretreatment is caused by hydrolysis and solubilization of hemicellulosic sugars, as shown in Table 2, for averaged reaction times of 5–20 min at 160°C. Results for pretreatments at 140°C with reaction times of 30–60 min were very similar.

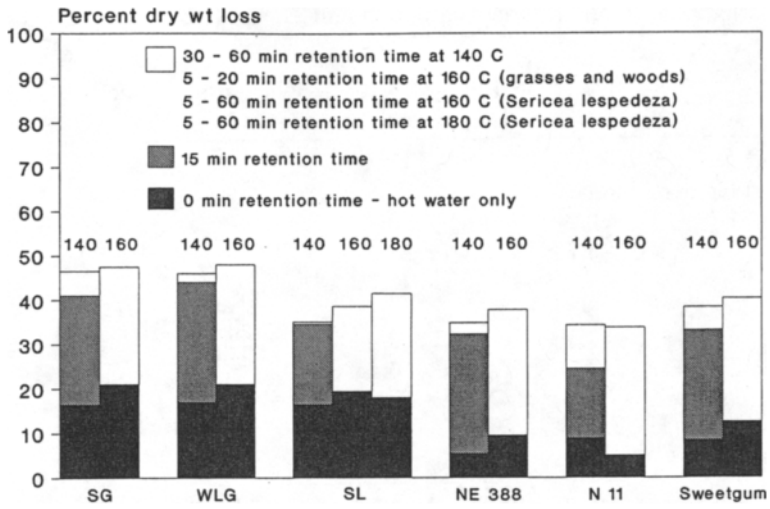

Fig. 1. Pattern of dry weight loss during acid pretreatment of hardwood and herbaceous samples (SG=Switchgrass; WLG=Weeping lovegrass; SL=Sericea lespedeza).

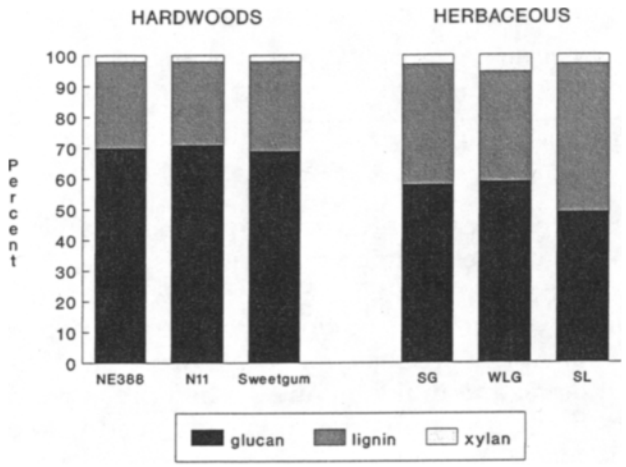

Fig. 2. Average residue compositions of hardwood and herbaceous samples after pretreatment at 160°C.

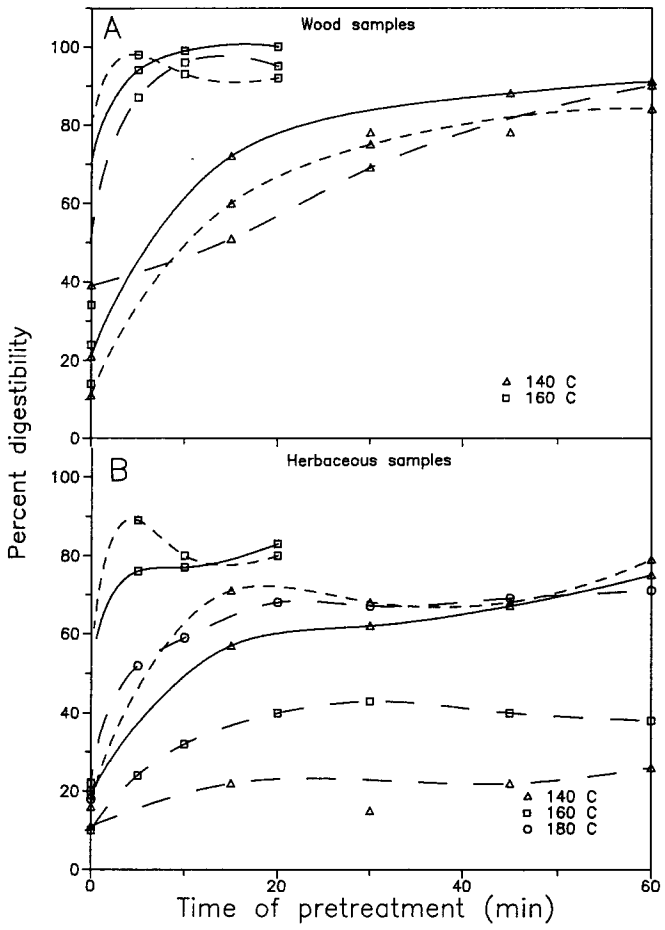

Fig. 3. A: Changes in enzymatic digestibility of cellulose in poplar NE 388 (———), poplar N11 (—— ——), and Sweetgum (— — — —) wood samples as a function of reaction time in dilute acid pretreatment. B: Changes in enzymatic digestibility of cellulose in Switchgrass (———), Weeping lovegrass (— — — —), and Sericea lespedeza (—— ——) as a function of reaction time in dilute acid pretreatment.

The lignin was solubilized only slightly (15–18% 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 very slight with only 5–10% solubilized from both poplars. Glucan was solubilized to a slightly higher extent (14–16% loss) from the sweetgum 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 fermentations. All sugars can be recovered in aqueous solutions after pretreatment

and eventually fermented to ethanol or other products. This situation contrasts with pulping, where only the solids are of value and solubilization of sugars from polysaccharides causes losses in pulp yield. Actual recoveries of hemicellulosic sugars in liquids from pretreatments at 140°C (30–60 min reaction time) and 160°C (5–10 min rt) were approximately 80–90% for xylose and 90–100% for galactose, mannose, and arabinose. Pretreatments at 160°C for 20 min were too severe, because higher destruction of sugars was observed. Small amounts of furfural (ca 0.04–0.08 wt%) and acetic acid (ca 0.3 wt%) also accumulated in liquids from these pretreatments. Overall, material balances for glucan and xylan were similar for short reaction times with recoveries being calculated at 94–103% from the starting biomass.

The composition of pretreated wood solids changes very little after removal of hemicelluloses. Average compositions for samples are shown in Fig. 2. The main component (ca. 70%) is cellulose, followed by lignin. Traces of xylan (ca. 2%) also remained in many pretreated samples.

The enzymatic digestibility of cellulose in all pretreated hardwoods rose dramatically after dilute acid prehydrolysis (Fig. 3). Cellulose digestibility in all three pretreated woods reached 90–100% after 5–10 min of pretreatment at 160°C, and slightly lower (80–90%) digestibilities were observed after 30–60 minutes at 140°C. The development of high enzymatic digestibilities of cellulose in all three woods pretreated at 160°C coincided with hydrolysis and solubilization of xylan, as we observed previously for dilute acid pretreatment of aspen wood and wheat straw (*5*). The same relationship was evident for poplar hybrid NE388 and sweetgum pretreated at 140°C. Wood of poplar hybrid N11 showed more of a linear response to pretreatment time at 140°C (Fig. 3), which indicates that additional reactions, such as lignin condensation, may contribute to development of porosity and, thus, enhanced cellulose accessibility during dilute acid pretreatment (*3*).

Herbaceous Crops

Chemical analysis of herbaceous plants is much more difficult than wood analysis. The problems are caused by much higher content of minerals, proteins, soluble sugars, and other components that can interfere with determination of cell wall components. We tested both the standard wood chemistry procedure and Van Soest's method of forage analyses (*18*). The wood chemistry procedure was retained for pretreatment investigations because it gave us direct determination of individual sugars in biomass. The Klason lignin procedure allows direct estimate 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 does not represent only lignin, but also aliphatic waxy compounds, acid insoluble ash (e.g., silica) and part of protein, this determina-

tion is very useful for investigation of dilute acid pretreatment. Klason "lignin" components are also generally insoluble in dilute acid pretreatment and represent a fairly good sum of water insoluble plant components that are not carbohydrates by nature. The fate of carbohydrates during pretreatment and enzymatic hydrolysis can then be followed separately from the rest of plant material. The analytical results for starting materials are shown in Table 1.

The pretreatment conditions for herbaceous samples were the same for hardwoods, except a slightly higher (0.5%, v/v) acid concentration was used to maintain pH = 1.35–1.45 during hydrolysis, and pretreatment of the legume (sericea lespedeza) was also investigated at 180°C because it did not respond very well at lower temperatures. The patterns of dry weight loss during dilute acid pretreatment of herbaceous crops at 140 and 160°C were very similar to those observed with hardwoods, except the content of water soluble extractives was much higher in herbaceous crops (Fig. 1 and Table 2). The dry weight losses during dilute acid pretreatment were caused by hydrolysis and solubilization (90–100%) of hemicelluloses and minor (approximately 18% for switchgrass and weeping lovegrass) solubilization of glucan (cellulose) and "lignin" (approximately 3% for switchgrass and 12% for weeping lovegrass). The solubilized sugars were again recovered from liquid fractions in high yields, together with minor amounts of furfural (ca. 0.05%) and acetic acid (ca. 0.3%). The average compositions of solids, after 5–20 min of pretreatment at 160°C, are shown in Fig. 2. The cellulose content of pretreated grasses is slightly lower than hardwoods and lower still in pretreated legume (Fig. 2). The lower cellulose contents in pretreated herbaceous crops are a direct consequence of the lower cellulose content in the starting materials (Table 1). The enzymatic digestibility of cellulose in pretreated grasses and legume is shown in Fig. 3. These results show that the two grasses respond to dilute acid pretreatment in a manner very similar to the hardwoods and wheat straw samples we have investigated so far. The enzymatic digestibility of cellulose in two grasses pretreated at 140°C was approximately 80% and increased to 85–90% for materials pretreated at 160°C.

The legume, Sericea lespedeza, appears to be much more resistant to dilute acid pretreatment (Figs. 3–5). The enzymatic digestibility of cellulose in legume pretreated at 140 and 160°C increased only to approximately 30 and 40%, respectively. Considerable digestibility (approx. 70%) of cellulose in this legume was only achieved after 20 min pretreatment at 180°C. The development of high enzymatic digestibility of cellulose still coincides with the complete hydrolysis of xylan bonds (Fig. 4), as it does for pretreatments at 140 and 160°C. However, the strong temperature effects on digestibility of cellulose in the range of 140 to 180°C, which do not seem to be related to the removal of hemicelluloses, indicate that other changes in cell wall structure and porosity have a strong influence on the effectiveness of dilute acid pretreatment of this legume. It should be also pointed out that dilute

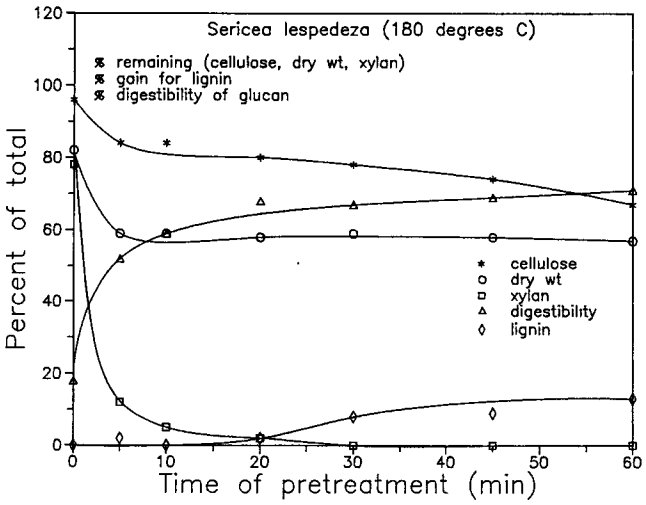

Fig. 4. Pattern of physical and chemical changes and enzymatic digestibility of Sericea lespedeza pretreated at varying times at 180°C.

acid pretreatment at 180°C caused appreciable hydrolysis of cellulose (Fig. 4), which was not reflected in the dry weight loss, but caused an increase in the apparent Klason lignin values (Fig. 4). Such an increase can be caused by the formation of hydroxymethylfurfural and its condensation into insoluble furan resins. The hydrolysis of cellulose during pretreatment at 180°C could cause serious losses of glucose from the system. The losses are significant for longer reaction times at 180°C, but they are not very large (<10%) for reaction times between 5 and 20 min since 65–75% of glucose was recovered in the liquid part of hydrolyzates for these reaction times. It has been observed by other workers (*21–24*) that the cell wall structure and composition of herbaceous legumes may be significantly different from grasses. Perhaps these differences manifested themselves during dilute acid pretreatment of sericea lespedeza.

Finally, we have also performed a limited comparative investigation of differences between rates of enzymatic hydrolysis of pretreated hardwoods and grasses. These results are shown in Fig. 5. The pretreated grasses reached slightly lower digestibility of cellulose than the pretreated hardwoods, but they seem to digest 2–3 times faster than hardwood particles. Additional studies at narrower particle size ranges are needed to establish if cellulose fibers in pretreated grasses are inherently more digestible than 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.

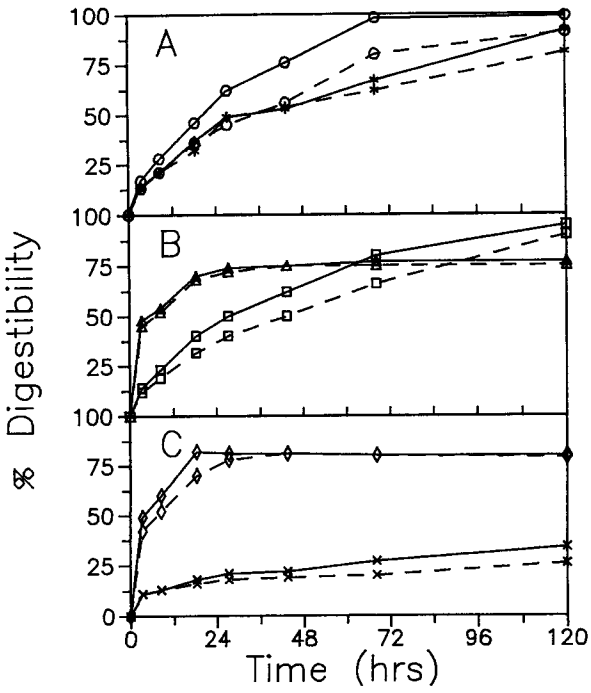

Fig. 5. Kinetics of enzymatic release of glucose from wood and herbaceous samples pretreated at 140°C for 60 min (- - -) and 160°C for 10 min (——) in dilute acid. A depicts NE388 (○) and Sweetgum (*) digestion; B depicts N11 (□) and Switchgrass (△) digestion; and C depicts Weeping lovegrass (◇) and Sericea lespedeza (x) digestion.

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