0273-2289/00/84–86/0005/$18.25

Fundamental Factors Affecting Biomass Enzymatic Reactivity

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Abstract

Poplar wood was treated with peracetic acid, KOH, and ball milling to produce 147 model lignocelluloses with a broad spectrum of lignin contents, acetyl contents, and crystallinity indices (CrIs), respectively. An empirical model was identified that describes the roles of these three properties in enzymatic hydrolysis. Lignin content and CrI have the greatest impact on biomass digestibility, whereas acetyl content has a minor impact. The digestibility of several lime-treated biomass samples agreed with the empirical model. Lime treatment removes all acetyl groups and a moderate amount of lignin and increases CrI slightly; lignin removal is the dominant benefit from lime treatment.

Index Entries: Lignocellulose; enzymatic digestibility; lignin; acetyl groups; crystallinity; correlation; pretreatment; lime.

Introduction

Lignocellulose is the most abundant biomass that can be converted into liquid fuels by enzymatic hydrolysis and/or microbial fermentation *(1,2)*. Because lignocellulose is water insoluble, the heterogeneous reactions involved in biomass conversion processes require direct physical contact between enzymes and substrates (i.e., cellulose and hemicellulose) *(3)*.

Pretreatments are necessary to enhance biomass digestibility by altering biomass structural features *(4–10)*. Theoretically, if the role of structural features that determine digestibility can be modeled, then it is possible to predict the enzymatic digestibility of lignocellulose and to design more effective pretreatments.

Table 1 summarizes data from previous studies on the physical and chemical structural features that affect lignocellulose digestibility *(11–40)*. Lignin content and cellulose crystallinity have received the most attention.

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Table 1
Summary of Relationships Between Biomass Structural Features and Digestibility

		Structural feature													
		Physical						Chemical							
Biomass	Digestibility	Crystallinity	DP[a]	Pore volume	Surface area	Particle size	Accessibility	Lignin	Hemicellulose	Cellulose	Ash	Acetate	CSM[b]	Relationship between structural features and digestibility[c]	Ref.
Cellulose	Enzymatic	✔												Inverse	*11*
Cellulose	Enzymatic	✔	✔											CrI, inverse; DP, n/c	*12*
Forages	Ruminant							✔						$D = 100 - 6\,L$	*13*
Forages	Ruminant							✔						$D = 110.4 - 1716/(100 - L)$	*14*
Cellulose	Enzymatic			✔	✔									Inversely linear	*15*
Hardwoods	Ruminant							✔						Linear	*16*
Birch, pine, fir, oak	Ruminant							✔						DL, exponential (hardwoods) and sigmoid (softwoods)	*17*
Ryegrass straw	Ruminant					✔								n/c	*18*
Cellulose	Enzymatic	✔												Inverse	*19*
Grass straws	Ruminant							✔	✔	✔			✔	$D = 97.51 - 1.36\,L - 0.61C$	*20*
Cellulose	Enzymatic	✔												Inverse	*21*
Cellulose	Enzymatic	✔			✔									CrI, inverse; surface area, n/c	*22*
Newspaper, corn stover, pine, cellulose	Enzymatic		✔											n/c	*23*
Wheat straw	Enzymatic	✔						✔						Inverse	*24*
Wheat straw	Enzymatic	✔			✔			✔						$D = 2.044\mathrm{SSA}^{0.988}(100 - \mathrm{CrI})^{0.257}L^{-0.388}$	*25*
Bagasse	Ruminant	✔												Inverse	*26*

Bagasse, wheat straw woods, cellulose	Enzymatic	✔	✔									DP, inverse; CrI, n/c	27
Poplar, pine, mixed hardwoods	Enzymatic	✔		✔	✔							CrI, n/c; pore volume and SSA, linear	28
Cellulose	Enzymatic	✔					✔[d]					Inverse	29
Rice straw	Enzymatic						✔[d]					Linear	30
Cellulose	Enzymatic	✔		✔		✔	✔[f]					CrI and pore volume, strong; particle size and accessibility, weak	31
Poplar	Enzymatic			✔								Linear	32
Newspaper, cardboard	Enzymatic	✔				✔						CrI, inverse; particle size, n/c	33
Poplar, wheat straw	Enzymatic										✔	Inverse	34
Cellulose, bagasse	Enzymatic	✔	✔		✔	✔						CrI, linear (cellulose), n/c (bagasse); SSA, linear (both); particle size and DP, n/c (both)	35
Poplar	Enzymatic						✔	✔			✔	Lignin and acetate, inverse; xylan, n/c	36
Mixed hardwoods	Enzymatic	✔			✔		✔					$D = 0.444(100 - \mathrm{CrI})^{0.293}(G/L)^{0.247}\mathrm{SSA}^{0.827}$	37
Cellulose, wheat straw	Enzymatic	✔					✔					$D = 122 - 0.21\mathrm{CrI} + 0.59DL - 0.013\mathrm{CrI}^2 - 0.011DL^2 + 0.015\mathrm{CrI}\cdot DL$	38
Woods	SSF[e]						✔	✔	✔	✔		$D = 200.8 - 5.67L$	39
Corn fiber	Enzymatic					✔						n/c	40

[a]Degree of polymerization.
[b]Cell-soluble matter.
[c]*D*, digestibility; CrI, crystallinity index; *L*, lignin content; *C*, cellulose content; *G*, glucan content; SSA, specific surface area; *DL*, extent of delignification; n/c, no correlation.
[d]Accessibility to cadoxen.
[e]Simultaneous saccharification and fermentation.
[f]Accessibility to formylation.

Lignin, a biologically resistant, netlike polymer surrounding cellulose and hemicellulose *(41)* correlates inversely with digestibility *(13,14,16,17,20, 24,25,36–39)*. In contrast, studies on crystallinity diverge. Although many researchers indicated that crystallinity correlates inversely with digestibility *(11,12,21,22,24–26,29,31,33,35,37,38)*, others suggested that other physical features might play a more important role, such as degree of polymerization *(27)*, pore volume *(28)*, surface area *(19,27,28)*, and particle size *(19,27)*. Bouveng *(42)* reported that about 70% of xylan residues contain acetyl groups. It is thought that acetyl groups sterically hinder xylanase activity. Deacetylation increased swellability *(8)* and enzymatic digestibility of poplar wood and wheat straw *(34,36)*.

Although the literature presents correlations between structural features and biomass digestibility, many studies have the following limitations:

1. The numbers of tested samples were small, reducing the predictive ability of these correlations.
2. A narrow spectrum of structural features was investigated.
3. In some studies, cross effects between structural features may have occurred during a pretreatment; that is, the pretreatment conditions that changed one structural feature might also change others. For example, NaOH pretreatments removed not only lignin but also removed acetyl groups from hemicellulose *(16,36,43)* and reduced crystallinity *(25)*.
4. The models reported previously have been applied only to biomasses from which they were derived; therefore, their predictive ability is unknown.

Our previous studies on switchgrass, bagasse, wheat straw, and poplar wood show that lime is an effective pretreatment agent *(44–46)*. However, why lime works remains unknown: What structural features change to render lime-treated biomass digestible? When answering this question, we will gain insight into pretreatments in general and be able to design more effective pretreatments.

Three structural features were studied as the independent variables: lignin content, acetyl content, and crystallinity. These three features were selected because they can be directly manipulated by pretreatment processes. To prepare 147 model lignocellulosic samples, poplar wood was selectively delignified using peracetic acid, deacetylated using KOH, and decrystallized using ball milling to create a broad spectrum of lignin contents, acetyl contents, and crystallinities. Cross effects were minimized. The model lignocelluloses were used to develop empirical correlations between these three structural features and enzymatic digestibility. The predictive ability of these correlations was tested for a variety of lime-treated biomasses (bagasse, switchgrass, and poplar wood) and ball-milled α-cellulose.

Materials and Methods

Substrates

Hybrid poplar, switchgrass, and bagasse were ground using a Thomas-Wiley laboratory mill (Arthur H. Thomas Company, Philadelphia, PA) and sieved through a 40-mesh screen.

Selective Delignification

Peracetic acid *(47)* was used as a delignifying reagent according to the following conditions: peracetic acid loading, 0.1–5.0 g/g of dry biomass; time, 24–48 h; temperature, 25°C; and biomass concentration, 0.05–0.1 g of solid/g of liquid.

Selective Deacetylation

Kong et al. *(36)* selectively deacetylated poplar wood with dilute KOH. The effects of deacetylation conditions were studied based on these conditions. In our study, the conditions explored were as follows: KOH loading, 0.1–1.5 mmol/g of dry biomass; time, 24–48 h; temperature, 25°C; and biomass concentration, 0.1 g of solid/g of liquid.

Selective Decrystallization

Ball milling decrystallized the biomass. The ball mill was built with two 1/6-hp, 156-rpm AC gearmotors (Dayton Electric Mfg. Co., Niles, IL). The rollers consisted of four 2.5-cm diameter × 63.5-cm long steel shafts enclosed with 3.8-mm od Buna-N rubber tubing (McMaster-Carr, Atlanta, GA). A 300-mL porcelain jar was charged with 9.525-mm zirconia grinding media (U.S. Stoneware, East Palestine, OH) to 50% of the jar volume. A sufficient amount of biomass was placed in the jar to fill the void volume between the media (about 0.12 mL/g of media). Then the jar was placed between the rollers and rotated at 74 rpm for various time periods (0–8 d) at room temperature.

Material Balances and Biomass Composition

Untreated and treated (delignified, deacetylated, decrystallized, or lime-treated) samples were repeatedly washed with fresh distilled water until the decanted water became colorless. The total dry wt of the sample was measured before and after treatment and washing. Dry weight measurement for material balances was described previously *(45)*.

Lignin content was determined as the summation of Klason lignin and acid-soluble lignin. Moisture, carbohydrate (glucan and xylan), Klason lignin, and acid-soluble lignin contents were measured using National Renewable Energy Laboratory standard procedures No. 001–004 *(48)*. Acetyl groups were measured using the transesterification method *(47,49)*. Throughout this article, the composition (lignin, acetyl, and carbohydrate contents) is expressed based on the dry wt of biomass, which was determined by drying the biomass at 105°C for 8 h.

Table 2
Treatment Conditions for Preparing Model Lignocelluloses

Condition	Delignification	Deacetylation	Decrystallization
Temperature	25°C	25°C	25°C
Time	24 h[a]	24 h	0, 3, and 6 d
Reagent or method	Peracetic acid	Potassium hydroxide	Ball milling[b]
Reagent loading	0, 0.1, 0.2, 0.3, 0.5, 1.0, and 5.0 g/g dry biomass	0, 0.07, 0.15, 0.35, 0.55, 0.75, and 1.5 mmol/g dry biomass	43 g grinding media/g dry biomass
Biomass concentration	0.1 g solid/g liquid[c]	0.1 g solid/g liquid	—

[a]Except that when peracetic acid loading is 5.0 g/g dry biomass, reaction time is 48 h.
[b]Grinding media, 9.525-mm zirconia; mill jar, 300-mL porcelain jar.
[c]Except that when peracetic acid loading is 5.0 g/g dry biomass, biomass concentration is 0.05 g solid/g liquid.

Crystallinity Measurements

Biomass crystallinity was measured by the XRD Laboratory, Department of Geophysics, Texas A&M University (College Station, TX) using a Rigaku Powder X-ray Diffractometer (Rigaku Denki, Danvers, MA). The specimen was prepared by placing approx 0.1 g of the milled biomass sample on a glass sample holder and then placing a drop of acetone on the sample to fix it on the holder. The sample was scanned at 2°/min from 2θ = 10° to 26° with a step size of 0.05°. The crystallinity was determined as the percentage of crystalline material in the biomass and expressed as the crystallinity index (CrI) *(50)*:

$$\mathrm{CrI} = [(I_{002} - I_{am})/\ I_{002}] \times 100 \quad (1)$$

in which I_{002} is the intensity of the 002 peak (at about 2θ = 22.5°) and I_{am} is the intensity at 2θ = 18.7°.

Preparation of Model Lignocelluloses

Table 2 summarizes the treatment conditions used to systematically prepare many model lignocelluloses with various lignin contents, acetyl contents, and CrIs. A large amount (about 4200 g) of poplar wood was divided into seven groups of about 600-g samples. Each 600-g sample was delignified by approx 0, 10, 20, 40, 60, 80, or 100% using peracetic acid under the recommended conditions. Each delignified sample was washed and dried and then divided again into seven subgroups of about 80-g samples that were deacetylated by approx 0, 10, 20, 40, 60, 80, and 100% using KOH under the recommended conditions. The delignified-and-deacetylated samples were washed and dried, and their lignin, acetyl, and carbohydrate contents were measured.

Each of these delignified-and-deacetylated samples was again divided into three groups and then ball-milled for 0, 3, and 6 d as suggested in Table 2.

Table 3
Water Solubility of Poplar Wood Components Before and After 6-d Ball Milling

Component	Raw composition (g component/g total)	Weight loss (%)[a] Washed only	Ball-milled[b] and washed	Amount removed by ball milling (%)[b]
Total	—	4.1	3.9	–0.2
Lignin	0.25	5.1	1.4	–3.7
Acetyl	0.03	2.8	2.2	–0.6
Glucan	0.43	4.0	3.9	–0.1
Xylan	0.18	7.3	8.9	1.6

[a]Weight percentage is based on the initial weight of each component.
[b]Grinding media, 9.525-mm zirconia; mill jar, 300-mL porcelain jar; time, 6 d; temperature, 25°C.

It was not necessary to remeasure the chemical composition after ball milling because, as indicated in Table 3, ball milling does not change biomass chemical composition, even under the most severe condition (i.e., 6 d). In total, there were 147 (7 × 7 × 3) model lignocellulosic samples; the CrI of each sample was measured.

Enzymatic Hydrolysis

Biomass was enzymatically hydrolyzed at 50°C, pH 4.8, for 3 d in a 100-rpm air-bath shaker, with a cellulase loading of 5 filter paper units (FPU)/g of dry biomass and a cellobiase loading of 28.4 cellobiase units (CBU)/g of dry biomass. The activity of cellulase (Cytolase CL enzyme, lot no. 17-92262-09, Environmental BioTechnologies, Santa Rosa, CA) was 91.8 FPU/mL using the filter paper assay *(48)*. The activity of cellobiase (Novozym 188, batch no. DCN00024, Novo Nordisk, Franklinton, NC) was 250 CBU/mL based on Novo's assay, which was performed by the company. The biomass concentration was 0.05 g/mL.

Samples (about 4 mL) were taken at time zero, 1 h, and 3 d. Time-zero samples were taken to measure background glucose and xylose concentrations from the enzymes and any soluble sugars in the biomass. One-hour samples were taken to indicate the initial digestion rates, and 3-d samples were to indicate the extent of digestion. After removal, the samples were boiled for 15 min in sealed tubes to denature the enzymes and thus prevent further hydrolysis. Then the glucose and xylose concentrations were measured using high-performance liquid chromatography (HPLC). The enzymatic digestibility of each sample was expressed as the percentage of glucan and xylan converted to soluble sugars.

Sugar Measurements

Glucose and xylose were measured using HPLC as described previously *(45,46)*. Because no other sugars were detected, in this article, *total sugar* denotes the summation of glucose and xylose.

Lime Pretreatment

Switchgrass and bagasse were treated with lime (calcium hydroxide) under various conditions as described previously *(45,46)*. Because of its high lignin content, poplar wood was treated with lime and oxygen at various pressures *(44)*.

Data Analysis

Ideally, we would use software that automatically fits the data to various built-in four-dimensional (4D) (lignin content, acetyl content, CrI, and digestibility) equations; unfortunately, such software was not available. However, TableCurve 3D software (Version 3.0, SPSS [Chicago, IL]) can automatically fit three-dimensional (3D) data to its built-in bank of about 453 million equations. To reduce the number of dimensions from four to three, the data sets *(51)* were sorted into several groups with similar acetyl content, the least influential independent variable. (The average acetyl content was used to characterize the acetyl content of the group.) For a group with similar acetyl content, TableCurve 3D was able to find an empirical function and determine parameters that fit the enzymatic digestibility to the lignin content and CrI. The fitted parameters of each group depend on the average acetyl content of each group. Those 3D parameters that depended on acetyl content were fit using a polynomial; SigmaPlot (Version 4.0, SPSS) was used to find the appropriate order and parameters in the polynomial. The best-fit polynomial was then substituted into the 3D equation obtained from TableCurve 3D, giving a 4D equation with the three independent variables (lignin, CrI, and acetyl). This procedure defined the form of the empirical equation. To obtain better parameter values, SigmaPlot was used to fit all 147 data sets to the 4D equation. This approach eliminated the errors that might result from the sorting step. Correlations were developed for both 1-h and 3-d digestibility.

Results and Discussion

Selectivity of Treatment Methods

Figure 1 illustrates the effects of peracetic acid loading on lignin and acetyl removals from poplar wood. It shows that under these conditions, lignin removals can be controlled from 8 to 95% by adjusting peracetic acid loading and biomass concentration. Figure 1 also shows that peracetic acid delignification under these conditions is fairly selective because only a small portion of acetyl was removed (0–14%).

Figure 2 illustrates the effects of KOH loading on acetyl and lignin removals from poplar wood. The acetyl removals distributed quite evenly from 14 to 97%, whereas the lignin removals were only 5–15%. This indicates that KOH deacetylation under these conditions is fairly selective.

Figure 3 shows the effects of milling time on CrI. Within 8 d, the CrI of ball-milled poplar wood and α-cellulose (Sigma, St. Louis, MO) were

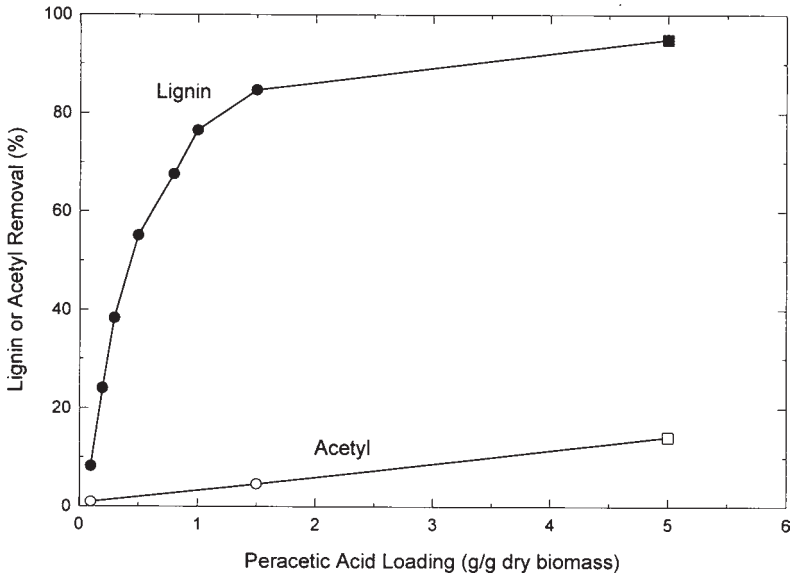

Fig. 1. Selective delignification. Treatment conditions: peracetic acid loading, 0.1–5 g/g dry biomass; time, 24 h (except for ■ and □, 48 h); temperature, 25°C; biomass concentration, 0.1 g solid/g liquid (except for ■ and □, 0.05 g solid/g liquid).

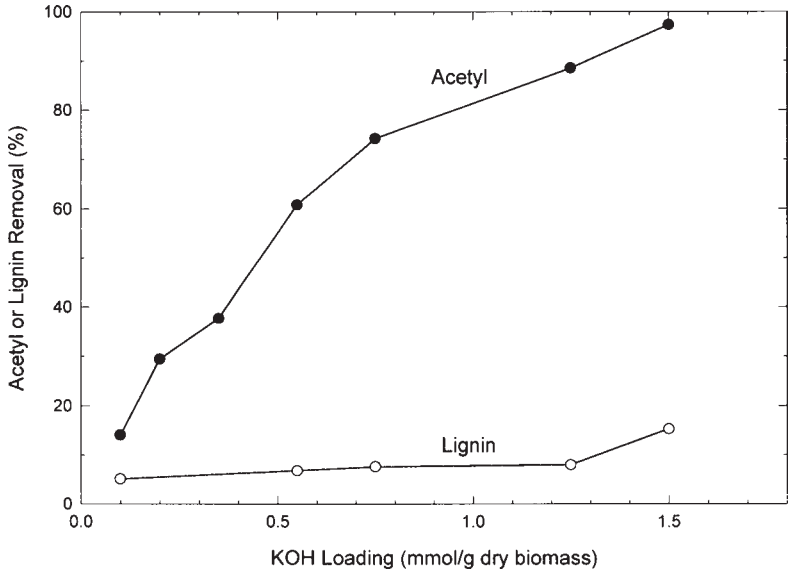

Fig. 2. Selective deacetylation. Treatment conditions: KOH loading, 0.1–1.5 mmol/g dry biomass; time, 24 h; temperature, 25°C; biomass concentration, 0.1 g solid/g liquid.

reduced from 54 to 10% and from 67 to 6%, respectively. Table 3 shows that ball milling is selective because none of the poplar wood components were removed by 6-d ball milling except for a slight amount of xylan (1.6%).

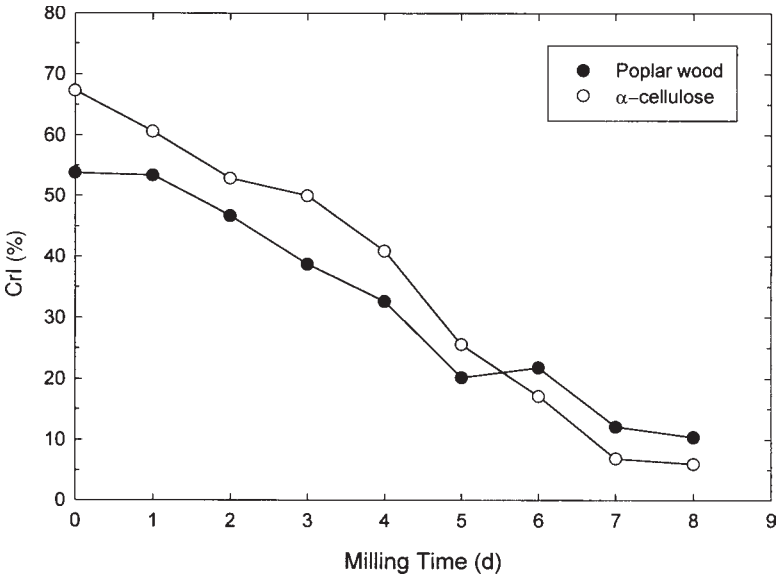

Fig. 3. Effects of milling time on CrI of poplar wood and α-cellulose.

Ball milling not only decrystallized the model lignocelluloses but also reduced their particle size. It is possible that any benefits from ball milling attributed to lower CrI actually result from a smaller particle size. However, particle size studies on poplar wood and switchgrass *(44,45)* show that below 40 mesh, particle size has little effect on biomass digestibility. This is consistent with other studies on ryegrass straw *(18)*, pure cellulose *(31)*, newspaper and cardboard *(33)*, bagasse *(35)*, and corn fiber *(40)*.

Structural Features of Model Lignocelluloses

The 147 model lignocelluloses contained the following structural features: lignin content, 0.7–26.3%; acetyl content, 0.1–3.1%; and CrI, 9.1–63.9% *(51)* (*see* Fig. 4). Slight cross effects occurred during severe deacetylation. At high KOH loadings (i.e., 1.5 mmol/g of dry biomass) needed to remove most acetyl groups (91% on average), some lignin (about 14%) was also removed.

For crystalline (not ball-milled) samples, the CrI generally increased with more severe delignification and/or deacetylation conditions because amorphous material (i.e., lignin and acetyl groups) was removed *(50)*.

Enzymatic Digestibility of Model Lignocelluloses

The detailed numerical data show that enzymatic digestibility increases at lower lignin contents, acetyl contents, and CrI *(51)*. From these data, we can answer several questions:

1. Do the initial hydrolysis rates (i.e., 1-h conversions) correlate with the ultimate sugar yields (3-d conversions)?

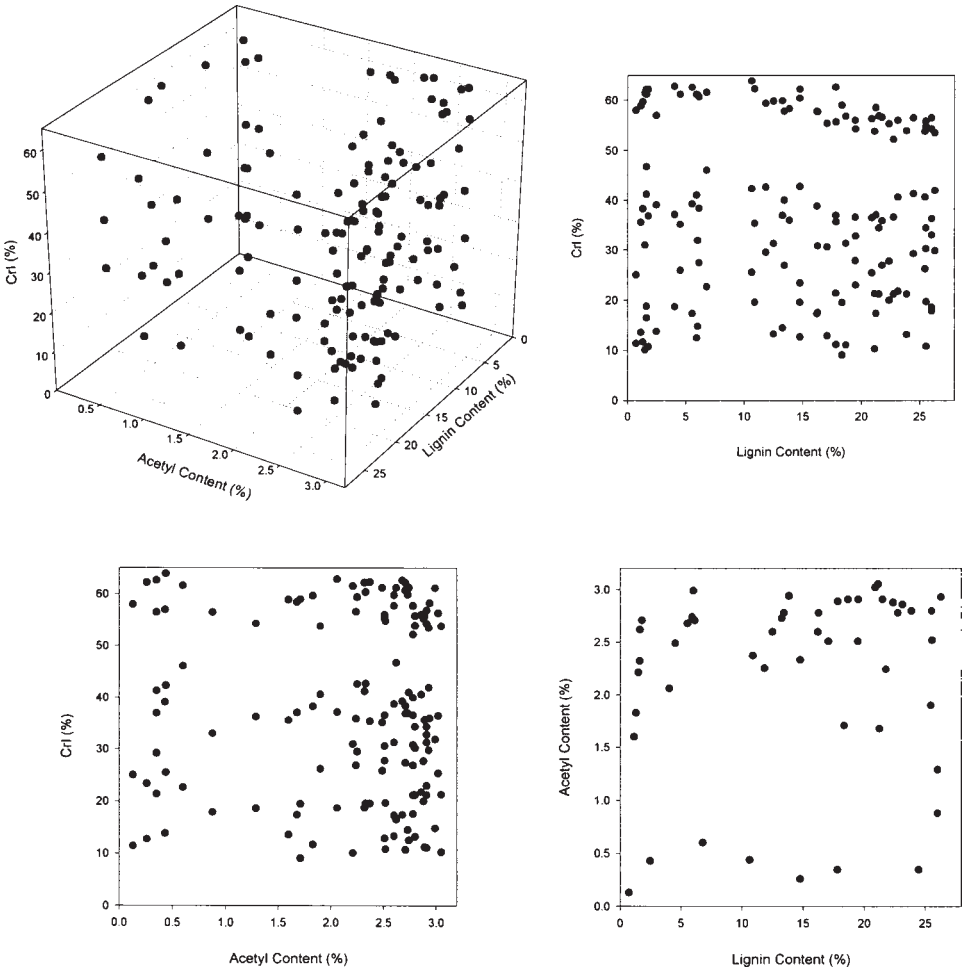

Fig. 4. Distribution of the structural features of the model lignocelluloses.

2. Do they give similar information?
3. What is the relative importance of the three structural features?
4. As barriers to enzymatic hydrolysis, how do these features relate to each other?
5. Specifically, are these enzyme barriers in series or in parallel?
6. Is it necessary to remove all these barriers to render the biomass enzymatically digestible (i.e., serial relationship), or is it sufficient just to remove one or some of them (i.e., parallel relationship)?

Correlation Between Initial Hydrolysis Rate and Ultimate Sugar Yield

Figure 5 shows that although the 1-h and 3-d total sugar conversions are correlated, there is significant scatter; therefore, they may provide different information. For example, some biomass samples had a low initial hydrolysis rate but a high 3-d sugar yield. Hence, it is important to study both 1-h and 3-d conversions.

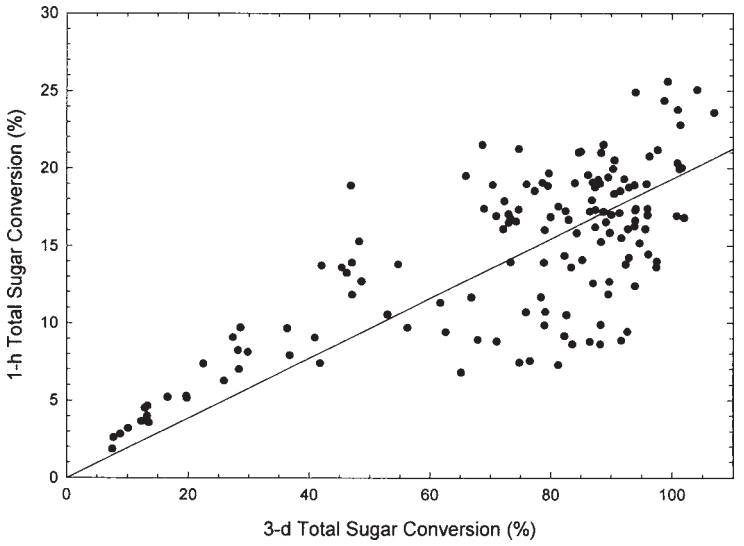

Fig. 5. Correlation between 1-h and 3-d total sugar conversions.

Effects of Lignin Content, Acetyl Content, and CrI on Ultimate Sugar Yield

Figure 6 illustrates the effects of the three structural features on 3-d total sugar conversions for high-acetyl (2.7–2.9%), low-acetyl (0.1–0.6%), high-lignin (24.5–26.3%), and low-lignin (0.7–1.8%) poplar wood (*see* Table 4 for summary). The data show that either lignin removal or decrystallization results in high 3-d digestibility; acetyl removal had a small effect. Low lignin is sufficient to obtain high digestibility regardless of CrI or acetyl content; low crystallinity is sufficient regardless of lignin or acetyl content.

Because enzymatic hydrolysis requires direct physical contact between enzyme and substrate *(3)*, enzymes must diffuse from the bulk aqueous solution to the particle surface, diffuse through physical barriers such as lignin, adsorb on the substrate surface, and then catalyze the hydrolysis. Lee and Fan *(52)* suggest that diffusive mass transfer of enzymes does not control hydrolysis rate; instead, it depends on the quantity of enzymes adsorbed on the substrate and their effectiveness. Furthermore, they suggest that effectiveness is a function of CrI.

Our data agree with Lee and Fan's *(52)* suggestions. We can interpret the results in Fig. 6 as follows: For samples with both low lignin and low CrI, many enzymes can adsorb onto the substrate. Furthermore, they are very effective and rapidly digest the biomass. For samples with low lignin and high CrI, many enzymes can adsorb on the substrate but they are not as effective owing to high crystallinity; however, sufficient enzymes are adsorbed so that the biomass is digested after a relatively long time (3 d). For samples with high lignin and low CrI, although the amount of enzymes adsorbed on the substrate is small (because lignin is a barrier and it can

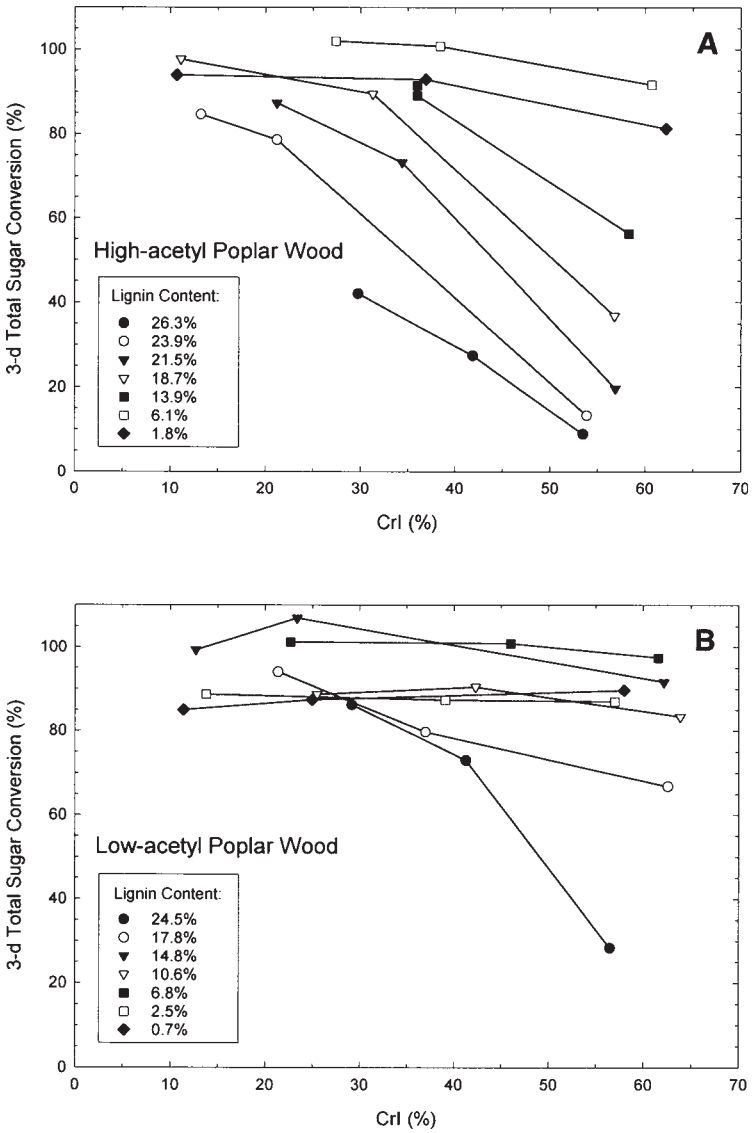

Fig. 6. *(continued on the next page)* Effects of lignin content and CrI on 3-d total sugar conversion of poplar wood for **(A)** high-acetyl samples (2.7–2.9%) and **(B)** low-acetyl samples (0.1–0.6%).

serve as a "sink" onto which enzymes unproductively adsorb), the enzymes adsorbed on the substrate are so effective owing to low crystallinity that high digestion can occur within 3 d. However, for samples with both high lignin and high CrI, enzymes are blocked by or adsorbed onto lignin allowing only a little to adsorb on the substrate surface. In addition, the enzymes adsorbed on the substrate are not effective because of the high crystallinity. In this case, the digestion is poor, even given a long reaction time.

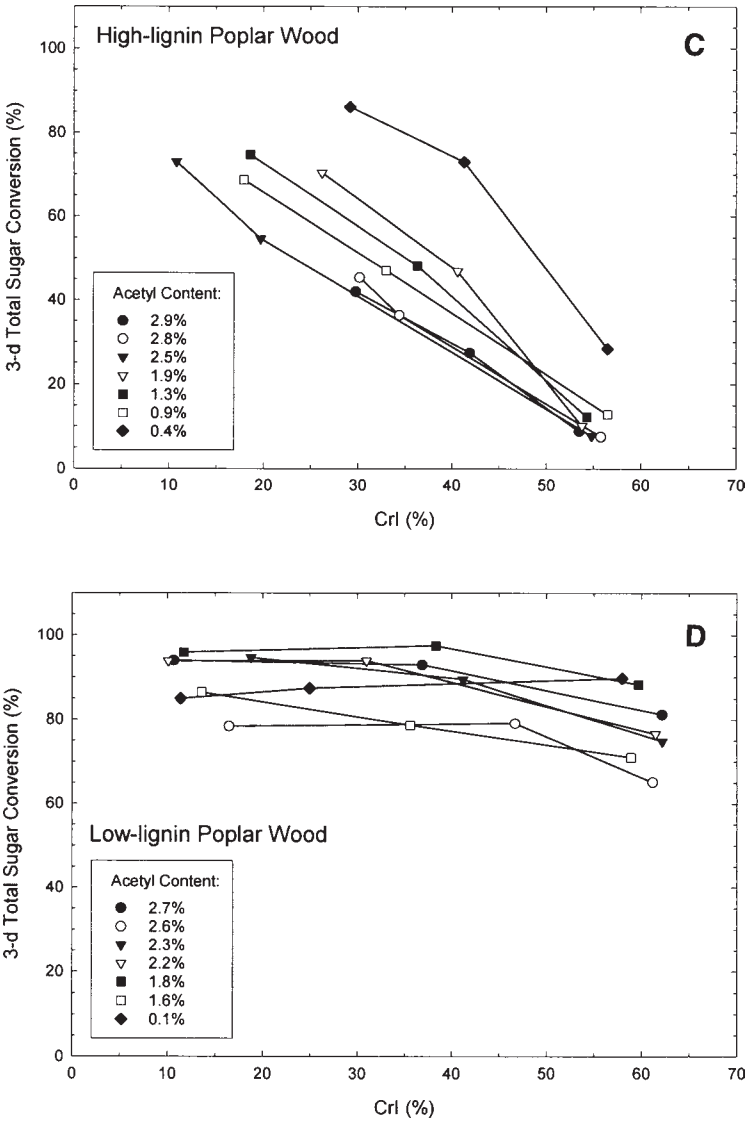

Fig. 6. *(continued from the previous page)* Effects of lignin content and CrI on 3-d total sugar conversion of poplar wood for **(C)** high-lignin samples (24.5–26.3%) and **(D)** low-lignin samples (0.7–1.8%).

Effects of Lignin Content, Acetyl Content, and CrI on Initial Hydrolysis Rate

The picture is different at shorter hydrolysis times. Figure 7 shows that CrI significantly affected initial hydrolysis rates (i.e., 1-h conversion) not only for the high-lignin samples but also for the low-lignin ones. High hydrolysis rates were obtained from the low-CrI samples regardless of lignin and acetyl contents, indicating that enzyme effectiveness depends significantly on CrI. For the low-lignin, high-CrI samples, although many

Table 4
Effects of Lignin Content, Acetyl Content, and CrI on Digestibility[a]

Lignin content		CrI		Acetyl content		3-d Digestibility		1-h Digestibility	
High	Low	High	Low	High	Low	High	Low	High	Low
✔		✔		✔			×		×
✔		✔			✔		×		×
✔			✔	✔		×		×	
✔			✔		✔	×		×	
	✔	✔		✔		×			×
	✔	✔			✔	×			×
	✔		✔	✔		×		×	
	✔		✔		✔	×		×	

[a]Summarized information from Figs. 6 and 7.

enzymes were adsorbed, they were not very effective on the high-crystallinity substrate, resulting in a low hydrolysis rate. Table 4 summarizes these observations.

A Schematic Model

Figure 6D shows that for low-lignin biomass, acetyl removal had no effect; that is, high digestibility was obtained even for high-acetyl biomass. However, for high-lignin biomass, Figs. 6C and 7C show that extensive acetyl removal increased conversions significantly. This implies that the relationship between lignin and acetyl barriers may be parallel, rather than serial.

Figure 8 shows a schematic model consistent with the data presented in Figs. 6 and 7. In this model, enzymes flow through pipes before reaching the substrate tank. The flow through each pipe is regulated by a valve. (Note: In this model, the valves are imperfect and leak.) The enzyme flow in the wide pipe is controlled by a large valve—lignin content—whereas that in the narrow pipe is controlled by a small valve—acetyl content. When the lignin valve is opened (i.e., most lignin is removed), enzymes can easily flow through the wide pipe and arrive at the substrate tank to adsorb on the substrate surface. In this case, fully opening the acetyl valve (i.e., removing all acetyl groups) has little effect on the enzymes flowing to the substrate tank because the acetyl pipe is small compared to the lignin pipe. In contrast, if the lignin valve is closed (i.e., none or little lignin is removed), enzymes can hardly flow through the wide pipe; however, if the acetyl valve is open, some enzymes can still flow through the narrow pipe and arrive at the substrate tank. If both valves are closed, few enzymes are able to squeeze through and reach the substrate tank.

After the enzymes arrive at the substrate tank, they begin to work. How fast they work (i.e., the enzyme effectiveness) depends on the substrate crystallinity. If the substrate is highly crystalline, the enzyme effec-

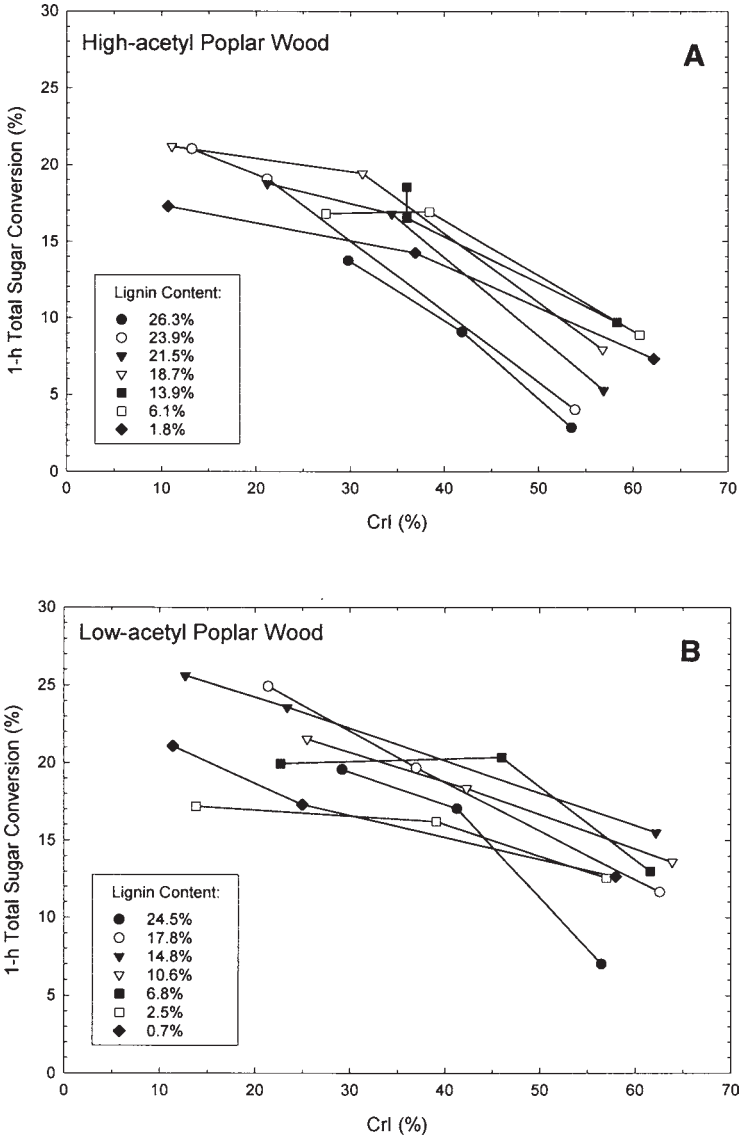

Fig. 7. *(continued on the next page)* Effects of lignin content and CrI on 1-h total sugar conversion of poplar wood for **(A)** high-acetyl samples (2.7–2.9%) and **(B)** low-acetyl samples (0.1–0.6%).

tiveness is low and the enzymes work slowly. In contrast, if the substrate is amorphous, the enzyme effectiveness is high and the enzymes adsorbed on the substrate work rapidly.

In this model, the ultimate extent of hydrolysis depends on two factors: how many enzymes arrive at the substrate tank and how fast they work. If there are many enzymes in the substrate tank, they may finish their job after a long time (e.g., 3 d) regardless of how fast they work. However, if just a few enzymes are present in the substrate tank, fast-working enzymes

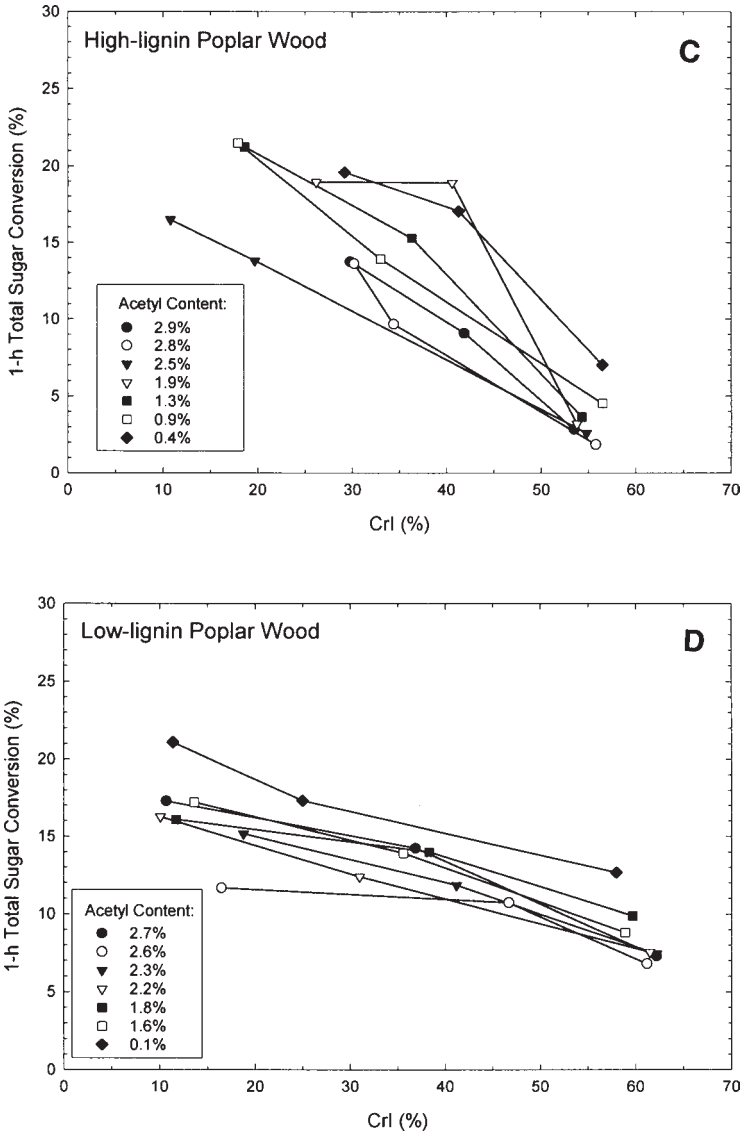

Fig. 7. *(continued from the previous page)* Effects of lignin content and CrI on 1-h total sugar conversion of poplar wood for **(C)** high-lignin samples (24.5–26.3%) and **(D)** low-lignin samples (0.7–1.8%).

may finish the job in time, but slow-working enzymes may not; in this case, enzyme effectiveness becomes important to the ultimate extent of hydrolysis.

Responses of Glucan and Xylan to Structure Changes

Figure 9 shows the effects of acetyl and lignin removals on 3-d glucan and xylan conversions. Figure 9A shows that 89% acetyl removal increased the glucan and xylan conversions by 2.3 and 7.3 times, respectively, indicating that deacetylation had more significant effects on hemicellulose digestibility than on cellulose digestibility. This observation agrees with

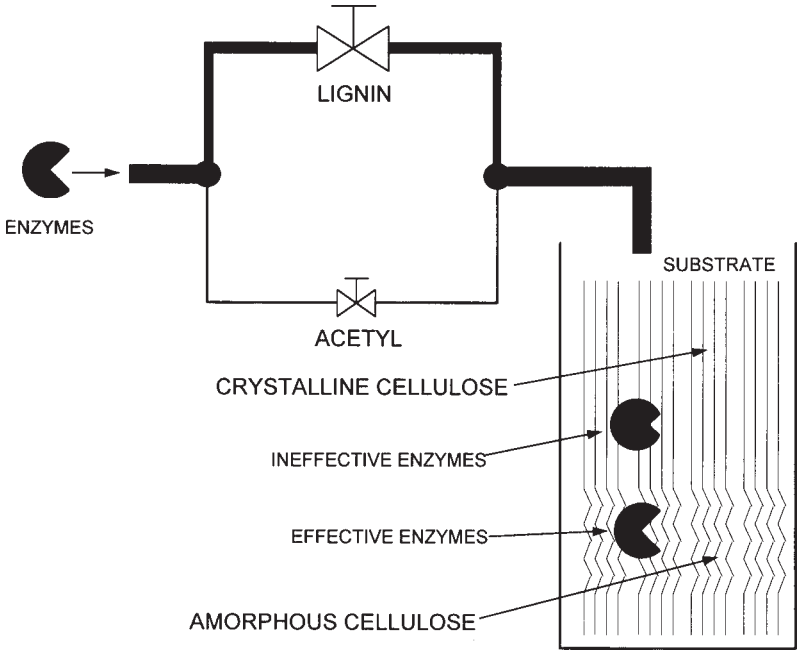

Fig. 8. A schematic model for the effects of lignin, acetyl groups, and crystallinity on enzymatic hydrolysis.

the findings of Grohmann et al. *(34)* for poplar wood and wheat straw. Figure 9B shows that 95% lignin removal increased the 3-d glucan and xylan conversions by 7.9 and 14.9 times, respectively. Clearly, glucan and xylan hydrolysis responds differently to structural features.

Correlations Between Enzymatic Digestibility and Structural Features

Because glucan and xylan respond differently to structural features, separate correlations for glucan and xylan must be developed. The proposed correlations have the following functionality:

$$X_G = f\left(\frac{\text{lignin content}}{\text{glucan content}}, \frac{\text{acetyl content}}{\text{glucan content}}, \text{CrI}\right) \quad (2)$$

$$X_X = f\left(\frac{\text{lignin content}}{\text{xylan content}}, \frac{\text{acetyl content}}{\text{xylan content}}, \text{CrI}\right) \quad (3)$$

in which X_G is the 1-h or 3-d glucan conversion (%) and X_X is the 1-h or 3-d xylan conversion (%). Rather than expressing lignin content as a percentage of the total biomass, it was expressed as a ratio to the glucan or xylan. This is logical if one views lignin as a barrier or enzyme sink that prevents enzymes from reaching carbohydrate, whether it is glucan or

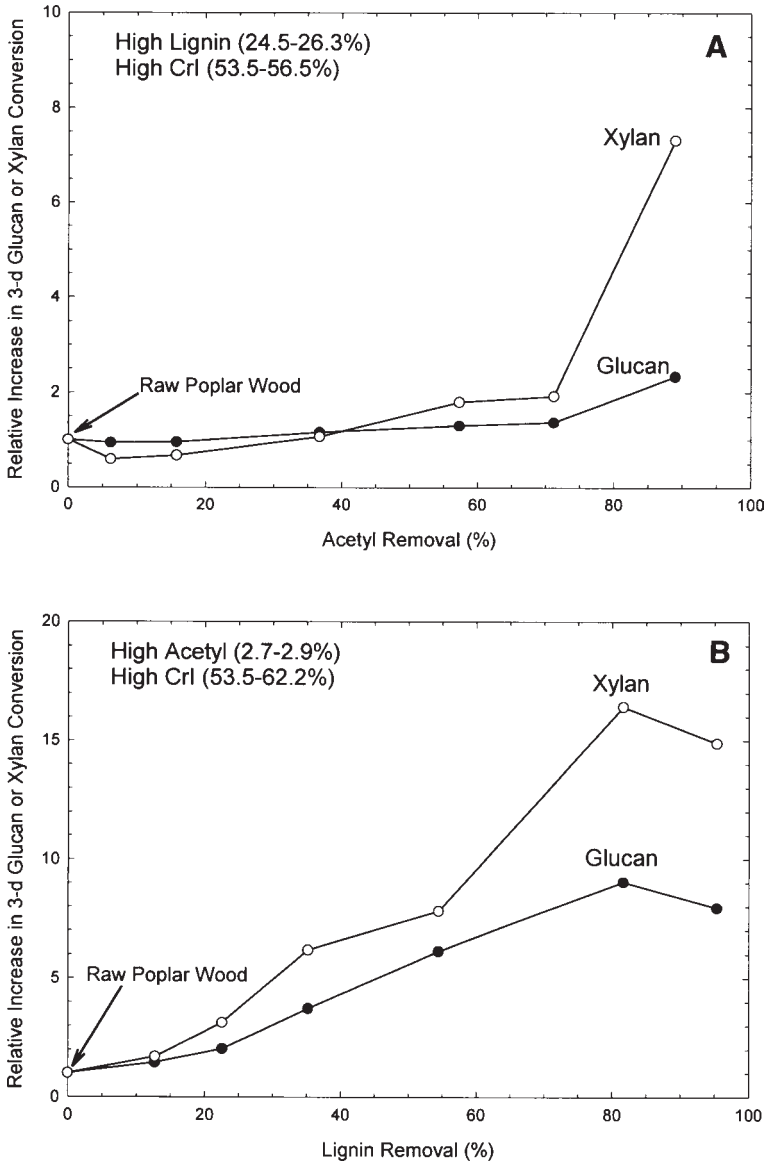

Fig. 9. Effects of acetyl or lignin removal on relative increases in 3-d glucan and xylan conversions of high-crystallinity poplar wood with **(A)** high-lignin content and **(B)** high-acetyl content.

xylan. A similar argument can be made for expressing acetyl content as a ratio to either glucan or xylan.

Because the enzymatic hydrolysis results show that acetyl content is the least influential independent variable, the 147 data sets were sorted into seven groups based on the acetyl/carbohydrate ratios. Each group contained between 9 and 33 data sets with similar acetyl/glucan or acetyl/xylan

ratios. TableCurve 3D determined that the following equations fit the sorted groups best:

For 1-h and 3-d glucan conversion

$$X_G = \frac{a}{1+\exp\left(\frac{b-\frac{L}{G}}{c}\right)} + \frac{d}{1+\exp\left(\frac{e-\mathrm{CrI}}{f}\right)} + \frac{g}{\left[1+\exp\left(\frac{b-\frac{L}{G}}{c}\right)\right]\left[1+\exp\left(\frac{e-\mathrm{CrI}}{f}\right)\right]} \quad (4)$$

For 1-h and 3-d xylan conversion

$$X_X = a' + b'\left(\frac{L}{X}\right) + c'\mathrm{CrI} + d'\left(\frac{L}{X}\right)^2 + e'\mathrm{CrI}^2 + f'\left(\frac{L}{X}\right)\cdot\mathrm{CrI} \quad (5)$$

in which L/G = (lignin content/glucan content) × 100; L/X = (lignin content/xylan content) × 100; and CrI = crystallinity index (%). For each sorted group, the parameters in Eqs. 4 and 5 were obtained using TableCurve 3D.

For Eq. 4, only parameters a, d, and g changed significantly with the acetyl/glucan ratios whereas others remained approximately the same. Using SigmaPlot, these parameters were fit to the acetyl/glucan ratios using cubic functions. Substituting these cubic functions into Eq. 4 gives

$$X_G = \frac{a_0 + a_1\left(\frac{A}{G}\right) + a_2\left(\frac{A}{G}\right)^2 + a_3\left(\frac{A}{G}\right)^3}{1+\exp\left(\frac{b-\frac{L}{G}}{c}\right)} + \frac{d_0 + d_1\left(\frac{A}{G}\right) + d_2\left(\frac{A}{G}\right)^2 + d_3\left(\frac{A}{G}\right)^3}{1+\exp\left(\frac{e-\mathrm{CrI}}{f}\right)} + \frac{d_0 + d_1\left(\frac{A}{G}\right) + d_2\left(\frac{A}{G}\right)^2 + d_3\left(\frac{A}{G}\right)^3}{\left[1+\exp\left(\frac{b-\frac{L}{G}}{c}\right)\right]\left[1+\exp\left(\frac{e-\mathrm{CrI}}{f}\right)\right]} \quad (6)$$

in which A/G = (acetyl content/glucan content) × 100.

Equation 6 is valid in the region

$$0 < \frac{L}{G} < 70 \quad (7)$$

$$0 < \frac{A}{G} < 7.5 \quad (8)$$

$$9 < \mathrm{CrI} < 65 \quad (9)$$

and

$$0.042\left(\frac{L}{G}\right) - \left(\frac{A}{G}\right) + 5.5 > 0 \quad (10)$$

Equation 6 is an empirical correlation between the 1-h or 3-d glucan conversion and the three structural features (L/G, A/G, and CrI). All the 147 data sets were fit to this equation and revised parameter estimates were

Table 5
Parameters of Correlations for 1-h and 3-d Glucan and Xylan Conversions

	1-h Sugar conversion		3-d Sugar conversion	
Parameter	Glucan (Eq. 6)	Xylan (Eq. 11)	Glucan (Eq. 6)	Xylan (Eq. 11)
a_0 or a_0'	111.8	280.9	101.9	79.5
a_1 or a_1'	–58.6	–269.7	–17.5	0.002
a_2 or a_2'	15.6	0.0006	4.6	0.4
a_3	–1.3	—	–0.4	—
b or b'	–85.9	0.03	40.9	0.4
c or c'	–54.5	0.2	–7.0	0.5
d or d'	—	–0.0001	—	–0.001
d_0	37.0	—	108.6	—
d_1	–1.7	—	–6.0	—
d_2	0.7	—	–0.7	—
d_3	–0.1	—	0.1	—
e or e'	42.6	–0.002	40.8	–0.005
f or f'	–9.0	–0.001	–8.1	–0.01
g_0	–205.3	—	–122.7	—
g_1	77.5	—	37.0	—
g_2	–29.6	—	–9.6	—
g_3	3.6	—	1.0	—

obtained using SigmaPlot (Table 5). The parameters obtained previously were used as the initial guesses for the nonlinear regression.

For Eq. 5, only parameter a' changed with the acetyl/xylan ratios. It was fit to an exponential growth function that was then substituted into Eq. 5, giving

$$X_X = a_0' + a_1' \exp\left[a_2'\left(\frac{A}{X}\right)\right] + b'\left(\frac{L}{X}\right) + c'\mathrm{CrI} + d'\left(\frac{L}{X}\right)^2 + e'\mathrm{CrI}^2 + f'\left(\frac{L}{X}\right)\cdot \mathrm{CrI} \quad (11)$$

in which A/X = (acetyl content/xylan content) × 100.

Equation 11 is valid in the region

$$0 < \frac{L}{X} < 170 \quad (12)$$

$$0 < \frac{A}{X} < 22 \quad (13)$$

$$9 < \mathrm{CrI} < 65 \quad (14)$$

and

$$0.044\left(\frac{L}{X}\right) - \left(\frac{A}{X}\right) + 15 > 0 \quad (15)$$

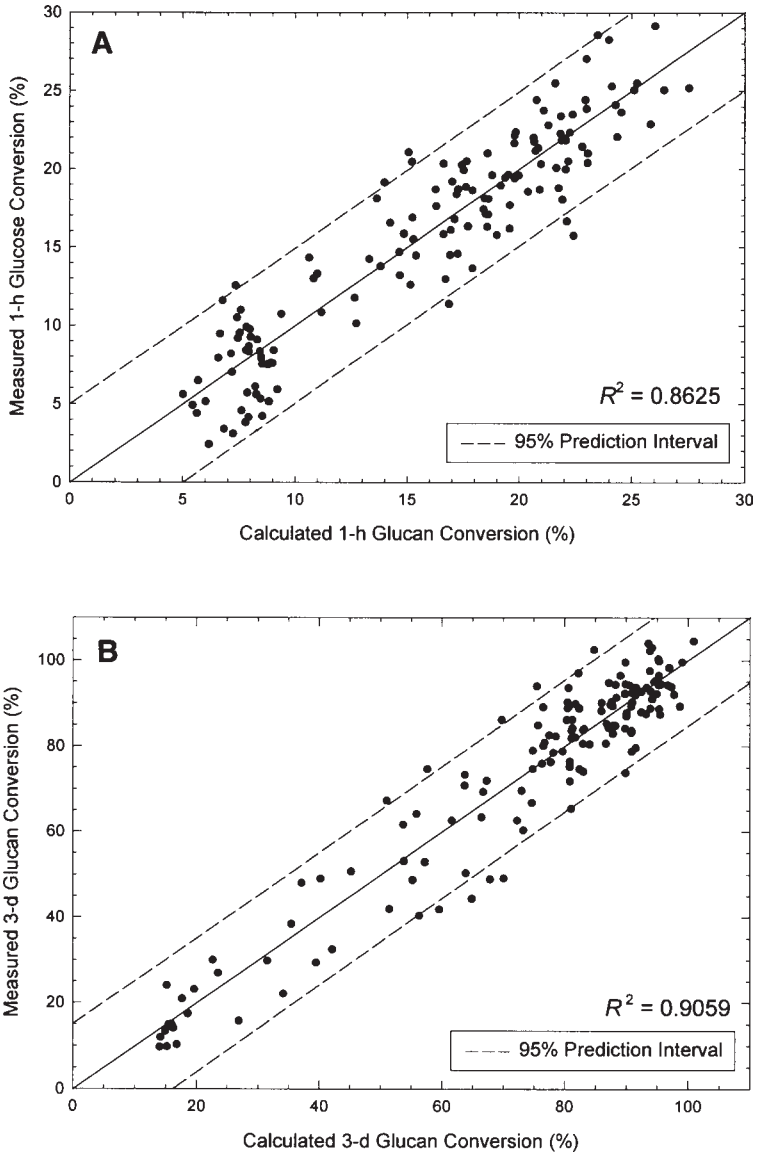

Fig. 10. Correlation between glucan conversions and L/G, A/G, and CrI for model lignocelluloses: **(A)** 1-h conversion; **(B)** 3-d conversion. Calculated glucan conversions were obtained using Eq. 6.

Equation 11 is the empirical correlation between the 1-h or 3-d xylan conversion and the three structural features (L/X, A/X, and CrI). Again, all the 147 data sets were fit to these equations and revised parameter estimates were obtained using SigmaPlot (Table 5).

Figure 10 compares the measured 1-h and 3-d glucan conversions to those calculated using Eq. 6. The coefficients of determination (R^2) were

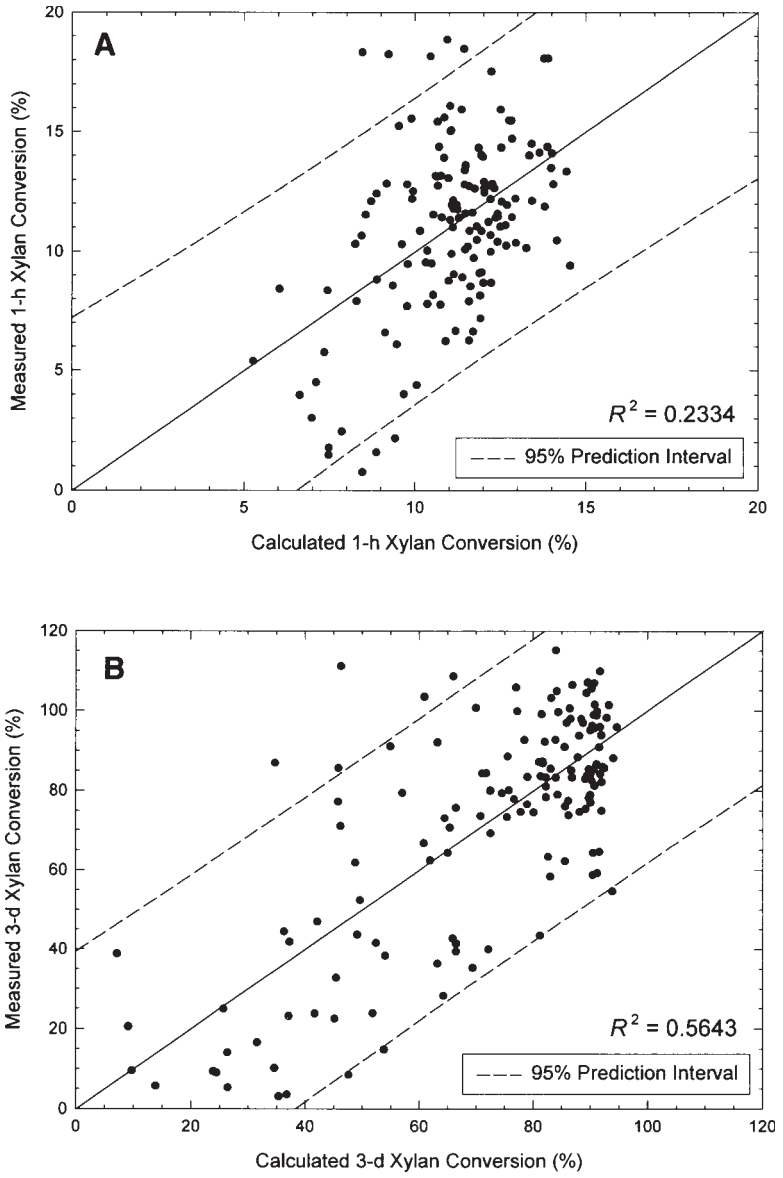

Fig. 11. Correlation between xylan conversions and L/X, A/X, and CrI for model lignocelluloses: **(A)** 1-h conversion; **(B)** 3-d conversion. Calculated xylan conversions were obtained using Eq. 11.

0.8625 and 0.9059 for 1-h and 3-d glucan conversions, respectively, which is satisfactory. Similarly, Fig. 11 compares the measured 1-h and 3-d xylan conversions with those calculated using Eq. 11. The R^2 values for the 1-h and 3-d xylan conversions were 0.2334 and 0.5643, respectively, which is less satisfactory.

Total sugar conversions were then calculated from glucan and xylan conversions as follows:

$$X_T = \frac{X_G \times \frac{G}{0.9} + X_X \times \frac{X}{0.88}}{\frac{G}{0.9} + \frac{X}{0.88}} = \left[1 + \frac{0.9}{0.88}\left(\frac{X}{G}\right)\right]^{-1} X_G + \left[1 + \frac{0.88}{0.9}\left(\frac{G}{X}\right)\right]^{-1} X_X \quad (16)$$

in which X_T is the 1-h or 3-d total sugar conversion (%); G and X are the glucan and xylan contents (%), respectively; and 0.9 and 0.88 are the conversion factors of glucose and xylose to equivalent glucan and xylan, respectively. Figure 12 compares the measured 1-h and 3-d total sugar conversions with those calculated by Eq. 16 with Eqs. 6 and 11 substituted for X_G and X_X, respectively. The R^2 values were 0.7969 and 0.8387, respectively, which is satisfactory.

If the enzymatic digestibility were completely determined by L/G, A/G, L/X, A/X, and CrI, the data points in Fig. 12 should have all fallen on the diagonal. The scattering of the data points may result from five possible causes:

1. Lignin content, acetyl content, and CrI do not completely determine enzymatic digestibility; other structural features may also determine the digestibility.
2. Lignin content, acetyl content, and CrI do completely determine enzymatic digestibility, but the empirical correlations (Eqs. 6 and 11) are not able to accurately capture the relationship between these variables.
3. CrI is an imperfect measure of cellulose crystallinity because of the presence of amorphous lignin and hemicellulose.
4. The 1-h and 3-d conversions are imperfect measures of initial reactivity and ultimate digestion, respectively.
5. Lignin content, acetyl content, and CrI do completely determine enzymatic digestibility, but the random errors in the measurements corrupt the correlations.

Analysis of random error propagation *(53,54)* shows that at the 95% confidence interval (CI), the measured and calculated 3-d total sugar conversions were 0.80 and 0.23%, respectively. These numbers are so small that random measurement errors could not account for the scattering. Therefore, the other four factors are likely candidates.

Although the empirical correlations (Eqs. 6 and 11) may be imperfect and may not include all the structural features that determine the enzymatic digestibility, these equations seem to capture the most important factors that determine enzymatic digestibility: lignin content, acetyl content, and CrI. Figure 12 shows that at the 95% CI, the 1-h and 3-d total sugar conversions can be predicted with a precision of ±5 and ±20%, respectively. Considering the complexity of lignocellulose structure and enzyme mecha-

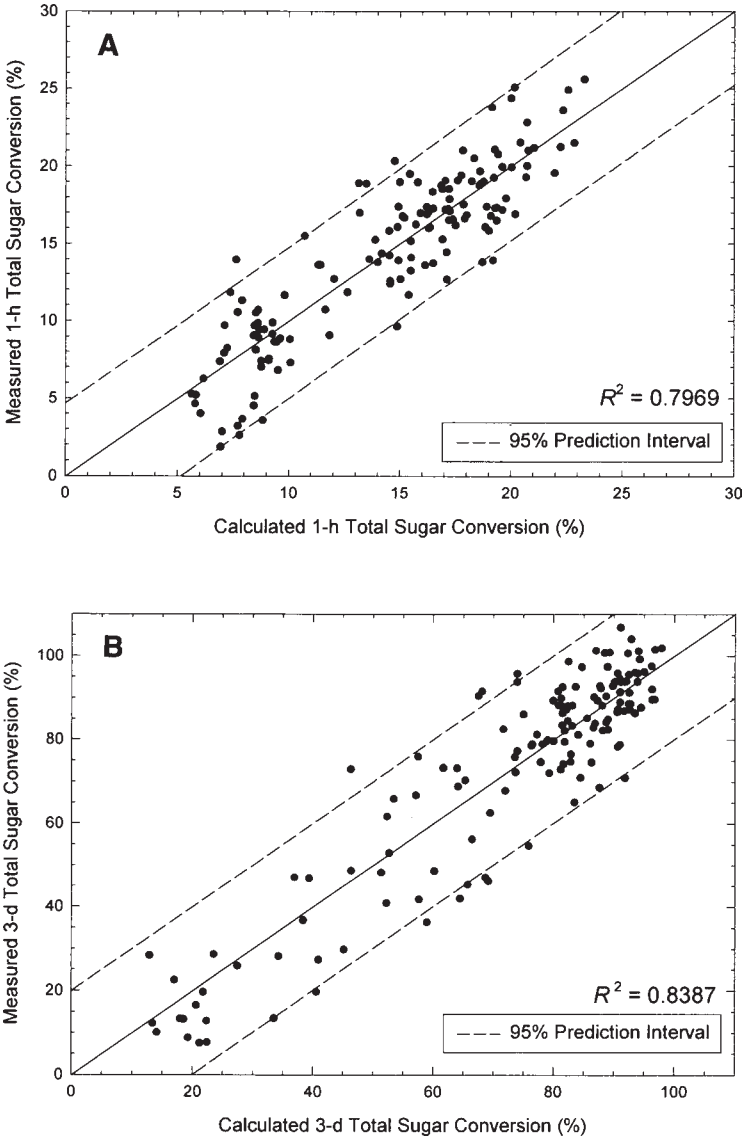

Fig. 12. Correlation between total sugar conversions and L/G, A/G, L/X, A/X, and CrI for model lignocelluloses: **(A)** 1-h conversion; **(B)** 3-d conversion. Calculated total sugar conversions were obtained using Eqs. 6, 11, and 16.

nism, it is fairly satisfactory that one can predict the enzymatic digestibility within such a precision.

Predictive Ability of the Correlations

Switchgrass, poplar wood, and bagasse were treated with lime under various pretreatment conditions (Table 6). Equations 6, 11, and 16 were tested for their predictive ability using these lime-treated biomasses, as

Table 6
Structural Features and Carbohydrate Contents of Lime-Treated[a] Biomass[b]

Sample[c]	Lime loading (g $Ca[OH]_2$/g dry biomass)	Time (h)	Temp (°C)	Oxygen pressure (bar absolute)	Structural feature			Carbohydrate content[d]		Removal[e]	
					Lignin content (%)[d]	Acetyl content (%)[d]	CrI (%)	Glucan (%)	Xylan (%)	Lignin (%)	Acetyl (%)
Switchgrass											
Raw	0	0	—	—	22.3	2.2	46.1	31.4	16.5	—	—
S1	0.1	1	80	No extra O_2	19.0	0.2	53.8	46.5	28.3	37.8	93.9
S2	0.1	2	80	No extra O_2	19.1	0.1	51.9	42.7	23.2	39.1	95.7
S3	0.05	2	120	No extra O_2	22.5	0.4	51.1	42.3	29.2	19.1	87.0
S4	0.1	2	120	No extra O_2	18.4	0.1	53.6	41.6	24.8	43.1	95.9
Poplar wood											
P1	0.1	6	150	No extra O_2	26.1	0.2	64.3	53.6	16.7	17.6	95.2
P2	0.1	6	150	7.9	14.7	0.2	64.8	71.3	16.6	63.8	96.2
P3	0.1	3	150	14.8	16.5	0.3	66.7	53.1	13.3	55.6	93.7
P4	0.1	6	150	14.8	8.0	0.4	67.8	62.3	9.6	82.6	93.2
Bagasse											
Raw	0	0	—	—	24.2	2.8	50.9	40.7	22.3	—	—
B1	0.1	1	120	No extra O_2	18.3	0.2	56.4	45.9	24.9	29.2	94.3

[a]Water loading, 9 mL/g dry biomass for switchgrass and poplar wood and 10 mL/g dry biomass for bagasse.
[b]Particle size, –40 mesh.
[c]S, switchgrass; P, poplar wood; B, bagasse.
[d]Based on dry wt at 105°C.
[e]Based on the initial weight of each component before treatment and wash.

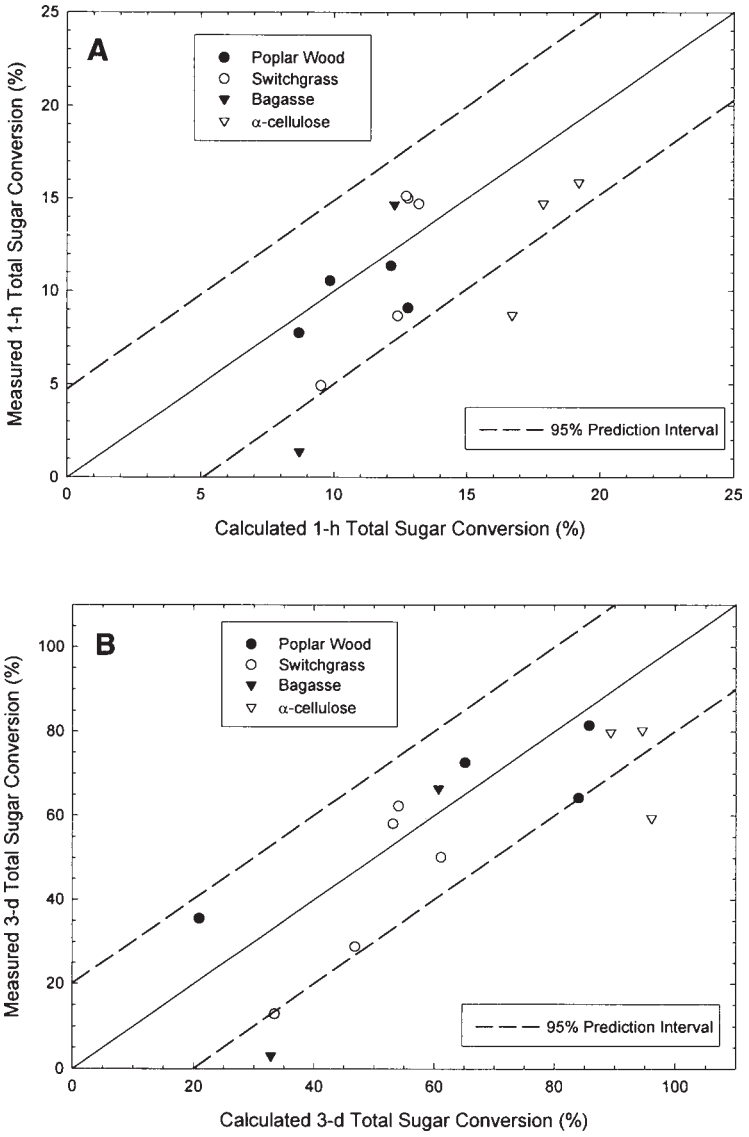

Fig. 13. Prediction of Eqs. 6, 11, and 16 on total sugar conversions for lime-treated switchgrass, poplar wood, bagasse, and ball-milled α-cellulose: **(A)** 1-h conversion; **(B)** 3-d conversion.

well as ball-milled α-cellulose. Figure 13 shows that the predicted enzymatic digestibility agrees satisfactorily with the measured data within ±5 and ±20% for the 1-h and 3-d total sugar conversions, respectively. However, the correlations slightly overestimate the actual data.

Effects of Lime Pretreatment on Enzymatic Digestibility

Table 6 summarizes the lignin, acetyl, and carbohydrate contents; CrI; and lignin and acetyl removals of the lime-treated biomasses. Lime effec-

tively removed acetyl groups under all pretreatment conditions. Using a standard lime loading of 0.1 g of $Ca(OH)_2$/g of dry biomass, 93–96% of acetyl groups were removed. Tarkow and Feist *(43)* reported that dilute NaOH (1%) removed 85% of acetyl from sugar maple at 40°C within 5 min. This indicates that acetyl groups are readily reactive with alkali even at mild conditions.

Compared with acetyl removal, lignin removal depended more significantly on the pretreatment conditions. Lignin removal increased with increasing lime loading, pretreatment time, pretreatment temperature, and oxygen pressure. In the case of poplar wood, oxygen is essential for delignification. Only 18% of lignin was removed without oxygen, whereas 83% of lignin was removed with oxygen.

As with the model lignocelluloses, the CrI of lime-treated biomass also increased slightly at more severe pretreatment conditions, likely because removal of amorphous materials, such as lignin and acetyl groups, increased the CrI.

The schematic model presented in Fig. 8 explains why lime is an effective pretreatment agent. Lime pretreatment fully opens the acetyl valve and moderately opens the lignin valve, allowing a substantial amount of enzymes to flow through the pipes and arrive at the substrate tank. Although high substrate crystallinity results in low enzyme effectiveness, lime pretreatment works because enough enzymes reach the carbohydrate polymers that the amount of enzymes adsorbed on the substrate is sufficient to achieve high digestion in a 3-d period.

Figure 14 shows the 3-d total sugar conversions calculated by Eqs. 6, 11, and 16 at a variety of lignin contents. Boundaries show the digestibility at 0% and 3% acetyl content, representing lime-treated and untreated biomasses, respectively. Figure 14 has both a typical biomass crystallinity (60%) and carbohydrate composition (glucan/xylan ratio = 45:20). It shows that lignin removal increases digestibility significantly whereas acetyl removal has a smaller effect; however, extensive acetyl removal helps reduce the required lignin removal. For 3% acetyl biomass, the lignin content must be reduced to 8% to increase the digestibility from 10 to 80%; however, for acetyl-free biomass, reducing the lignin content to 16% is sufficient. Therefore, it is not necessary to remove all lignin to render biomass digestible. In fact, for a high-lignin (25%), high-CrI (60%), acetyl-free biomass, only 43% lignin removal is needed for it to become highly digestible (80%).

Selecting an Appropriate Pretreatment Method

The results reported earlier show that lignin content and CrI are the dominant factors that determine enzymatic digestibility (Table 4). Figure 15 illustrates the 3-d total sugar conversion as a function of CrI at various lignin contents. The sugar conversions were calculated using Eqs. 6, 11, and 16 with a typical acetylated biomass composition (glucan/xylan ratio = 45:20; acetyl/xylan ratio = 3:20). Again, Figure 15 shows that either low

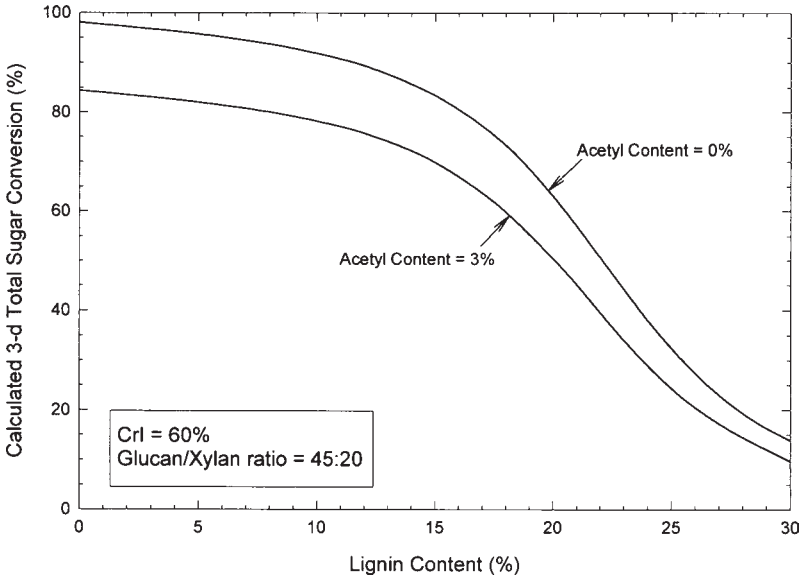

Fig. 14. Calculated 3-d total sugar conversions of crystalline biomass as a function of lignin content at 0 and 3% acetyl contents using Eqs. 6, 11, and 16.

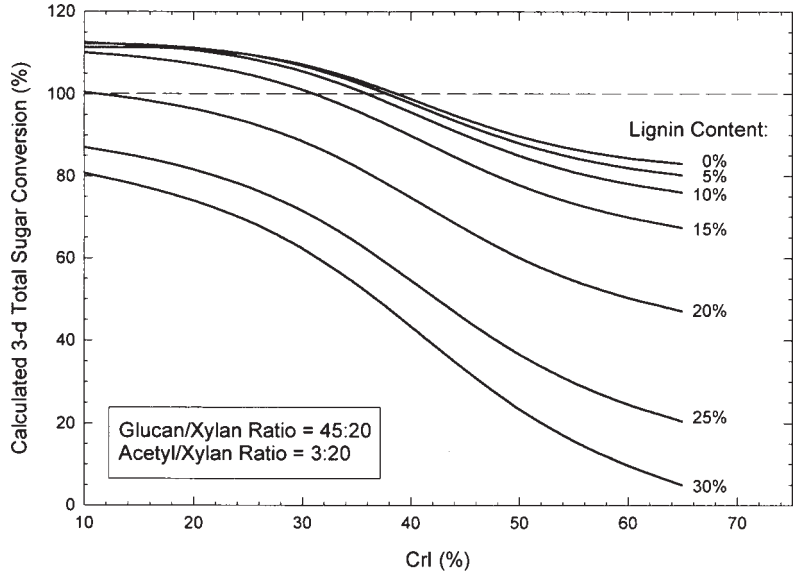

Fig. 15. Calculated 3-d total sugar conversions of acetylated biomass as a function of CrI at various lignin contents using Eqs. 6, 11, and 16.

lignin or low CrI is sufficient to obtain high digestibility. Therefore, pretreatments that can significantly reduce either lignin content or CrI should render biomass digestible. However, selecting a delignifying or decrystallizing pretreatment depends on economics and the type of biomass.

For example, with low-lignin biomass such as rice straw (12% lignin) *(55)*, it may be more economically feasible to use a delignifying pretreatment because only slight delignification is necessary to render it digestible.

For a biomass with a typical composition (25% lignin and 60% CrI), a pretreatment that can either reduce the CrI to <22% or reduce the lignin content to <10% will achieve 80% digestibility. Ball milling can reduce the CrI effectively *(22,24)*, but it is too energy intensive to be economically feasible. Delignifying agents such as ethanol, phenol, and ethylene glycol are effective *(55)* but may be too expensive for industrial applications. Our previous study on the oxidative lime pretreatment of poplar wood shows that lime plus oxygen can reduce the lignin content from 28 to 9% *(44)*.

Figure 15 shows that if the CrI and the lignin are both reduced to <31 and <15%, respectively, the model predicts that enzymatic digestibility exceeds 100%, which is impossible. The predictions of >100% conversion imply that if a pretreatment can significantly reduce both lignin content and CrI, the treated biomass will become so digestible that the enzyme loading can be reduced below 5 FPU/g of dry biomass.

Conclusion

Poplar wood was selectively delignified, deacetylated, and decrystallized using peracetic acid, KOH, and ball milling, respectively. Treatment conditions such as reagent loading, temperature, and biomass concentration were carefully controlled so that cross effects were minimized. The extent of delignification, deacetylation, and decrystallization was also controlled by treatment conditions. In total, 147 model lignocelluloses with a broad spectrum of lignin contents (0.7–26.3%), acetyl contents (0.1–3.1%), and CrI (9.1–63.9%) were prepared.

The enzymatic hydrolysis results show that among the three structural features, lignin content and CrI have the greatest effects on digestibility. *Low lignin content is sufficient to obtain high digestibility regardless of acetyl content or CrI; low CrI is sufficient regardless of lignin or acetyl content*. However, for high-CrI and moderate-lignin biomass, extensive acetyl removal increased digestibility significantly. Lignin and acetyl contents function as parallel barriers for enzymatic hydrolysis. CrI has more significant effects on initial hydrolysis rates than on ultimate sugar yields, indicating that CrI is responsible for the effectiveness of enzymes adsorbed on the substrate.

Material balances on lime-treated biomass show that lime removes essentially all acetyl groups (93–96%) and moderate lignin (26–32% for switchgrass and 13% for poplar wood), unless oxygen is used. The proposed schematic model shows that lime pretreatment is effective because it fully opens the acetyl valve and moderately opens the lignin valve, allowing a substantial amount of enzymes to flow through pipes and arrive at the substrate tank.

Delignification, deacetylation, and decrystallization impact glucan and xylan digestibility differently. Delignification and deacetylation have

greater effects on hemicellulose digestibility because lignin and acetyl groups are attached to the hemicellulose matrix. Decrystallization has greater effects on cellulose digestibility because cellulose is crystalline and hemicellulose is not.

Empirical correlations for enzymatic digestibility were developed for the model lignocelluloses using lignin/glucan ratio, lignin/xylan ratio, acetyl/glucan ratio, acetyl/xylan ratio, and CrI as the independent variables. The R^2 values were 0.7933 and 0.8387 for 1-h and 3-d total sugar conversions, respectively. The 95% prediction intervals show that the correlations can predict the 1-h and 3-d total sugar conversions of a biomass sample within a precision of 5 and 20%, respectively. The predictive ability of the correlations was tested on lime-treated biomass (switchgrass, poplar wood, and bagasse) and ball-milled α-cellulose. The agreement between the measured and calculated values shows that the correlations are satisfactory and that the three structural features—lignin content, acetyl content, and CrI—are major factors that determine enzymatic digestibility.

This research provides a reference for selecting an appropriate pretreatment. For industrial applications, a pretreatment that can significantly reduce either lignin content or CrI will effectively render biomass digestible. However, the selection of a pretreatment (delignifying or decrystallizing) depends on economics and the type of biomass. Lime pretreatment is sufficient for moderate-lignin biomass such as switchgrass and bagasse. For woody biomass, oxidative lime pretreatment is effective.

Acknowledgment

We gratefully acknowledge the support of U.S. Department of Agriculture Grant 96-35500-3237.

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