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Enzymatic Saccharification and Fermentation of Xylose-Optimized Dilute Acid–Treated Lignocellulosics

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Abstract

The cellulose reactivity of two lignocellulosic feedstocks, switchgrass and poplar, was evaluated under straight saccharification (SS) and simultaneous saccharification and fermentation (SSF) conditions following dilute sulfuric acid pretreatments designed for optimum xylose yields. The optimum pretreatment conditions, within the constraints of the experimental system (Parr batch reactor), were 1.2% acid, 180°C, and 0.5 min for switchgrass and 1% acid, 180°C, and 0.56 min for poplar. The cellulase enzyme preparation was from *Trichoderma reesei* and fermentations were done with *Saccharomyces cerevisiae*. Time courses for SS were monitored as the sum of glucose and cellobiose; those for SSF as the sum of glucose, cellobiose, and ethanol. Percentage conversions under SS conditions were 79.1% and 91.4% for the pretreated poplar and switchgrass feedstocks, respectively. Analogous values under SSF conditions were 73.0% and 90.3% for pretreated poplar and switchgrass, respectively.

Index Entries: Lignocellulosic; poplar; switchgrass; corn stover; simultaneous saccharification and fermentation; cellulase; cellulose; xylose.

Introduction

Lignocellulosic biomass is an abundant renewable resource, with an annual production of approx 4×10^{10} tons (*1*), and has obvious potential as starting material for the production of biochemicals. Thus, there continues to be widespread interest in developing processes for the conversion of lignocellulosics to value-added biochemicals. Processes of this type generally require that the polysaccharide fraction of the biomass, primarily cellulose and hemicellulose, be hydrolyzed to its constituent monomeric sugars; the resulting sugars subsequently being converted to target products. The target product that has received the most attention to date is fuel ethanol.

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In general, biomass-to-ethanol conversion processes include (a) an initial "pretreatment" step to increase the reactivity/enzyme susceptibility of the cellulose component of the feedstock, (b) an enzymatic saccharification step for the hydrolysis of the cellulose in the pretreated feedstock, and (c) a fermentation step to convert the resulting liquid-phase sugars to ethanol. Traditional two-stage biomass-to-ethanol processes include "separate hydrolysis and fermentation" (SHF) operations to perform steps "2" and "3" (*2*). In SHF processes, the cellulose is first enzymatically hydrolyzed to glucose, and the resulting glucose is converted to ethanol via microbial fermentation. An alternative approach is to use a one-stage process, known as "simultaneous saccharification and fermentataion" (SSF) to perform steps "2" and "3" (*3*). In SSF processes, cellulose hydrolysis and glucose fermentation occur in one operation. In conventional SSF the cellulolytic enzyme is produced outside the SSF reactor to which it is added; however, efforts are now underway to obtain fermenting yeast that can produce the enzyme *in situ* (*4*). Major benefits of SSF processes over SHF processes are the reportedly higher specific activities of the cellulolytic enzymes due to removal of inhibitory saccharification products via yeast fermentation *(5)*, and the need for only a single saccharification/fermentation reactor.

The efficiency of SHF and SSF operations depends strongly on the physical and chemical properties of the feedstock (*6–20*). This is the primary reason for including the "pretreatment" step (step "1" above) in biomass-to-ethanol processes. The focus of traditional pretreatment processes is the optimization of cellulose saccharification (*21–28*). One such approach is to incubate the feedstock at elevated temperatures (>140°C) in aqueous sulfuric acid (0.2–1.5% H_2SO_4) for relatively short periods of time (<5 min); processes of this nature are referred to as "dilute-acid pretreatments" (*19,21, 25–32*). Several acids have been evaluated for such processes, including dilute hydrochloric, nitric, carbonic, and sulfuric acids Sulfuric-based pretreatments have received the most attention owing to their being relatively inexpensive and having relatively high hemicellulose recoveries and cellulose digestibilities *(33)*. Efficiencies for dilute acid–pretreatments are improving as novel processing technologies are applied, as has been the case in recent years with the application of nonuniform temperature processes *(34)* and the application of flowthrough reactors *(34–36)*. Several alternative pretreatment strategies, that do not rely on the addition of an acid catalyst, also show promise for biomass-to-ethanol processes; included in this group are neutral hot water- *(37)*, lime- *(38)*, and ammonia-based *(39,40)* processes.

The economics of ethanol production require that pretreatment processes make the vast majority of the cellulose component of the feedstock susceptible to enzymatic saccharification, while simultaneously generating maximum sugar yields (primarily xylose) as a result of the hydrolysis of the feedstock's hemicellulose component (primarily xylans). The xylose resulting from this process can itself serve as a substrate for the production of ethanol or other biochemicals. The primary focus of the majority of studies

dealing with dilute-acid pretreatments has been to remove xylan and maximize the availability of the cellulose in SHF and SSF processes *(26,41)*, not necessarily the optimization of xylose yields. Previous studies in our laboratory have focused on maximizing xylose yields during dilute-acid pretreatments of common lignocellulosic feedstocks *(42)*. Pretreatment conditions ranged from 140° to 180°C and from 0.6 to 1.2% acid. Maximum xylose yields over this range were >80% of theoretical for each of the feedstocks.

The objective of the present study was to determine if pretreatment conditions chosen to optimize xylose yields were also effective in enhancing cellulose reactivity. Thus, the performance of switchgrass and poplar feedstocks, pretreated based on optimum xylose yields, were tested under saccharification (cellulase enzyme susceptibility) and SSF conditions.

Materials and Methods

Switchgrass and hybrid poplar feedstocks were received from the National Renewable Energy Laboratory (NREL, Golden, CO). Cellulase enzyme derived from *Trichoderma reesei* was from Environmental Biotechnologies, Inc., CA. *Saccharomyces cerevisiae* (D_5A) is an NREL strain genetically derived from Red Star Brewers Yeast. Yeast extract and peptone were from Difco (Detroit, MI). Glucose, α-cellulose, and glucose oxidase/peroxidase were purchased from Sigma Chemical Company (St. Louis, MO).

Preparation of Dilute-Acid Pretreated Solids

Optimum pretreatment conditions (percentage sulfuric acid, temperature, and time), based on maximum xylose yields, were determined previously: 1.2% acid, 180°C, and 0.5 min for switchgrass and 1% acid, 180°C, and 0.56 min for poplar *(42)*. Following the pretreatment, slurries were washed with distilled water in a large funnel with Whatman No.5 filter paper. Solid residues were collected and stored at 4°C until assayed (within 4 d).

Enzyme Saccharification

Enzyme saccharification was performed according to NREL standard procedures *(14)* in 20-mL glass scintillation vials. Cellulose contents of reaction mixtures were adjusted to 1% with 50 m*M* sodium citrate (pH 5.0) buffer containing 0.04% tetracycline and 0.03% cycloheximide as preservatives. Reactions were initiated by the addition of cellulase [60 filter paper units (FPU)/g cellulose] to give a total 10 mL working volume. Vials were capped tightly and incubated at 50°C with gentle rotation at 68 rpm for a period of 8 d or until the generation of soluble sugar became negligible. At predetermined times, a 0.5 mL slurry was withdrawn from the reaction mixture and boiled for 5 min to terminate the reaction. The slurry was centrifuged at 12,000*g* for 5 min, and the glucose content of the supernatant

was quantified enzymatically (glucose oxidase/peroxidase). The cellobiose concentration of the supernatant was determined from the amount of glucose liberated as a result of exhaustive β-glucosidase (cellobiase) treatment. The percentage conversion of original cellulose was obtained from the sum of the glucose and cellobiose generated during saccharification and the amount of cellulose in the original reaction mixture. α-Cellulose was used as a control in all enzyme saccharification experiments.

Simultaneous Saccharification and Fermentation (SSF)

SSF was performed similar to that described in NREL Standard Procedure No. 008 *(43)*. Yeast inocula were grown in two stages. In the first stage, a frozen stock culture of *S. cerevisiae* D_5A was inoculated into 100 mL of 1% yeast extract, 2% peptone, and 2% glucose (YPD liquid medium, pH 5.0) and incubated at 38°C and 150 rpm for 8–12 h in a 250-mL baffled shake flask. In the second stage, 10 mL of the first stage culture was added to 90 mL of 1% yeast extract, 2% peptone, and 5% glucose (pH 5.0) and incubated at 38°C and 150 rpm for 8–12 h in a 250-mL baffled shake flask. The SSF reaction was initiated in separate 250-mL Erlenmeyer flasks containing previously autoclaved (121°C for 20 min) dilute acid–pretreated solids and raw biomass at pH 5.0 containing 3% cellulose in the final reaction mixture, by addition of 10 mL of sterile 10X YP medium (10% yeast extract and 20% peptone, pH 5.0), 10 mL of the second stage yeast inoculum, and an enzyme load of 25 FPU/g cellulose, in a final volume of 100 mL. The flasks were fitted with water traps containing sterile water to minimize ethanol evaporation while allowing release of carbon dioxide, and incubated at 38°C in an environmental shaker bath at 150 rpm for a period of 8 d or until ethanol production became negligible. At predetermined times, 4 mL of slurry were withdrawn aseptically and centrifuged at 12,000*g* for 5 min. The supernatants were then filtered using 0.22 μm Nylon membrane filters. The glucose and cellobiose contents of the supernatants were determined as described above under "Enzymatic Saccharification." Ethanol in the supernatant was measured by gas chromatography using an HP Porapak Q capillary column (10 m, 0.53 mm) using an FID Detector. The internal standard for ethanol determination was 1 g/L isopropanol. Percentage cellulose conversion was obtained by comparing the sum of the products, glucose, cellobiose, and ethanol to the amount of cellulose in the original reaction mixture:

$$\%\ \text{Cellulose conversion} = \frac{(\text{moles glucose}) + (\text{moles cellobiose})(2) + (\text{moles Ethanol})(0.5)}{\text{moles glucose monomer equivalents in cellulose}}$$

As in the SHF experiments, α-cellulose was used as a control in all experiments. Bacterial contamination was monitored by plating fermented slurries on YPD medium containing 0.03% cycloheximide. Reaction flasks contaminated with bacteria were discarded.

Results and Discussion

Compositional data for the raw and pretreated feedstocks, along with mass balance data for the corresponding pretreatements, are provided in Table 1. A comparison of the glucan (cellulose) and xylan (hemicellulose) composition of the raw versus pretreated feedstocks provides chemical evidence for the severity of the pretreatments and demonstrates that xylan is the predominant macrocomponent removed from the feedstock as a result of dilute acid pretreatment. This is consistent with xylans being markedly more susceptible to acid-catalyzed hydrolysis/dissolution than cellulose *(44)*. The relative reactivity of the xylan and glucan components, coupled with the relatively high xylan content of the switchgrass feedstock, explains the higher percentage of original dry matter retained in the poplar "prehydrolyzed solids." The amount of each feedstock's original glucan retained in the prehydrolyzed solid is of particular importance to this study because this is the target substrate for SSF and straight saccharification experiments; the percentage glucan retained in the poplar prehydrolyzed solid was shown to be significantly higher than the corresponding value for switchgrass. Xylose recoveries in prehydrolysates from both feedstocks were greater than 80%.

The 168-h time course for conversion of the cellulose component of each feedstock, in terms of the extent of cellulose/glucan conversion under SSF conditions, is presented in Fig. 1. The extent of "cellulose con-version" is calculated as the sum of glucose, cellobiose, and ethanol in the liquid phase relative to the amount of cellulose in the reaction mixture when saccharification/fermentation was initiated. The calculations account for two moles of ethanol per mole cellulose-derived glucose. A portion of the glucose consumed by the yeast will necessarily be used for cell maintenance and growth, thus total conversion values will be less than 100%. The presented data demonstrate that rates of cellulose conversion differed; the cellulose component of the pretreated switchgrass was the more readily hydrolyzed. The enzyme loads used in this set of experiments were relatively high, NREL's current reference protocol specifies a cellulase load of 10 or 15 FPU/g cellulose *(43)*; the higher enzyme levels used here are not expected to maximize, and may somewhat obscure, differences in rates/extents of biomass saccharification. SSF cellobiose (<2 mg/mL) and glucose (<1 mg/mL) concentrations peaked early (<24 h) in the process and then declined appreciably. Total yields, in reference to the percentage of cellulose converted, are presented in Table 2. Yields were approx 90% and approx 73% of theoretical for the pretreated switchgrass and poplar, respectively. These yields are with respect to the amount of cellulose in the dry pretreated solids. It is also informative to consider yields with respect to the amount of cellulose in the original dry feedstock prior to pretreatment. In this case, SSF yields were similar, approx 70% for poplar and approx 73% for switchgrass, owing to a lower percentage of the switchgrass

Table 1
Mass Balance Data for Switchgrass and Poplar Pretreatments[a]

	Feedstock composition (%, dry wt. basis)		Composition of prehydrolyzed solids (%, dry wt. basis)		Parcentage of original component recovered in prehydrolyzed solid		Parcentage of original component recovered in prehydrolyzed liquid	
Component	Switchgrass	Poplar	Switchgrass	Poplar	Switchgrass	Poplar	Switchgrass	Poplar
Dry matter	100	100			52.6	74.0	43.8	30.0
Glucan	32.2	39.8	49.7	54.9	81.3	102.0	17.9	5.8
Xylan	20.3	14.8	2.2	1.5	5.7	7.3	80.8	81.9
Galactan	0	0	—	—	—	—	—	—
Arabinan	3.7	1.2	—	—	—	—	68.4	—
Mannan	0.4	2.4	—	0.8	—	24.7	—	51.9
Ash	7.1	1.3	7.8	0.2	57.9	11.3	71.5	185.9
Klason lignin	19.5	26.9	34.9	35.9	94.2	98.8	NA	NA
Acid soluble lignin	3.7	2.2	1.1	1.4	17.6	47.1	102.0	98.0
Uronic acid	1.1	2.4	0.34	1.2	17.3	37.0	61.2	72.0

[a]Pretreatment conditions: for switchgrass, 1.2% acid, 180°C, 0.5 min; for poplar, 1.0% acid, 180°C, 0.56 min.

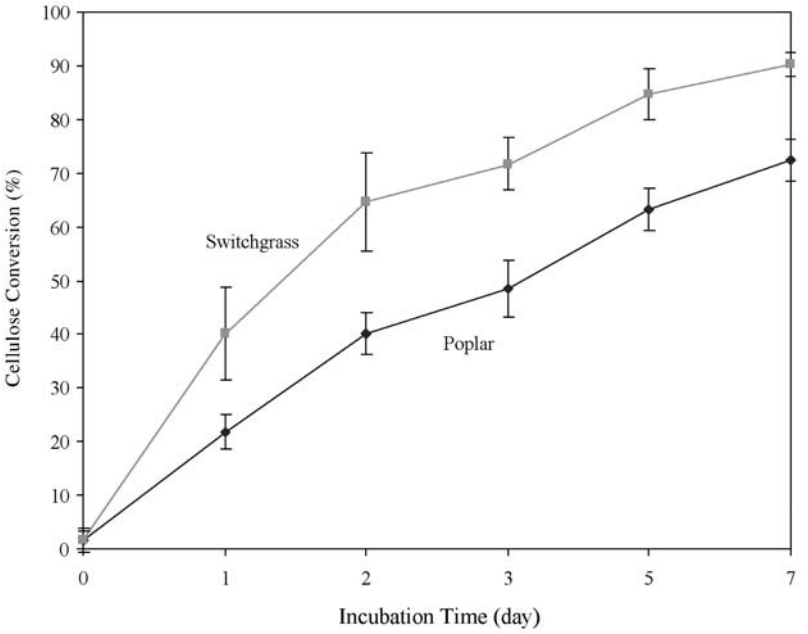

Fig. 1. Time course for cellulose conversion from pretreated switchgrass and poplar feedstocks under SSF conditions. Data points represent means with standard deviations for three experiments. Symbols: ◆, poplar pretreated with 1% sulfuric acid, 180°C, 0.56 min; ■, switchgrass pretreated with 1.2% sulfuric acid, 180°C, 0.5 min. SSF conditions as described in text.

glucan being retained in the prehydrolysate solid (and thus not included in the SSF cellulose-conversion yield). Another perspective of the data may be obtained by summing glucan yields from SSF with glucan recoved in the prehydrolysate liquid stream; this scenario demonstrates that the "overall" percentage of recoverable glucan is significantly higher for switchgrass (>90%). Switchgrass ethanol yields in 7-d SSF experiments were also higher, approx 14.0 g/L, than those for poplar, approx 11.5 g/L.

The reactivity of the cellulose component of each of the feedstocks was also tested under straight saccharification conditions, where enzyme loads and reaction temperatures are higher (see "Methods"). Figure 2 shows the time course for poplar cellulose conversion (saccharification); the time course is presented in reference to that observed under SSF conditions. As expected, rates of cellulose conversion are substantially higher under straight saccharification conditions. The same trend was observed with the switchgrass feedstock (data not shown), although maximum cellulose conversion under straight saccharification conditions was achieved earlier (<24 h). The extent of enzyme-catalyzed cellulose hydrolysis was similar under the two experimental conditions (SSF and straight saccharificaiton) for both feedstocks; under both scenarios poplar yields were low compared to those for switchgrass. The similarity in the amount

Table 2
Cellulose Conversions in Straight Saccharification and SSF Experiments With Dilute-Acid Pretreated Poplar and Switchgrass Feedstocks

Sample	Pretreatment condition (H_2SO_4, temp, time)	% cellulose conversion (straight saccharification)[a]	% cellulose conversion (SSF)[b]
Poplar (pretreated)	1.0%, 180°C, 0.56 min	79.1 ± 3.8[c]	73.0 ± 3.2[c]
Poplar (raw)	no pretreatment	10.0 ± 2.1[d]	6.8 ± 0.7[d]
Switchgrass (pretreated)	1.2%, 180°C, 0.5 min	91.4 ± 3.0[c]	90.3 ± 2.5[c]
Switchgrass (raw)	no pretreatment	18.0 ± 4.1[d]	24.0 ± 4.3[d]

[a][(Glucose + cellobiose) / cellulose in reaction mixture at time zero] × 100.
[b][(Ethanol + glucose + cellobiose) / cellulose in reaction mixture at time zero] × 100.
[c]Means ± standard deviation for three experiments.
[d]Means ± standard deviation for two experiments.

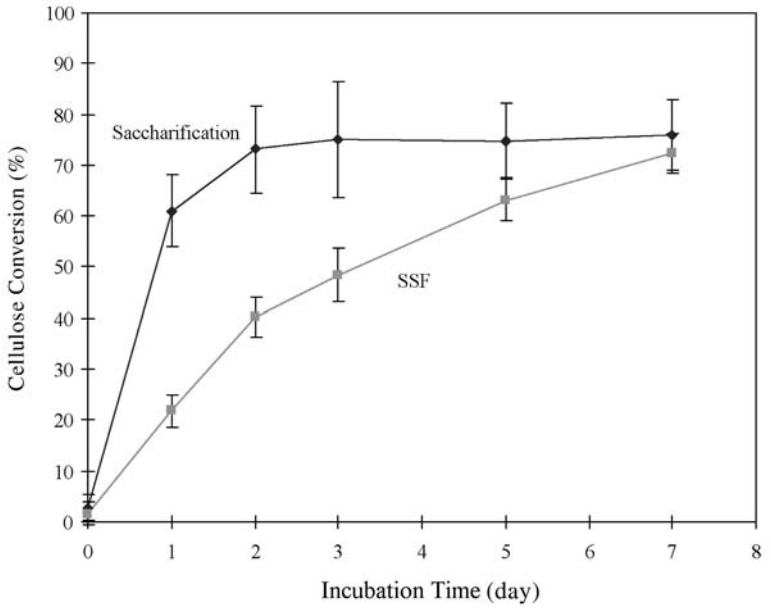

Fig. 2. Comparison of time courses for cellulose conversion for pretreated poplar (pretreatment at 1% sulfuric acid, 180°C, 0.56 min) under straight saccharification and SSF conditions. Data points represent means with standard deviations for three experiments. Symbols: ◆, straight saccharification; ■, SSF. SSF and straight saccharification conditions as described in text.

of cellulose converted in SSF and straight saccharification experiments for both the switchgrass and poplar feedstocks, as presented here, is not consistent with previous reports showing higher extents of cellulose conversion with SSF compared to straight saccharification *(2,45,46)*. The rationale given for enhanced cellulose conversion in SSF experiments is that yeast utilization of saccharification products lowers product inhibition of the cellulases relative to that encountered during straight saccharification, presumably allowing the reaction to proceed further. Product inhibition of this type, if of a competitive nature, is not likely to lower cellulose conversion values in the current saccharification experiments because reaction times were extended well beyond those corresponding to the initiation of the product plateau (<2 d, see Fig. 2 for poplar).

Decreases in the rates of cellulose conversion over the course of SSF experiments may be due to several factors, including reductions in enzyme activity, yeast viability, and/or enzyme-susceptible cellulose. Table 3 summarizes enzyme activity and yeast cell viability data from representative SSF experiments with the pretreated feedstocks. Data obtained with a model cellulose substrate are included for comparative purposes. The cellulose conversion data, in this case based on quantifying the cellulose content of the residual feedstock remaining at the end of

Table 3
Cellulose Conversion, Residual Cellulose, Cellulase Activity and Yeast Viability in SSF Experiments with Poplar, Switchgrass, and Cellulose Feedstocks

Sample	Pretreatment	Cellulose converted (%)			Cellulase activity (relative values)[d]		Yeast viability (CFU/mL)[e]	
		SSF[a]	Additional saccharification[b]	residue[c]	Day 0	Day 8	Day 0	Day 8
Poplar	1% H_2SO_4, 180°C, 0.56 min	73.0 ± 3.2	4.7 ± 2.9	15.5 ± 3.3	3.1 ± 0.0	2.7 ± 0.2	7.3×10^7	1.7×10^5
Poplar	None	6.8 ± 0.7	1.5 ± 0.0	92.4 ± 4.0	5.9 ± 0.2	4.3 ± 0.1	7.3×10^7	1.3×10^6
Switchgrass	1.2% H_2SO_4, 180°C, 0.5 min	90.3 ± 2.5	0.7 ± 0.1	6.5 ± 3.9	4.0 ± 0.1	3.8 ± 0.1	7.3×10^7	7.1×10^4
Switchgrass	None	24.0 ± 4.3	1.0 ± 0.0	79.8 ± 3.3	6.1 ± 0.0	4.8 ± 0.5	7.3×10^7	1.2×10^5
α-Cellulose		91.2 ± 1.2	1.0 ± 0.2	nd[f]	6.6 ± 1.3	5.9 ± 0.3	7.3×10^7	2.9×10^4
Enzyme control					6.8	6.29		
Enzyme + yeast control					7.2	5.8	7.3×10^7	6.5×10^5

[a]Percentage of cellulose converted in standard 7-d SSF treatment.
[b]Percentage cellulose converted as result of straight saccharification treatment subsequent to SSF.
[c]Cellulose remaining in residue following sequential SSF and straight saccharification treatments.
[d]Activity based on glucose liberated from filter paper substrate in standard FPU assay (mg glucose/mL reaction mixture).
[e]Colony forming units determined by plating on yeast dextrose medium.
[f]Not determined.

the SSF experiment, are in general agreement with the values obtained by summing saccharification and metabolic end products (glucose + cellobiose + ethanol, as done in Fig. 1). The "additional saccharification" values give an indication of the amount of enzyme-susceptible cellulose remaining in the feedstock residue at the completion of the SSF phase of the experiments ("enzyme-susceptible" in this case is defined as that cellulose that is susceptible to saccharification under straight saccharification conditions, see "Methods"). The amount of enzyme-susceptible cellulose remaining in the pretreated switchgrass feedstock at the completion of the SFF reaction/fermentation phase was found to be less than 1% of the total cellulose in the original reaction mixture. The corresponding value for the pretreated poplar was higher, but was still a relatively small percentage of the total cellulose (<5%). The "cellulase activity" values demonstrate that a significant fraction of the enzyme activity added to initiate the SSF process quickly associates with the solid phase (i.e., values for soluble enzyme activity in reaction mixtures containing pretreated feedstocks, shortly after initiating the reaction, are low relative to the corresponding enzyme controls in which no feedstock is present). Enzyme adsorption is not likely to be limited to the cellulose component of the feedstocks, because the α-cellulose containing samples actually showed less enzyme adsorption than the pretreated feedstocks. These data also indicate that the enzyme was stable under SSF conditions, as enzyme activities associated with the liquid phase changed little over the eight day SSF experiment. The "yeast viability" data indicate that they remain capable of metabolic activity over the course of the experiment. Taken together, the data of Table 3 suggest that the primary factor that limits the utilization of the cellulose remaining in the feedstocks at the end of SSF experiments is due to the recalcitrant nature of the cellulose; not limitations in enzyme activity or yeast cell viability. Analogous experiments under straight saccharification conditions provided similar results (data not presented).

SSF and straight saccharification experiments differ with respect to enzyme load, temperature, and the presence of glucose-utilizing yeast. We attempted to ascertain the relevance of enzyme and temperature differences by comparing progress curves for saccharification of the pretreated poplar and switchgrass feedstocks under different enzyme/temperature combinations (Figs. 3A,B). Saccharification rates were clearly fastest when enzyme and temperature conditions corresponded to those of straight saccharification (higher temperature, higher enzyme load). Comparison of the reaction time courses suggests that temperature is the more significant of the contributing factors, this based on the observation that reaction rates tended to be faster for the low enzyme/high temperature treatment compared to the high enzyme/low temperature treatment. All treatments resulted in essentially the same extent of cellulose conversion by the completion of the experiment. Similar progress curves were obtained when this experiment was done with a model cellulose (α-cellulose, data

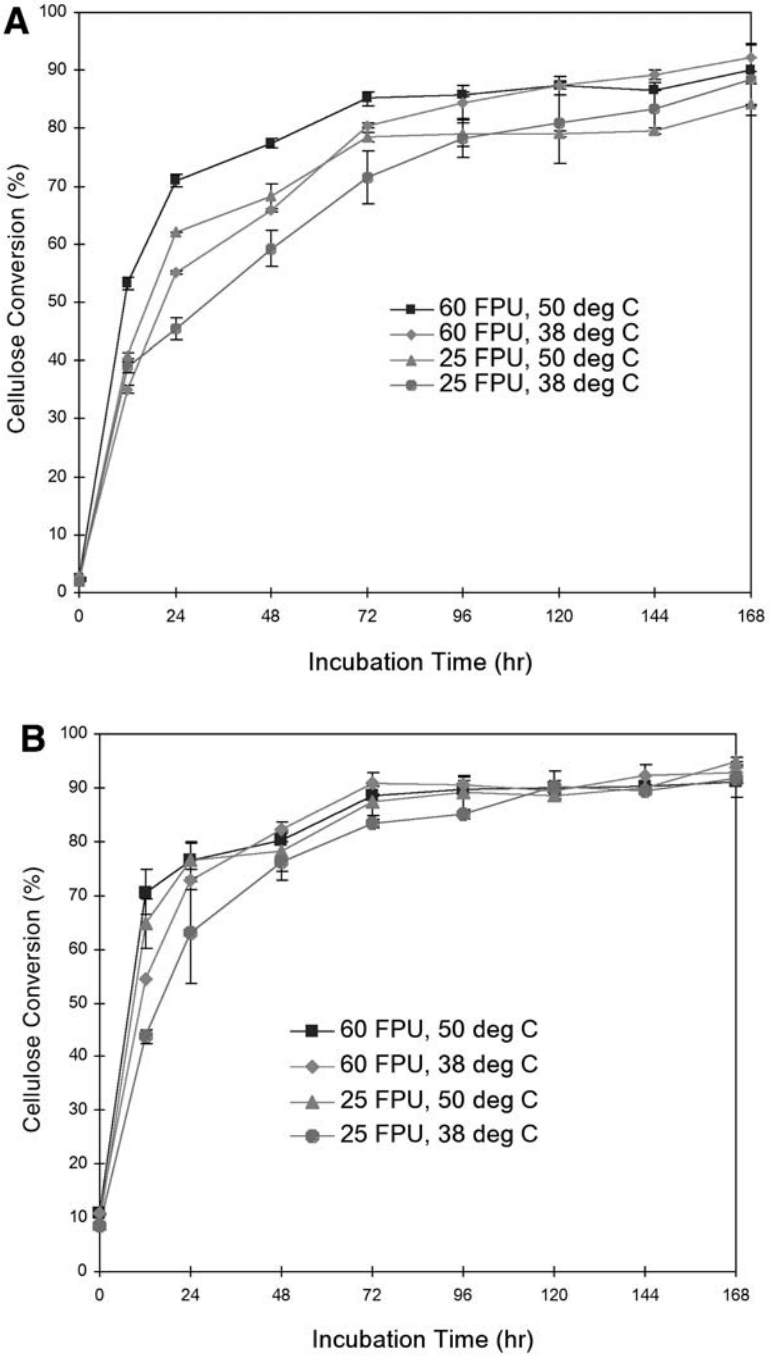

Fig. 3. Time courses for cellulose conversion at enzyme load (FPU per gram cellulose) and temperature combinations common to straight saccharification and SSF experiments. **(A)** pretreated poplar (0.9% sulfuric acid at 180°C for 0.6 min), **(B)** pretreated switchgrass (0.9% sulfuric acid at 180°C for 3 min). Data points represent means with standard deviations from two determinations. Symbols: ■, 60 FPU and 50°C; ◆, 60 FPU and 38°C; ▲, 25 FPU and 50°C; ●, 25 FPU and 38°C.

not shown). The results illustrate the importance of developing heat tolerant organisms for increased reaction rates in SSF applications.

The cellulose conversion parameters obtained in this study, in which the pretreatment was optimized for xylose yield (as dissolved xylose monomer in prehydrolysate liquid), may be compared with previously published SSF *(2,41,45,46)* and straight saccharification *(2,21,23,32,45,46)* studies employing similar equipment. The switchgrass results from this work are comparable, indicating that pretreatments designed for xylose recovery are likewise appropriate for cellulose conversion. The situation with the poplar feedstock appears more complicated. The rates and extents of cellulose conversion for the pretreated poplar are somewhat below previously published maxima. A positive relationship between cellulose reactivity and extents of xylan dissolution via dilute-acid preteatment has been observed *(23,26)*. This presents an interesting trade-off, to maximize xylose yields or to maximize cellulose reactivity (presumably via maximum xylan removal). The key point being that maximum xylose yields are not based solely on xylan hydrolysis/dissolution. Xylose yields are based on simultaneous synthetic and degradative acid-catalyzed reactions *(42)*. Hence, pretreatment conditions for maximum xylose yields will not necessarily coincide with conditions for maximum xylan removal and cellulose reactivity. It can be assumed that product market opportunities and improved processing technologies will ultimately dictate optimization strategies.

Conclusion

The results obtained in this study provide information on the reactivity of the cellulose fraction of prehydrolyzed poplar and switchgrass feedstocks resulting from dilute acid pretreatments previously determined to optimize xylose yields in a stirred batch reactor pretreatment system (*42*). Cellulose reactivity was tested under saccharification (cellulase enzyme susceptibility) and SSF conditions. The pretreated switchgrass feedstock performed well under both conditions, while the glucose and ethanol yields from the pretreated poplar were somewhat low compared to those reported in a similar study in which the conditions of the dilute-acid pretreatment were chosen based on optimizing cellulose reactivity rather than xylose yields. The presented data indicate that the primary factor that limits the utilization of the cellulose remaining in the feedstocks at the end of SSF experiments is due to the recalcitrant nature of the cellulose.

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