0273-2289/02/98-100/0641/$13.50

Cellulase Adsorption and an Evaluation of Enzyme Recycle During Hydrolysis of Steam-Exploded Softwood Residues

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Abstract

The sugar yield and enzyme adsorption profile obtained during the hydrolysis of SO_2-catalyzed steam-exploded Douglas-fir and posttreated steam-exploded Douglas-fir substrates were determined. After hot alkali peroxide posttreatment, the rates and yield of hydrolysis attained from the posttreated Douglas-fir were significantly higher, even at lower enzyme loadings, than those obtained with the corresponding steam-exploded Douglas-fir. The enzymatic adsorption profiles observed during hydrolysis of the two substrates were significantly different. Ultrafiltration was employed to recover enzyme in solution (supernatant) and reused in subsequent hydrolysis reactions with added, fresh substrate. These recycle findings suggested that the enzyme remained relatively active for three rounds of recycle. It is likely that enzyme recovery and reuse during the hydrolysis of posttreated softwood substrates could lead to reductions in the need for the addition of fresh enzyme during softwood-based bioconversion processes.

Index Entries: Cellulases; Douglas-fir; ultrafiltration; adsorption; enzyme recycle; hydrolysis; bioconversion.

Introduction

The bioconversion of wood and agricultural wastes to fuel-grade ethanol has the potential to produce a value-added product from renewable residual materials from other industrial processes, while concurrently reducing greenhouse gas emissions. Although significant advances have been made in the bioconversion of hardwood and agricultural residues,

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progress in softwood feedstocks still remains limited. The inherent nature and distribution of the lignin in softwoods, and its subsequent recalcitrance following steam explosion restrict the enzymatic conversion of the cellulosic polymeric polysaccharides to monomeric sugars required for efficient and effective fermentation. Consequently, high enzyme loadings and relatively long incubation periods are generally required for effective cellulose saccharification to be achieved. These limitations therefore make softwood feedstocks unattractive from an economic perspective, although there is an abundance of these waste residues readily available in many areas around the world, particularly in Canada, the United States, and Scandinavia.

Over the years, various pretreatment strategies have been evaluated in an attempt to increase the efficacy of the enzymatic hydrolysis of softwood-derived lignocellulosics, and therefore reduce the cost of the overall bioconversion process *(1)*. However, todate, no viable process for softwood conversion has been established. A more viable approach to improving the practicality and economic feasibility of employing softwood-derived substrates in this process may be the recovery and reuse of cellulases from the reaction suspension as well as the residual substrates. It has been shown that the recovery of cellulases from both the supernatant and hydrolyzed residue during hydrolysis of steam-pretreated hardwood substrates is possible *(2,3)*, with estimated savings of 130–427% *(3)*.

Ultrafiltration has proven to be an efficient way to recover cellulases, as well as to continuously remove the end products generated during hydrolysis that could potentially inhibit hydrolysis reactions *(4–6)*. For example, using steam-exploded *Eucalyptus viminalis* wood chips that were alkaline peroxide treated, cellulolytic enzymes were successfully recycled over five consecutive reactions by readsorption of enzyme on the fresh substrates and ultrafiltration, without the addition of fresh β-glucosidase, for effective hydrolysis *(7)*.

Previous work on cellulase recycle has shown that the reuse of cellulases can manifest shorter incubation times, as well as increase the efficiency and decrease the cost of enzymatic hydrolysis *(3,7–9)*. However, the presence of lignin has been shown to play an important role in limiting the efficiency of enzymatic hydrolysis of lignocellulosic material *(9,10)*, and therefore, the efficacy of enzyme recycle. It was previously reported that the adsorption of cellulases onto steam-pretreated hardwoods was influenced by the nature of lignin *(11)*. Further, when three different strategies were used to recycle cellulases for the hydrolysis of steam-exploded birch, the results indicated that lignin was the most important factor influencing the efficiency of cellulase recycle *(12)*. It has also been suggested that different pretreatment condition may affect the cellulase adsorption characteristics onto a substrate *(13,14)*. However, very little is currently known about these interactions.

During the enzymatic hydrolysis of cellulosic substrates, several factors restrict the sustained catalytic activity of the cellulase mixture. It has been suggested that these limitations are owing to both substrate- and

enzyme-related factors *(15)*. It has been difficult to evaluate the reuse and/or recycle of cellulases, primarily because our current knowledge of the characteristics of cellulase adsorption onto lignocellulosic substrates is insufficient. Researchers generally use sugar yield or determination of the enzyme activity in the supernatant of the resultant hydrolysate to express the efficiency of the reused enzyme.

The objective of the present study was to determine the adsorption profiles of a cellulase preparation added to different Douglas-fir substrates, derived under various reaction conditions, during enzymatic hydrolysis. We also investigated the feasibility of using an ultrafiltration step for recycling cellulolytic enzymes involved in the hydrolysis of softwood substrates.

Materials and Methods

Substrates

Avicel PH101, a microcrystalline cellulose, was used as a model substrate, while SO_2-impregnated, medium-severity (4.5 min, 195°C, 4.5% [w/w] SO_2 to oven-dried wood) steam-exploded Douglas-fir (DF) represented the lignocellulosic substrates. Following explosion, the water-insoluble fraction of the DF substrate was extracted by hot alkali peroxide (DFP) at 80°C (pH 11.5) for 45 min *(16)* to remove a large portion of the recalcitrant lignin, and serve as a second lignocellulosic material. The composition of the DF and DFP substrates was 52.0% cellulose, 46.1% lignin, and 1.9% hemicellulose, and 91.8% cellulose and 8.2% lignin, respectively.

Enzymes

Hydrolysis experiments were performed using a complete *Trichoderma reesei* cellulase system (Celluclast; Novozymes, Denmark) supplemented with a commercial β-glucosidase (Novozym 188; Novozymes) at a ratio of 1:1.75 filter paper unit:cellobiase unit (FPU:CBU) to avoid end product inhibition owing to the accumulation of cellobiose. The celluclast contained 49 mg of protein/mL, as measured by a Bio-Rad protein assay (Bio-Rad, Hercules, CA), and contained the following hydrolytic activities: 80 FPU/mL of filter paper activity, 52 IU/mL of carboxymethylcellulase (CMCase), 226 IU/mL of xylanase, and 50 IU/mL β-glucosidase. The protein content and activities of Novozym 188 were as follows: 44 mg/mL, 5 FPU/mL, 34 IU/mL of CMCase, 94 IU/mL of xylanase, and 500 IU/mL of β-glucosidase. The enzyme activities were measured using standard procedures as described previously *(17)*.

Hydrolysis Experiments

Batch hydrolysis reactions were carried out in 300-mL Erlenmeyer flasks containing 2, 5, or 10% substrate (w/v) in 100 mL of 50 m*M* acetate buffer (pH 4.8) supplemented with 40 µg/mL of tetracycline and 30 µg/mL

of cycloheximide. The reaction mixtures were incubated at 45°C in a shaking water bath maintained at 150 rpm. Substrates were preincubated for 10 min at 45°C prior to the addition of enzyme, to allow the reaction medium to equilibrate. Aliquots of 0.5 mL were taken at different time points, and the samples were immediately chilled on ice and centrifuged at 5000*g* for 10 min. The sugar and protein content of the supernatant were determined.

Determination of Cellulase Adsorption Kinetics and Isotherms

Adsorption isotherms were established by varying the amounts of cellulase protein added to the different cellulosic substrates (10 mg/mL). Free cellulase was determined by measuring the amount of protein in the supernatant after equilibrium was attained. Bound cellulase was calculated as the difference between free protein and the total protein initially added to the reaction medium. The experimental data were fit to the Langmuir adsorption isotherm using the following linearized form of the equation:

$$P/P_{ads} = 1/P_{\max}K_p + (1/P_{\max})P$$

in which P is the concentration of unadsorbed cellulases (mg of cellulases/mL), P_{ads} is the concentration of adsorbed cellulases (mg of cellulases/mg of cellulose or substrate), $P_{\max}$ is the maximal adsorbed cellulases (mg of cellulases/mg of cellulose or substrate), and K_p is the equilibrium constant (mL/mg of cellulases).

Recycle of Enzymes

Cellulosic substrates were hydrolyzed for 24 h as described previously. The hydrolysate was filtered through glass filter paper (GF/A; Whatman), and then passed through an Amicon ultrafiltration cell containing a 5000 mol wt cutoff polyethersulfone membrane (NMWL5000; Millipore, Bedford, MA). The retentate was collected and added to fresh substrate (Avicel, DF, DFP) and buffer to carry out the subsequent rounds of hydrolysis for 24 h. The process was repeated for multiple rounds. The sugar content and enzyme activity (filter paper activity, CMCase, β-glucosidase) of the hydrolysate and retentate were determined after each round of hydrolysis.

Substrate Composition

The chemical composition of the substrates was determined using a modified Klason lignin method derived from the TAPPI Standard Method T222 om-98 *(18)*. Briefly, 0.2 g of sample was incubated at 20°C with 3 mL of 72% H_2SO_4 for 2 h with mixing every 10 min. The reaction was then diluted with 112 mL of deionized water (final acid concentration of 4% H_2SO_4) and transferred to a serum bottle. The solution was subjected to autoclaving at 121°C for 1 h and filtered through a medium coarseness sintered glass filter for gravimetric determination of acid-insoluble lignin.

The concentration of the wood sugars (arabinose, galactose, glucose, xylose, and mannose) in the filtrate was quantified using high-performance liquid chromatography (HPLC), while acid-soluble lignin was determined by absorption measurements at 205 nm *(19)*. The HPLC system (Dionex DX-300; Dionex) was equipped with an ion-exchange PA1 (Dionex) column, a pulsed amperometric detector with a gold electrode, and a Spectra AS 3500 autoinjector (Spectra-Physics). Prior to injection, samples were filtered through 0.45-mm HV filters (Millipore) and a volume of 20 µL was loaded. The column was preequilibrated with 250 m*M* NaOH and eluted with deionized water at a flow rate of 1.0 mL/min.

Determination of Protein and Sugar

Total protein was measured using the Bio-Rad Protein Assay using bovine serum albumin as the standard, per the manufacturer's instructions. The amount of unadsorbed protein in the supernatant was reported as a percentage of the amount of protein present in the substrate blank. The amount of sugars released during enzymatic hydrolysis was determined using a YSI 2700 Select Biochemistry Analyzer (YSI, Yellow Springs, OH).

Results and Discussion

Effect of Enzyme Loading on Hydrolysis of Different Substrates at 2% Consistency

Generally, softwoods have been considered the worst-case scenario as a feedstock for the bioconversion process because of their highly recalcitrant lignin, which consequently influences the efficiency of enzymatic hydrolysis. However, previous work *(20,21)* has indicated that a compromise in the pretreatment conditions (medium severity) between those optimized for high hemicellulose recovery and efficient cellulose hydrolysis will probably be required, in order to consider softwood residues as a potential feedstock for this process. Substrates produced via pretreatment at medium severity (~R_o = 3.76) contain a high lignin content, which limits the cellulase enzyme accessibility to cellulose when compared with substrates pretreated under higher severity, which are more accessible but have lower recovery of the hemicellulose-derived sugars. By contrast, pretreatment at lower-severity conditions generally liberates hemicellulose-derived sugars optimally, but generates a solid residue that is not readily amenable to the hydrolysis of cellulose.

As indicated in Fig. 1, an enzyme loading of 60 FPU/g of cellulose was necessary to achieve ~85% conversion of Douglas-fir medium severity pretreated feedstock (DF) within 72 h of incubation. Even when the enzyme loading was increased to 120 FPU/g of cellulose, complete hydrolysis could not be attained within the reaction time (Fig. 1). At lower enzyme loadings (i.e. ~10–20 FPU/g of cellulose), <50% of the solid residue was hydrolyzed, illustrating the recalcitrance of this substrate. Previously, it was shown that

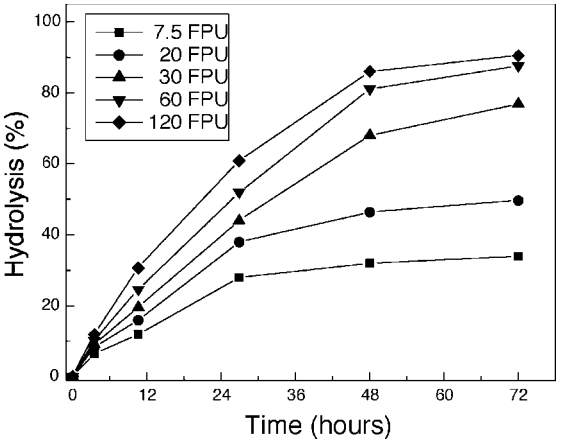

Fig. 1. Hydrolysis profiles of DF over time at different enzyme loadings (FPU/g cellulose), 2% solids concentration (w/v), and 45°C.

this substrate is characterized by a 50% residual lignin content, which was in a highly condense form *(22)*.

These and previous results have indicated that the removal of the residual lignin is a major process obstacle that needs to be addressed before softwood residues can be readily hydrolyzed. We have recently shown *(16)* that lignin removal from steam-pretreated softwood substrates can be accomplished by an innovative, hot alkaline peroxide posttreatment step. Briefly, following extensive water washing, the steam-exploded water-insoluble lignocellulosic is treated with hydrogen peroxide at pH 10.5 and 80°C for 45 min. When employing the posttreated residue (DFP) as a substrate for enzymatic hydrolysis, it was apparent that virtually all of the substrate was hydrolyzed within 48 h at an enzyme loading of 10 FPU/g of cellulose (Fig. 2). At a higher enzyme loading of 20 FPU/g of cellulose, >98% conversion could be achieved within 24 h of incubation. This hydrolysis rate was even faster than could be achieved with a pure cellulosic substrate (Fig. 3). At lower enzyme loadings (5 FPU/g of cellulose), roughly 80% conversion was attained within 48 h of reaction. A comparison of the initial rates of hydrolysis (samples at 1 h) of the three substrates (DF, DFP, Avicel) demonstrated that the DFP substrate could be hydrolyzed at a much faster rate than the steam-pretreated DF and the model substrate Avicel (Table 1).

The initial rate of hydrolysis, as defined by the 1-h sampling point, did not appear to be dependent on the amount of adsorbed cellulase protein. It was shown that, although increasing the enzyme loading significantly increased the initial rate of hydrolysis for the different substrates, there was not a correlation between initial rate of hydrolysis and the extent of protein adsorption (Table 1, Fig. 4). However, it was apparent that the DFP substrate showed not only a much higher initial rate of hydrolysis and conversion at the same enzyme loading, but was more readily hydrolyzed at lower enzyme loading than was the Avicel or DF substrate.

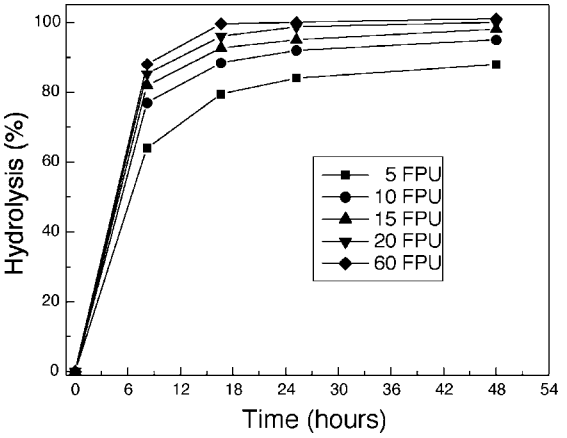

Fig. 2. Hydrolysis profiles of DFP over time at different enzyme loadings (FPU/g cellulose), 2% solids concentration (w/v), and 45°C.

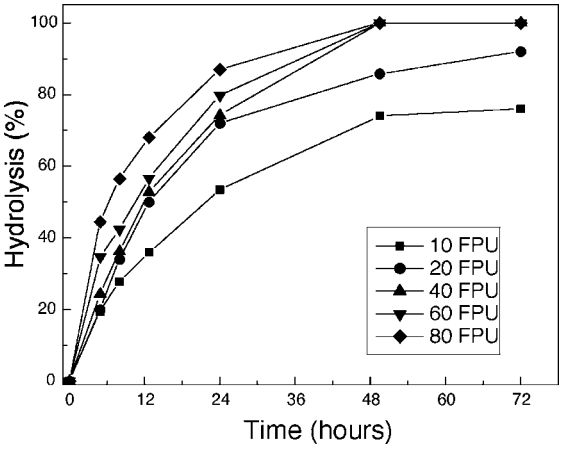

Fig. 3. Hydrolysis profiles of Avicel over time at different enzyme loadings (FPU/g cellulose), 2% solids concentration (w/v), and 45°C.

Table 1
Initial Rate of Hydrolysis of Lignocellulosic Substrates at 20 FPU/g Cellulose, 2% Solids Concentration, and 45°C

Substrate	Initial hydrolysis rate (mg/h/mg cellulose)[a]
Avicel	0.33 ± 0.003
Water-insoluble, medium-severity, steam-pretreated Douglas fir (DF)	0.20 ± 0.007
Hot alkali, peroxide-treated, medium-severity, steam-pretreated Douglas fir (DFP)	1.02 ± 0.006

[a] Defined as the total concentration of glucose liberated at a set time (mg/h) divided by the total amount of available cellulose polymer present in the initial reaction mixture (mg).

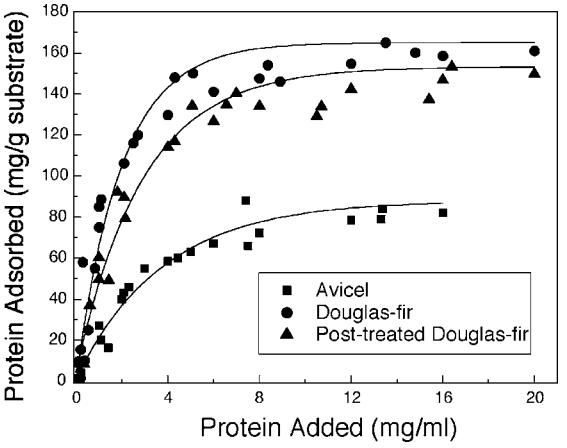

Fig. 4. Adsorption isotherm of Avicel, DF, and DFP at 4°C using varying concentrations of cellulase protein.

Table 2
Adsorption Parameters for Mixtures of Celluclast and Novozym 188 on Various Cellulosic Substrates

Substrate	Temperature (°C)	K_p (mL/mg protein)	P_{max} (mg/g substrate)	P_{max} (mg/g cellulose)
Avicel	4	0.3	95.2	95.2
DF	4	0.78	171.3	342.4
DFP	4	0.59	162.4	180.4

Cellulase Adsorption on Different Substrates

The adsorption capacity of the total proteins (Celluclast and Novozym 188) on the three cellulosic substrates was determined and is represented by Langmuir isotherms (Fig. 4). Although the DF and DFP substrates demonstrated similar adsorption saturation kinetics, they differed substantially from the adsorption profile observed with Avicel, and the maximum protein adsorbed was much higher (Table 2). The DF and DFP substrates demonstrated similar P_{max} values when calculated based on grams of substrate. However, these values differed significantly when they were based on gram of cellulose. The DF substrate was much less readily hydrolyzed than the DFP and Avicel. This was probably owing to the residual, condensed lignin *(22)*, since lignin has been shown to play an important role in influencing the efficiency of enzymatic hydrolysis of lignocellulosic material *(10)*. It has also been shown that enzymes are not only adsorbed to the cellulosic part of the substrate, but also remain adsorbed to the residual material that is void of any polysaccharides and contain primarily lignin *(23)*. However, the role of lignin and its influence on cellulase adsorption have still not been fully resolved. For example, although the DF substrate contained a higher residual lignin content, its maximum protein adsorption was very similar to that of the DFP (Table 2), which possesses a sig-

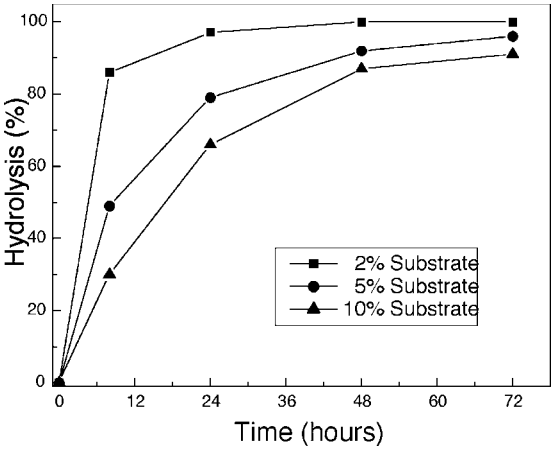

Fig. 5. Hydrolysis profiles of DFP at different solids concentrations (w/v) at 20 FPU/g of cellulose enzyme loading and 45°C.

nificantly lower lignin content (~50 vs ~10%). This suggests that lignin plays a significant role in adsorbing cellulases, while concurrently acting as a barrier to the cellulase enzymes and limiting the efficacy of hydrolysis. It is also apparent that the pretreated softwood substrates (DF, DFP) have a higher adsorption capacity than Avicel. Although the presence of lignin will contribute to this difference, it is likely that the drying and bleaching of Avicel, and consequently the limited "reswelling" of this substrate, influence the structure of the cellulose, adsorption capacity, and enzymatic hydrolysis.

Effect of Consistency on Hydrolysis

The concentration (w/v) of the substrate is an important factor in the enzymatic hydrolysis of lignocellulosic substrates, because it influences the rate, degree, and yield of hydrolysis, and thus significantly influences the technoeconomic potential of the overall process. For example, at a substrate concentration of 2% solids, the hydrolysis of DFP feedstock was quick and complete (Fig. 5). By contrast, by increasing the concentration to 5 or 10%, the hydrolysis was significantly slower and incomplete, even after prolonged incubation times (72 h). The degree of saccharification also has other implications. For example, previous attempts to employ enzyme-recycling strategies showed *(1,3,7)* that the more a substrate was hydrolyzed, the easier the enzymes were recovered. This was probably owing to the fact that the enzymes were still tightly associated with the recalcitrant, original substrate and were thus restricted from reacting with added, fresh substrate. Our results concur with the previous findings because the DFP substrate that was readily hydrolyzed demonstrated a constant percentage of enzyme associated with the solid phase, even as the lignocellulosic substrate was being saccharified (Fig. 6). For subsequent work, we therefore used a 2% solids concentration.

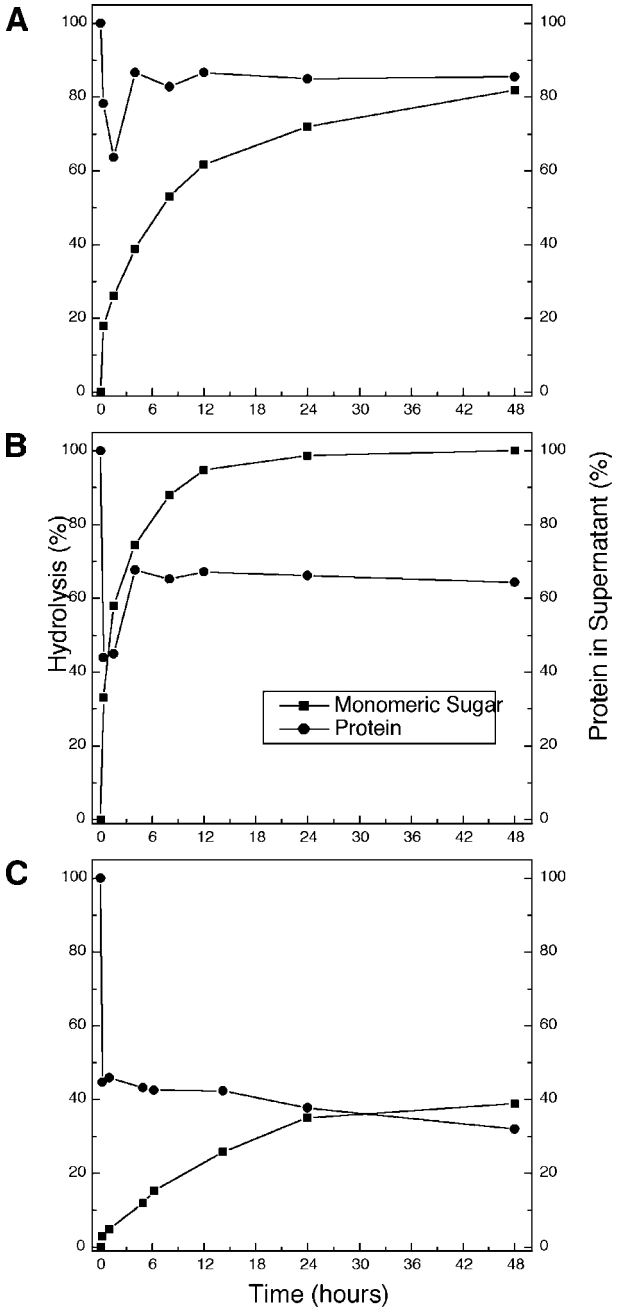

Fig. 6. Conversion and protein in supernatant profiles of **(A)** Avicel, **(B)** DFP, and **(C)** DF at 20 FPU/g of cellulose, 2% solids concentration, and 45°C.

Protein Association During Saccharification of Lignocellulosic Substrates

The amount of total protein adsorbed and desorbed during the course of hydrolysis at an enzyme loading of 20 FPU/g of cellulose for the three substrates (Avicel, DF, DFP) was compared in a separate experiment (Fig. 6). All three substrates rapidly adsorbed between 40 and 60% of the added protein within the first 20 min of incubation. The DF and DFP substrates initially adsorbed approximately the same amount of protein, while the Avicel adsorbed significantly less (~20% less) during the same period. Following the rapid adsorption that occurred in the first hour of the reaction, some of the protein was desorbed from both the Avicel and DFP substrates into the reaction supernatant, while the DF substrate continued to maintain and/or adsorb slightly more protein throughout the course of the reaction. This suggests that the lignin associated with the DF substrate has a higher binding affinity for the added enzyme, which restricts its release into solution. It is probable that the lower lignin content (8.2%) resulting from the hot alkali delignification stage, and therefore the more amorphous nature of the DFP substrate, are physical characteristics that enhance the release of adsorbed protein into the supernatant.

A similar result was observed when cellobiohydrolase proteins from *T. reesei* were used in studies evaluating model cellulose substrate hydrolysis *(24)*. The experimental data indicated that the release of enzyme to the liquid phase might be a result of the changes in cellulose or substrate structure during hydrolysis. For example, fragmentation may result in the particle size being reduced, thus increasing the overall available surface area, and therefore allowing the adsorption of more enzyme per gram of substrate. It was shown that significant amounts of the enzyme were associated with the unhydrolyzed residues, including both the completely hydrolyzed and partially hydrolyzed residues. If we assumed that all of the solubilized substrate is converted to monomeric glucose, the adsorption potential of the unhydrolyzed residue would increase as a function of increased hydrolysis, since the protein concentration in the reaction solution is constant throughout the reaction period.

It was previously reported that the adsorption profile of cellulases differed with various pretreated substrates *(25)*. Generally, it was observed that most of the enzymes were rapidly adsorbed onto the substrate within the first 10 min of contact at 30°C, and >40% of the cellulases remained adsorbed onto the unhydrolyzed residue after hydrolysis was terminated *(25)*. Similar results were reported when pretreated wood substrates were used *(3,26)*. When physicochemically pretreated substrates were used, it was shown that enzymes were rapidly and extensively adsorbed onto the substrate, and it was concluded that this was a function of increased accessibility *(27,28)*.

Even after complete hydrolysis of the DFP substrate, 35% of the starting protein still remained associated with the unhydrolyzed residue (lignaceous material). These results concur with previous findings indicating that pretreated lignocellulosic residues that contain significant quantities of lignin moieties clearly retain substantial amounts of enzyme, even when little target substrate remains *(29)*. Therefore, the alkaline peroxide posttreatments not only changed the substrate chemistry and morphology, but also influenced the adsorption/desorption profiles of the cellulase enzyme during hydrolysis.

Enzyme Recycle Strategies for Douglas-fir Substrates

Primary enzyme recycle experiments were conducted on the DF and DFP substrates at a 2% substrate concentration, at 20 FPU/g of cellulose for 24 h, after which the residue was separated by filtration and the supernatant was concentrated by ultrafiltration. The ultrafiltration retentate and the associated residue with bound enzymes were defined as the "recovered enzyme" and were used to conduct additional rounds of hydrolysis for 24 h. This was achieved by supplementing fresh substrate with the collected, concentrated protein. As expected, the conversion of both the DF and DFP substrates decreased during the three rounds of hydrolysis. However, the DFP substrate showed 74.6% conversion efficiency after three rounds of recycle with the initial enzyme solution, while the DF substrate demonstrated only a 20.9% conversion rate.

Clearly, the efficacy of the recycled enzyme system was significantly influenced by the characteristics of the substrates. However, it should be recognized that the composition of the feedstocks are constantly changing during each round of sequential hydrolysis. Thus, the conversion efficiency, as measured by sugars liberated from the solid residue, is not a true representation of the recovered enzymes' potential to hydrolyze new substrate. When the enzyme activities (filter paper activity, CMCase, β-glucosidases) in solution after hydrolysis for each of the 24-h recycle rounds were determined (Table 3), it was apparent that the enzyme activities in the solution dropped during each sequential hydrolysis round. It is difficult to evaluate the enzyme activity of adsorbed protein, and, therefore, it is hard to predict if the decrease in enzyme activity observed in solution truly reflects the enzyme activity of the bound enzyme. Mes-Hartree et al. *(30)* and Sutcliffe and Saddler *(10)* reported that steam-pretreated Aspen wood (240°C), and lignin derived from the hardwood substrates, adsorbed not only the cellulases responsible for filter paper activity and CMCase activity, but also the β-glucosidases. This was unexpected because β-glucosidases do not have a cellulose-binding domain, and therefore, should not exhibit a high affinity for solid cellulosic substrates. It was suggested that the noncellu-losic material might adsorb cellulases nonselectively, by hydrophobic interactions, and consequently influence the results of the enzyme assays.

Table 3
Enzyme Recovery from Residues and Supernatant
During Enzymatic Hydrolysis of DF and DFP Substrates
at 20 FPU/g Cellulose During Recycle Experiments

Recycle experiment				Enzymatic hydrolysis	Supernatant activity (%)		
Substrate	Recycle no.	Substrate (%)	Lignin (%)	Conversion (%)[a]	Filter paper activity	CMCase	β-Glucosidase
DFP	1	2.0	8.2	99.2	82.7	80.3	72.9
	2	2.3	15.1	93.2	64.8	56.8	71.8
	3	2.6	20.8	74.6	39.3	48.2	57.9
DF	1	2.0	46.1	43.6	40.6	44.2	38.0
	2	3.5	48.1	30.8	32.9	27.9	25.7
	3	5.1	57.7	20.9	17.9	20.1	19.4

[a]Percentage of the solid cellulosic polymer converted to soluble monomeric sugar.

Conclusion

It was shown that SO_2-catalyzed steam-pretreated Douglas-fir followed by a posttreatment with hot alkali peroxide improved the accessibility of cellulases to substrate, and increased the efficiency of enzymatic hydrolysis. Cellulase adsorption profiles differed when comparing the DF and DFP substrates. Initially, protein was quickly adsorbed and gradually released, however, the DFP maintained a relatively constant concentration of protein in solution, while the DF substrate continuously adsorbed protein during hydrolysis. This latter phenomenon was attributed to the higher residual lignin content of the substrate. Complete (100%), 93 and 86% hydrolysis of the cellulosic fraction of the DFP cellulose fraction could be achieved at 2, 5, and 10% consistency, respectively, at 20 FPU/g of cellulose after 48 h of reaction time. Significantly poorer results were obtained with the DF substrate, which was not subject to the hot alkali peroxide posttreatment and the Avicel (model substrate), which had previously been dried, but contained very little residual lignin.

An evaluation of enzyme recovery and reuse by ultrafiltration for three consecutive rounds of hydrolysis for 24 h at 45°C resulted in cellulose conversion rates of 97.2, 92.6, and 73.6%, respectively, when using DFP as a substrate. These results clearly indicate that it is possible to recover reasonable amounts of protein and reuse this enzyme during the hydrolysis of posttreated steam-exploded softwood substrates.

Acknowledgments

This work was supported in part by Natural Resources Canada.

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