BSA Treatment to Enhance Enzymatic Hydrolysis of Cellulose in Lignin Containing Substrates

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Abstract: Cellulase and bovine serum albumin (BSA) were added to Avicel cellulose and solids containing 56% cellulose and 28% lignin from dilute sulfuric acid pretreatment of corn stover. Little BSA was adsorbed on Avicel cellulose, while pretreated corn stover solids adsorbed considerable amounts of this protein. On the other hand, cellulase was highly adsorbed on both substrates. Adding a 1% concentration of BSA to dilute acid pretreated corn stover prior to enzyme addition at 15 FPU/g cellulose enhanced filter paper activity in solution by about a factor of 2 and beta-glucosidase activity in solution by about a factor of 14. Overall, these results suggested that BSA treatment reduced adsorption of cellulase and particularly beta-glucosidase on lignin. Of particular note, BSA treatment of pretreated corn stover solids prior to enzymatic hydrolysis increased 72 h glucose yields from about 82% to about 92% at a cellulase loading of 15 FPU/g cellulose or achieved about the same yield at a loading of 7.5 FPU/g cellulose. Similar improvements were also observed for enzymatic hydrolysis of ammonia fiber explosion (AFEX) pretreated corn stover and Douglas fir treated by SO_2 steam explosion and for simultaneous saccharification and fermentation (SSF) of BSA pretreated corn stover. In addition, BSA treatment prior to hydrolysis reduced the need for beta-glucosidase supplementation of SSF. The results are consistent with non-specific competitive, irreversible adsorption of BSA on lignin and identify promising strategies to reduce enzyme requirements for cellulose hydrolysis.

Keywords: bovine serum albumin (BSA); beta-glucosidase; cellulase; cellulose; enzymatic hydrolysis; lignin; simultaneous saccharification and fermentation (SSF)

INTRODUCTION

Conversion of cellulosic biomass sources including agricultural and forestry residues and herbaceous and woody plants to fuels and chemicals offers significant economic, environmental, and strategic advantages (Lugar and Woolsey, 1999; Lynd et al., 1996), and use of cellulase enzymes for breakdown of cellulose to glucose offers the high sugar yields vital to economic success (Lynd, 1996; Lynd et al., 1991; Wyman, 1999). However, cellulases act slowly, and high enzyme loadings are currently needed to realize reasonable rates and yields (Himmel et al., 1999; Wooley et al., 1999). Major strides have been announced recently in reducing cellulase costs so they are positioned for commercial use (American Chemical Society, 2005; American Institute of Chemical Engineers, 2004), but a better understanding of the interaction between enzymes and substrate and their influence on cellulase performance is still important to develop novel pretreatment and enzyme technologies that enhance cellulose hydrolysis.

Applying additives has shown promise in improving cellulase effectiveness, with most studies applying surfactants. In an early report, the non-ionic surfactant Tween 80 enhanced the enzymatic hydrolysis rate of newspaper cellulose by 33% (Castanon and Wilke, 1981). Subsequent research found that several cationic surfactants improved performance with cellulose (Avicel) and tissue paper, while anionic surfactants did not (Ooshima et al., 1986). Biosurfactants, including sohorolipid and Tween 80, enhanced the saccharification rate of cellulose in Sigmacell 100 and steam exploded poplar by as much as a factor of 7 while decreasing enzyme adsorption on cellulose (Helle et al., 1993). Others showed that incubation of enzymes with surfactants was more effective than adding surfactants to substrates (filter paper) prior to hydrolysis (Kaya et al., 1995). Tween 20 reduced thermal deactivation of cellulase and increased enzymatic cellulose and xylan conversion for lime pretreated corn stover by 42% and 40%, respectively, with loading on biomass found to be more important than the concentration in solution (Kaar and Holtzapple, 1998). Tween 80 enhanced enzymatic hydrolysis yields for steam exploded poplar wood by 20% in the simultaneous saccharification and fermentation (SSF) process (Ballesteros et al., 1998). Addition of Tween 20 reduced cellulase adsorption on solids and allowed a 50% reduction in cellulase loadings to obtain the same

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conversion for steam pretreated spruce (SPS) while having little effect on yields for delignified SPS (Eriksson et al., 2002). In the same study, 1.7% bovine serum albumin (BSA) produced about the same results as adding Tween 20 for SPS, but adding Tween 20 in addition to BSA did not increase cellulose conversion.

It has been shown that BSA adsorbs on lignin with the type of pretreatment affecting the amount of BSA taken up (Kawamoto et al., 1992). In addition, cellulase is adsorbed on cellulose, and cellulase and particularly beta-glucosidase are adsorbed non-productively on lignin (Sutcliffe and Saddler, 1986; Tatsumoto et al., 1988). We hypothesized that (1) BSA and other proteins will adsorb competitively and irreversibly on lignin and that (2) BSA or other proteins could be added prior to enzymatic hydrolysis to improve the effectiveness of cellulase. Thus, we initiated research in mid 2001 to obtain more information on how cellulase, beta-glucosidase, BSA, and other proteins adsorb on cellulose and pretreated biomass that contains cellulose and lignin and their effect on the enzymatic digestion of cellulose in each case, with progress reported at the 26th Symposium on Biotechnology for Fuels and Chemicals in May, 2004 and the AIChE Annual Meeting in November, 2004. This paper presents more recent results from this ongoing investigation using BSA as a model compound.

MATERIALS AND METHODS

Substrates

Insoluble solids from dilute acid pretreatment of corn stover graciously provided by the National Renewable Energy Laboratory (NREL) in Golden, Colorado from a large lot they obtained from Harlan, Iowa and maintained at controlled conditions were used as one of the substrates for these tests. The composition of the corn stover, as determined through NREL LAP procedures 001, 002, and 012 (Ehrman, 1994a,b; Ruiz and Ehrman, 1995), was found to be 37.8% glucan, 21.3% xylan, 1.6% arabinan, 3.8% mannan, 1.4% galactan, 17.8% lignin, and 7.8% ash by dry weight. This material was milled to pass through a 2 mm opening and then screened to obtain a -420 $+250$ μm fraction which was stored in plastic Ziploc bags and kept in a freezer (-20°C) as the source for all tests. The milled corn stover was soaked overnight in 1% (w/w) dilute sulfuric acid solution at 5% (w/w) solids. The presoaked slurry containing 40 g by dry weight of corn stover was transfered to a 1-L Parr reactor constructed of Carpenter-20 (Parr Instruments, Moline, IL) and fitted with a flat-blade impeller on a one-piece shaft operated at 100 rpm by a Parr DC motor drive (A1750HC, Parr Instruments). The reactor was lowered into a 4 kW model SBL-2D fluidized sand bath (Techne Co., Princeton, NJ) set at 320°C to the bottom of reactor head flange, which allowed for a rapid heat-up of the agitated contents to 140°C in about 2 min, and then was raised enough to maintain 140°C for 40 min (Brennan, 2003). Following pretreatment, the composition of the solids was 56% cellulose, 28.2% lignin, and 11.2% hemicellulose (including xylan, galactcan, arabinan, and mannan).

The University of British Columbia in Vancouver, Canada graciously supplied insoluble solids following pretreatment of Douglas fir at 195°C for 4.5 min with 4.5 % (w/w) SO_2, as previously described (Yang et al., 2002). This material had a composition of 56.3% cellulose, 46.1% lignin, and 8.2% hemcellulose. Michigan State University kindly provided insoluble solids produced by pretreating corn stover in the ammonia fiber explosion (AFEX) process at 90°C for 5 min with a 1:1 ammonia to corn stover ratio and a 60% moisture content. This material had a composition of 39.7% cellulose, 17.2% lignin, and 33.4% hemcellulose (Dr. Bruce E. Dale personal communication). Avicel PH101, containing more than 97% cellulose and less than 0.16% water soluble materials, was purchased from Sigma (St Louis, MO).

Enzymes and Organisms

The primary enzyme used in this study was a mixture of Celluclast 1.5 L cellulase (80 FPU/mL and 50 IU/mL β-glucosidase, Sigma) supplemented with commercial Novozyme 188 β-glucosidase (480 β-glucosidase CBU/mL and 0.5 FPU/mL, Sigma) at a ratio of 1:1.75 FPU:CBU activities to reduce end-product inhibition due to cellobiose accumulation. Celluclast 1.5 L and Novozyme 188 contained 49 mg protein/mL and 44 mg protein/mL, respectively, as measured by the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA). Enzyme activities were measured as described previously (Ghose, 1987). SSF experiments used this same enzyme formulation in combination with the D_5A strain of *Saccharomyces cerevisiae* (ATCC 200062) developed by NREL (Hayward et al., 1995).

Protein Addition and Treatment

To measure hydrolysis with just cellulase, 0.26 mg of cellulase was added per mL of a 2% suspension of Avicel or pretreated corn stover solids at time zero. The effect of BSA (Sigma) addition was first determined by adding enough BSA to pretreated corn stover to make a 1% (w/w) solution 1.5 h before and/or 10 h after cellulase addition. Additionally, cellulase at a loading of 15 FPU/g cellulose was added at the same time as enough BSA to make a 1% solution to a 2% suspension of pretreated corn stover. Finally, 1.2 mg of BSA was added per mL of suspension at time zero followed by 0.26 mg of cellulase one and a half hours later. In this case, the mixture was quickly transferred to a medium crucible (Fisher Scientific Company L.L.C, PA) before adding the enzymes to filter off the free liquid portion, and the solids were then washed three times with the supernatant (Yang and Wyman, 2003).

Cellulose Hydrolysis and SSF

Enzymatic hydrolysis of each substrate was conducted at a 2% solids concentration (2 g dry weight/100 mL) in 50 mM

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acetate buffer (pH 4.8) containing 40 μg/mL tetracycline and 30 μg/mL cycloheximide for cellulase loadings of 7.5–20 FPU/g cellulose. Flasks were preincubated at 50°C in water using an orbital shaker bath (3540, Barnstead International, Dubuque, IA) at 150 rpm for 10 min, and enzymes were added to start the hydrolysis after acclimation. Aliquots of 0.5 mL were taken at 0, 4, 24, 48, and 72 h; immediately chilled on ice; and centrifuged at 5,000*g* for 10 min (Yang and Wyman, 2004). Total sugar analyses were carried out on the resultant supernatants as described below.

All SSF runs were performed with a 2% (w/w) cellulose loading in 250 mL flasks using the D_5A strain of *S. cerevisiae* yeast according to standard methods described by NREL (Hayward et al., 1995). Cellulase loadings of 7.5–15 FPU/g cellulose were supplemented with β-glucosidase to achieve FPU:CBU ratios of 1:1.75 or 1:1. All SSF experiments were run for 7 days with sampling at 8, 24, 36, 48, 72, 96, 120, 144, and 168 h. Samples were analyzed for glucose, cellobiose, ethanol, and byproduct concentrations using HPLC analysis as described below.

Analytical Procedures

The sugar and acid insoluble lignin content of the solids were determined using the Klason lignin procedure published by NREL as LAP 003 (Templeton and Ehrman, 1995) and LAP 014 (Ruiz and Ehrman, 1996). Sugar concentrations in solution were measured by a high performance liquid chromatography system (Waters 2695, Milford, MA) equipped with a pulsed refractive index detector (Waters 2410, Milford, MA). The column was equilibrated with deionized water at a flow rate of 0.6 mL/min. An Aminex HPX-87P (Bio-Rad, Sunnyvale, CA) column was used for sugar separations and equilibrated with deionized water at a flow rate of 0.6 mL/min for the standard method for this analysis. The total concentration of protein in solution was measured by the Bio-Rad protein assay (Bio-Rad Laboratories) to determine how much dissolved protein was not adsorbed on substrate, but no differentiation was possible between BSA and cellulase other than by measuring the cellulase and beta-glucosidase activities in solution by standard methods (Ghose, 1987).

RESULTS

A mixture of Celluclast 1.5 L and Novozyme 188 at a ratio of FPU:CBU = 1:1.75 was first added to a 2% slurry of Avicel PH101 at an enzyme loading of 20 FPU/g cellulose and a temperature of 50°C. As shown in Figure 1, the protein concentration in solution dropped rapidly from about 0.26 mg/mL initially to about 0.1 mg/mL within just a few hours but did not change much after that, demonstrating that cellulase is adsorbed on cellulose, as expected. When BSA was added alone at a concentration of 1.2 mg/mL, its concentration in solution dropped only slightly from the initial value of about 1.2 mg/mL to about 1.15 mg/mL at the end of the 48 h period. In the third set of data in Figure 1, adding cellulase at a loading of 20 FPU/g cellulose after treating Avicel with 1.2 mg/mL BSA for 0.5 h resulted in an increase in dissolved protein concentration to 1.44 mg/mL, a rapid drop back to about 1.2 mg/mL within a few hours, and a slight increase in concentration after that. It is interesting to note that the difference between the protein concentration in solution for this latter case and the case when only BSA was added was about the same as the amount of dissolved protein in solution when only cellulase was applied.

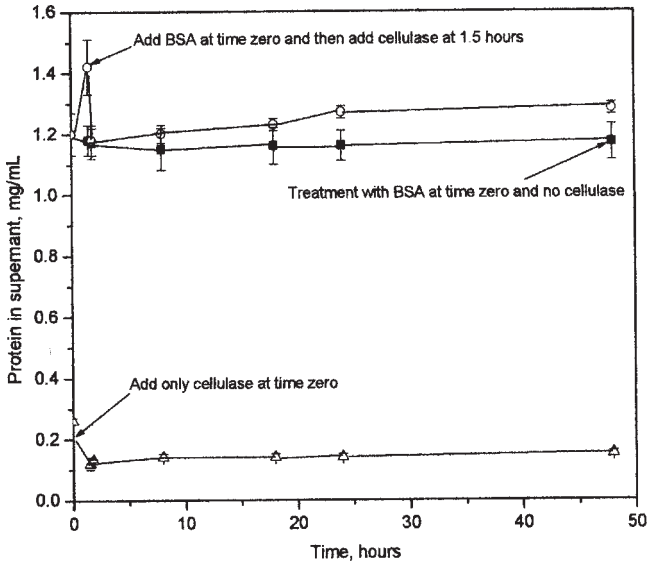

Figure 1. Concentration of protein in the supernatant at 50°C for addition of only cellulase (open triangles), only BSA (solid rectangles), and BSA followed by cellulase after 30 min (open circles) to a 2% Avicel PH101 slurry.

The same procedure was repeated for the insoluble solids produced by dilute acid pretreatment of corn stover, with the results shown in Figure 2. When just the enzyme mixture was added for an enzyme loading of 20 FPU/g substrate at 50°C, the protein concentration in solution dropped quickly from about 0.26 mg/mL to less than 0.1 mg/mL in just a few hours and then continued to drop to a very low concentration of 0.021 mg/mL after 20 h of enzymatic hydrolysis. Thus, as enzymatic hydrolysis of cellulose proceeded, more cellulase was continually adsorbed onto the pretreated corn stover solids even though less cellulose was available, unlike the results for pure cellulose (Avicel) in which enzyme was released into solution. For treatment of pretreated corn stover with 1.2 mg/mL of BSA, the dissolved protein concentration dropped from 1.2 mg/mL initially to less than 0.41 mg/mL in the first 1.8 h and then gradually dropped to 0.13g/mL after 48 h. However, when 0.26 mg/mL of enzyme mixture was added to the pretreated corn stover slurry 1.5 h after treating with BSA (1.2 mg/mL), the dissolved protein concentration dropped to about 0.83 mg/mL. Then the dissolved protein concentration jumped up to about 1.2 mg/mL at 1.8 h, dropped back rapidly to about 0.3 mg/mL within the next 6 h, and continued to drop more slowly after that to about 0.21 mg/mL after 48 h. As shown in Figure 3, adding just BSA or just cellulase exhibited similar adsorption behavior

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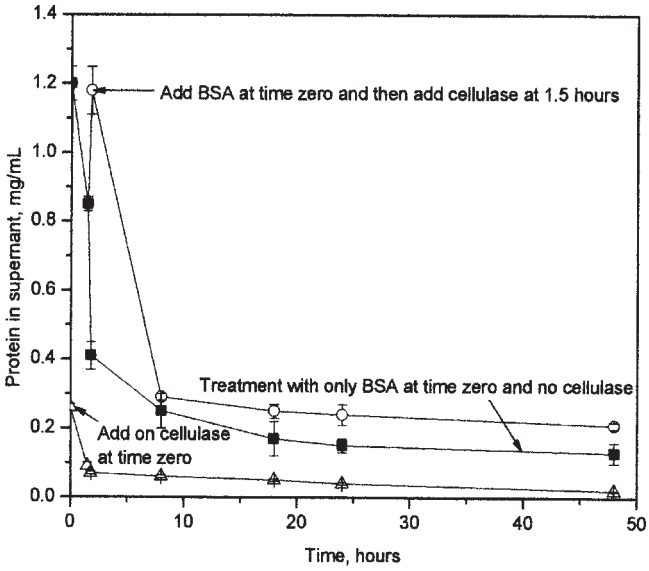

Figure 2. Concentration of protein in the supernatant at 50°C for addition of only cellulase (open triangles), only BSA (solid rectangles), and BSA followed 30 min later by cellulase (open circles) to a 2% slurry of the insoluble solids from pretreatment of corn stover at 140°C with 1 wt% sulfuric acid for 40 min in a batch reactor.

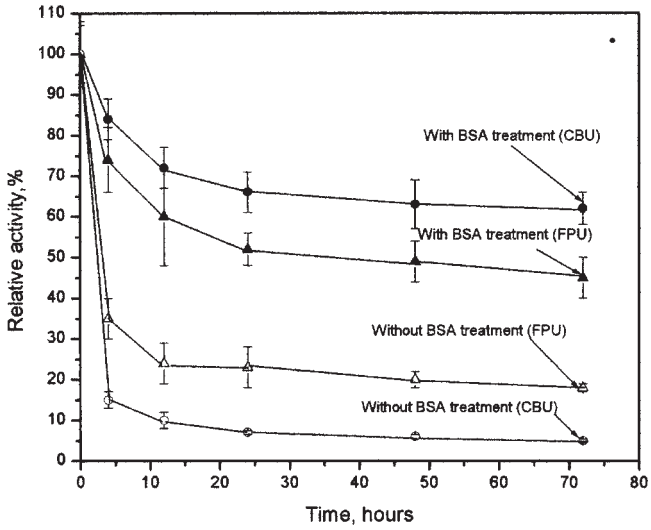

Figure 4. Changes in relative enzyme activities in the supernatant for hydrolysis of corn stover pretreated at 140°C for 40 min with 1 wt% sulfuric acid with and without BSA treatment prior to adding enzyme at a loading of 15 FPU/g cellulose.

with pretreated corn stover at 4°C, but much more cellulase than BSA was absorbed on Avicel.

To determine how addition of BSA prior to adding the standard cellulase/β-glucosidase enzyme mixture affected adsorption of enzyme, the cellulase activity in solution was measured by the filter paper assay for pretreated corn stover, as reported in Figure 4 for an enzyme loading of 15 FPU/g of cellulose. Based on a relative activity of 100% for the protein in solution at time zero, the cellulase activity dropped rapidly to about 20% of its initial value within 3 days when the enzyme was added to pretreated corn stover without prior treatment with BSA. On the other hand, when BSA was added to pretreated corn stover prior to the enzyme mixture, the activity in solution only dropped to about 50% of its initial value after 3 days. When followed in a similar fashion, β-glucosidase activity dropped to about 5% of its initial value when the enzyme mixture was added to pretreated corn stover alone but dropped far less to about 70% of the initial activity when the pretreated corn stover was treated with BSA prior to adding enzyme.

The time course of enzymatic hydrolysis of cellulose in Avicel and pretreated corn stover were followed for a cellulase loading of 15 FPU/g cellulose with and without BSA treatment. As shown in Figure 5, treatment with BSA

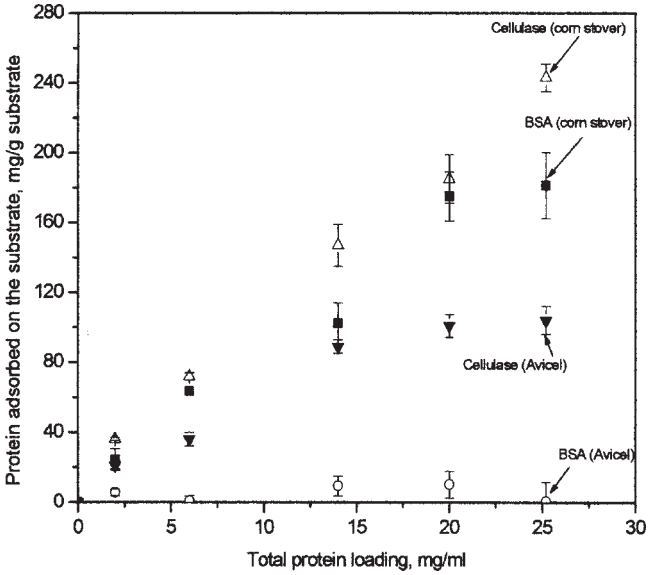

Figure 3. Cellulase and BSA protein adsorbed at 4°C on Avicel and corn stover pretreated at 140°C with 1 wt% sulfuric acid for 40 min in a batch reactor.

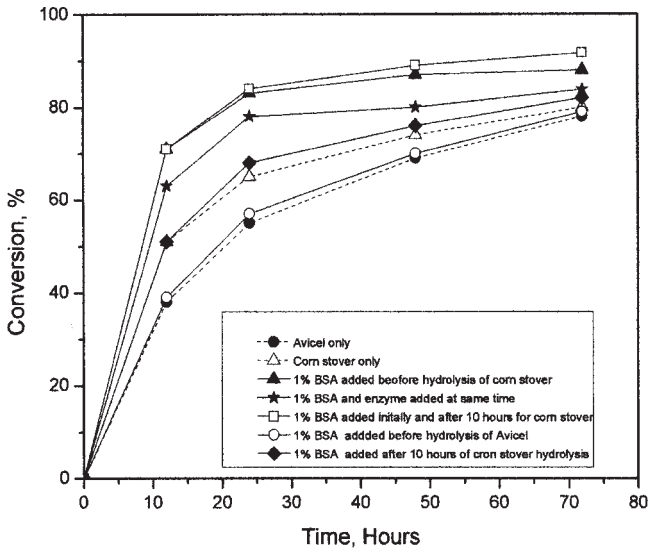

Figure 5. Hydrolysis of Avicel PH101 and pretreated corn stover without and with BSA treatment before, at the same time as, and after adding enzyme at a loading of 15 FPU/g cellulose.

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prior to cellulase addition had little effect on the conversion of Avicel throughout the reaction period. However, treatment of pretreated corn stover with BSA prior to cellulose addition greatly enhanced the rate of hydrolysis, and the conversion of cellulose at 72 h was improved to about 90% when treated with BSA versus only about 78% when the cellulase mixture was employed alone. Adding BSA after cellulase addition had little effect on performance at the initial stages of hydrolysis but did improve the final yield by a few percent. When cellulase and BSA were added at the same time, the initial hydrolysis rate was significantly improved, and the final yield was about 5% higher than for adding cellulase alone. In addition, Figure 5 shows that adding BSA to pretreated corn stover, which had been treated with BSA before adding cellulase and hydrolyzed for 10 h, enhanced the final yield by about 3%. It also shows that the final yield still improved by around 2% even when BSA was added to pretreated corn stover after 10 h of hydrolysis.

Table I summarizes data for the enzymatic hydrolysis of the cellulose in corn stover pretreated by dilute sulfuric acid and AFEX and in Douglas fir pretreated by sulfur dioxide. At the pretreatment conditions used, the lignin content ranged from 17.2% to 46.1% and the cellulose content varied from 39.7% to 56.3%, as shown in the Table. In the case of corn stover pretreated in a batch reactor at optimum conditions of 1 wt% H_2SO_4 at 140°C for 40 min, the conversion increased from 82.3% to 91.7% at 72 h for treatment with BSA prior to enzyme addition at 15 FPU/g cellulose, and the conversion was almost the same at 81.9% when half the amount of enzyme was added following BSA treatment. For corn stover pretreated by AFEX, BSA treatment increased the conversion from 76.6% to 82.5% at an enzyme loading of 15 FPU/g cellulose or achieved about the same conversion of 74.3% for a lower loading of 10 FPU/g cellulose. A stronger effect was observed for Douglas fir, a high lignin content softwood, in that cellulose conversion increased from 54.2% to 73.5% when the pretreated solids were treated with BSA prior to enzymatic digestion at a somewhat higher enzyme loading of 20 FPU/g cellulose. Furthermore, treating pretreated Douglas fir with BSA prior to enzymatic hydrolysis increased cellulose conversion to 59.7% with half the amount of enzyme.

Protein treatment of lignin containing pretreated biomass was also applied prior to cellulose conversion via the SSF route to biomass conversion (Spindler et al., 1989). As shown in Figure 6, BSA treatment of corn stover pretreated with dilute acid as above increased the ethanol yield in SSF from 83.2% to about 93.2% after 7 days for an enzyme mixture loading of 15 FPU/g cellulose. In addition, the rate of ethanol production was much faster for BSA treatment, reaching nearly the final yield value in only about 4 days versus the more than 7 days needed without BSA addition. Alternatively, BSA treatment gave similar results at an enzyme loading of 7 FPU/g cellulose to those possible with twice the enzyme loading but without BSA addition. Figure 6 also demonstrates that BSA treatment achieved better results with less β-glucosidase supplementation. For example, adding enzyme with a 1:1 ratio of cellulase to β-glucosidase activity to BSA treated corn stover gave slightly faster rates and higher yields than adding a 1:1.75 ratio to corn stover without BSA treatment for a 15 FPU/g cellulose enzyme loading in both cases.

DISCUSSION

A number of mechanisms have been offered to explain the effect of additives on enzymatic hydrolysis performance. Tween 80 was thought to reduce cellulase adsorption (Castanon and Wilke, 1981), and cationic surfactants were projected to alter the balance of endo- and exoglucanase adsorption (Ooshima et al., 1986). Others indicated that non-ionic surfactants (Park et al., 1992) and biosurfactants (Helle et al., 1993) promoted cellulase desorption after saccharification, and biosurfactants were also said to make cellulose more susceptible to enzymes (Helle et al., 1993). Tween 80 was projected to give more intimate contact of cellulase with cellulose (Ballesteros et al., 1998). More recently, surfactants and proteins were both viewed as preventing non-productive adsorption of cellulase on lignin (Eriksson et al., 2002). However, no benefits were found from

Table I. Effect of BSA addition on the enzymatic hydrolysis of corn stover cellulose pretreated with dilute acid and by ammonia fiber explosion (AFEX) and Douglas fir cellulose pretreated with sulfur dioxide.

Substrate	Pretreatment condition	Cellulose (% dry weight)	Lignin (% dry weight)	Xylan (% dry weight)	BSA prewash	Cellulase loading (FPU/g cellulose)	Percent conversion of total cellulose at 72 h
Corn stover	Dilute acid (1% H2SO, 140°C , 40 min)	56	28.2	11.2	None	15	82.3
					1% BSA	15	91.7
						7.5	81.9
Corn stover	AFEX (NH_3:Corn stover = 1:1, 60% moisture content, 90°C, 5min)	39.7	17.2	33.4	None	15	76.6
					1% BSA	15	82.5
						10	74.3
Douglas fir	Steam explosion (4.5% SO_2, 195°C , 4.5 min)	56.3	46.1	8.2	None	20	54.2
					1% BSA	20	73.5
						10	59.7

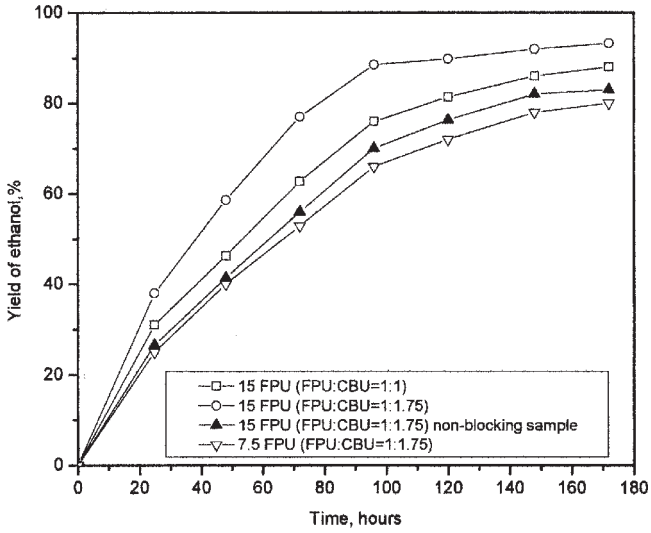

Figure 6. Effect of BSA treatment on ethanol yields for different enzyme loadings and β-glucosidase supplementation levels for simultaneous saccharification and fermentation (SSF) of corn stover cellulose pretreated with dilute acid.

adding Tween 20 to cellulose (Avicel), contrary to findings reported by others that would suggest surfactants act differently than proteins (Helle et al., 1993; Ooshima et al., 1986).

In this study, pretreated corn stover adsorbed even more cellulase than Avicel but had a high capacity for BSA as well, consistent with literature findings that report adsorption of a substantial amount of cellulase on both lignin and cellulose (Grohmann et al., 1989; Ooshima et al., 1990; Sutcliffe and Saddler, 1986; Tatsumoto et al., 1988). On the other hand, Avicel adsorbed substantial amounts of cellulase but little BSA. Thus, the adsorption of BSA on pretreated corn stover must be primarily due to its high affinity for lignin (Kawamoto et al., 1992). The measurement of activities in solution suggested that BSA attached to the lignin and significantly reduced non-specific adsorption of cellulase on lignin, leaving more cellulase free in solution. On this basis, we have termed proteins that behave in this way "lignin blockers." Furthermore, treatment with BSA prior to adding cellulase reduced adsorption of β-glucosidase substantially more than cellulase due to the greater capacity of lignin for β-glucosidase than the other cellulase components (Sutcliffe and Saddler, 1986; Tatsumoto et al., 1988).

Although the individual concentrations of BSA and cellulase could not be measured effectively, hydrolysis data indicated that BSA had little effect on the yields or rates of cellulose (Avicel) hydrolysis, probably because of its limited adsorption. More importantly, the greater concentrations of cellulase and β-glucosidase in solution that resulted from prior BSA treatment of lignin containing solids made more enzyme available for cellulose hydrolysis, and cellulose conversion was greatly enhanced by BSA treatment prior to enzyme addition at a given enzyme loading. Alternatively, the data also showed that BSA treatment prior to enzymatic hydrolysis achieved the same conversion with significantly lower cellulase loadings than possible with higher cellulase loadings without BSA treatment. These benefits were found for corn stover pretreated by dilute sulfuric acid and ammonia and for Douglas fir pretreated with sulfur dioxide. However, although AFEX pretreatment removed little lignin, BSA treatment had less effect on the cellulase efficiency for AFEX than for acid pretreatment. One possible explanation is that lignin treated with ammonia has less capacity for cellulase than lignin treated with dilute acid, a mechanism that appears consistent with the observation that AFEX pretreated solids need less cellulase to achieve similar hydrolysis performance to acid pretreatment (Dale et al., 1996).

Bovine serum albumin addition was particularly effective when added before cellulase, consistent with it being irreversibly bound to lignin. Yet, adding BSA with cellulase still resulted in some gain in both rates and yields. Furthermore, adding BSA after hydrolysis began also enhanced rates whether pretreated corn stover was treated with BSA prior to hydrolysis or not. The latter result could be attributed to BSA attaching to lignin exposed during hydrolysis and reducing non-productive cellulase adsorption later in the process. Alternatively or in addition to this, this observation could be due to BSA adsorbing on lignin following desorption of cellulase. In any event, these results support the idea that BSA attaches competitively to lignin in the presence of cellulase.

The significantly greater amounts of β-glucosidase available in solution to breakdown the powerful enzyme inhibitor cellobiose into glucose appeared particularly effective in improving cellulose hydrolysis rates and yields for prior addition of BSA to pretreated biomass in the SSF process. Consequently, treatment with BSA or other proteins that behave similarly has important implications in potentially reducing or eliminating the need for β-glucosidase supplementation as now practiced to achieve high hydrolysis yields (Brown and Torget, 1996; Spindler et al., 1989; Wyman et al., 1986). Beyond the potential utility of this result, this observation indicates that β-glucosidase supplementation is needed at least in part to compensate for its preferential adsorption by pretreated lignin. Thus, significant benefit could result from developing novel pretreatments that do not produce lignin with such a high capacity for cellulase and particularly β-glucosidase.

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