Bioresource Technology 101 (2010) 5941–5951

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Impact of surfactants on pretreatment of corn stover

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ARTICLE INFO

Article history:
Received 26 July 2009
Received in revised form 23 February 2010
Accepted 1 March 2010
Available online 20 March 2010

Keywords:
Surfactants
Tween-80
Lignocellulosics
Enzymatic hydrolysis
Adsorption

ABSTRACT

Lignin in pretreated cellulosic biomass can non-productively adsorb cellulase, resulting in loss of a significant portion of this expensive protein. In addition, lignin interferes with the path for cellulase action, slowing down hydrolysis. Thus, the effectiveness of enzymatic hydrolysis of pretreated lignocellulosic biomass can be significantly enhanced if lignin is removed or effectively modified before adding enzymes. In this study, the enzymatic digestibilities of solids resulting from using the surfactants Tween-80, dodecylbenzene sulfonic acid, and polyethylene glycol 4000 during water-only or dilute acid pretreatment of corn stover at 140–220 °C were evaluated. All of these surfactants increased lignin removal during pretreatment and reduced non-productive binding of enzymes on the biomass surface, but Tween-80 increased enzymatic hydrolysis yields and enhanced total sugar recovery more than the other two. Surfactant pretreatment was found to improve lignin solubility, which could improve cellulose digestibility by reducing unproductive binding to enzyme, and also appeared to enhance performance by modifying the biomass surface.

1. Introduction

Ethanol produced from lignocellulosic biomass has great potential to address vital energy security, trade deficit, environmental, and economic issues that are becoming more urgent in light of declining petroleum reserves and increasing international demand for transportation fuels. However, no commercial facilities are yet in operation, and technical advances can help compensate for risk of first applications by reducing costs and improving profitability (Wyman, 2007; Yang and Wyman, 2008). A dominant concern is the high price of enzymes and the costly pretreatments needed to achieve high sugar yields for enzymatic hydrolysis essential to economic success. For example, typical cellulase loadings of about 15 FPU/g cellulose used to achieve high yields of sugars from pretreated biomass could be translated into about 30 g of enzyme per liter of ethanol made, an extremely high and expensive dose. Thus, enzyme costs must either be reduced below about $2/kg protein or strategies developed to substantially reduce loadings (Himmel et al., 1999; Wingren et al., 2005; Wyman, 2007). One promising approach has been the addition of surfactants, especially non-ionic surfactants, after biomass pretreatment to improve enzymatic hydrolysis or reduce the amount of enzyme needed to achieve a given conversion (Castanon and Wilke, 1981; Eriksson et al., 2002; Kaar and Holtzapple, 1998). It was reported that applying two classes of non-ionic surfactants, NP and Tween, after ammonia–hydrogen peroxide pretreatment prior to enzymatic hydrolysis increased performance with decreased enzyme loadings (Kim and Chun, 2004). Kim et al. concluded that NP 20 was 10–20% more effective than Tween-20 and 80 when applied to recycled newspaper at 40 °C with a stirring speed of 400 rpm for 1 h before enzymatic hydrolysis (Kim et al., 2007).

Several explanations have been offered to explain how surfactants enhance enzymatic digestion: (1) surfactants alter the substrate structure and make it more accessible to enzymes (Helle et al., 1993; Kaar and Holtzapple, 1998); (2) surfactants stabilize enzymes and prevent their denaturation during hydrolysis (Kaar and Holtzapple, 1998; Kim et al., 1982); (3) surfactants increase positive interactions between substrates and enzymes (Eriksson et al., 2002; Kaar and Holtzapple, 1998; Malmsten and VanAlstine, 1996), and (4) surfactants reduce non-productive adsorption of enzymes (Eriksson et al., 2002). However, a mechanism that can consistently explain how surfactants improve enzymatic hydrolysis has yet to be developed.

Lignin is widely recognized to be an obstacle to efficient enzymatic hydrolysis. First, it unproductively adsorbs a large fraction of the cellulase, making it unavailable for enzymatic hydrolysis of cellulose (Lu et al., 2002). On top of that, lignin impedes enzyme access to cellulose and hemicellulose, resulting in extended reaction times to achieve high conversions (Lu et al., 2002; Sutcliffe and Saddler, 1986; Yang and Wyman Charles, 2006). Thus, it would be beneficial to remove or modify lignin in a way that reduces its

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doi:10.1016/j.biortech.2010.03.003

negative influence on enzymatic hydrolysis. However, pretreatment technologies that are effective for removing lignin, such as organic solvent methods, tend to be too expensive for making ethanol from cellulosic biomass competitively. Evidence with flow-through systems strongly suggested that lignin was released into solution during pretreatment with acid or just water/steam and then reacted to form compounds with limited solubility that precipitated back on the surface (Liu and Wyman, 2003; Yang and Wyman, 2004). This cyclical pattern was projected to disrupt lignin enough to improve enzymatic hydrolysis, but the lignin deposits still interfered with enzyme action and non-productively adsorbed enzyme (Donohoe et al., 2008). Therefore, an approach that can possibly reduce lignin condensation onto pretreated solids would benefit the subsequent enzymatic hydrolysis in terms of achieving higher yields with lower enzyme loading.

On this basis, we hypothesized that pretreating biomass with surfactants could capture some of the lignin released into the liquid phase by forming emulsions, thereby reducing the amount redeposited on biomass. However, only one study showed that 3.33 wt% of the non-ionic surfactant Tween-20 during pretreatment at 170–190 °C enhanced enzymatic hydrolysis of bagasse and decreased the amount of lignin remaining in the treated material by 22–27% compared to those just treated with water (Kurakake et al., 1994). Therefore, in this study, the effectiveness of surfactants as additives for biomass pretreatment with dilute acid or just water was evaluated. In addition, the effect of surfactant pretreatment on enzyme adsorption, lignin content, and hydrophobicity of the resulting solids was studied to gain new insights into mechanisms that could influence performance.

2. Methods

2.1. Materials

Corn stover was provided by the National Renewable Energy Laboratory (NREL, Golden, Colorado) from a lot they obtained from nearby Kramer farm (Wray, CO). The composition determined by NREL LAP procedures 001, 002, and 003 was about 37.7% glucan, 18.8% xylan, and 17.6% lignin, plus other sugars and ash. Avicel PH-101 cellulose was purchased from Sigma (St. Louis, MO, cellulose content > 97%, Lot & filling code: 1300045 32806P01). Genencor International (2600 Kennedy Drive, Beloit, WI, 53511) kindly supplied Spezyme CP cellulase (301-05330-205, 59 ± 5 FPU/ml, 123 ± 10 mg protein/ml), while Novozyme 188 (066K0676, 665 CBU/ml, 140 ± 5 mg protein/ml) was purchased from Sigma (St. Louis, MO) for β-glucosidase supplementation.

Three types of surfactants previously identified to be beneficial for treatment after conventional pretreatments but prior to adding enzymes were used in this research: Tween-80 (Polyoxyethylene Sorbitan Mono-Oleate, purity > 80%, Fisher Scientific, Lot No. 043457), dodecylbenzene sulfonic acid (DDBSA, purity > 85%, Acros Organics, New Jersey, USA, Lot # A0131295001), and polyethylene glycol 4000 (PEG, Hampton Research, 34 Jounery, Aliso Viejo, CA 92656-3317, USA, Lot# 260540). Unless otherwise stated, 3% (w/w) of one of these surfactants was added based on dry biomass weight for each pretreatment, with the result that about 0.015 g of surfactant was added to 0.5 g (dry) of biomass in a reaction tube.

2.2. Pretreatment

Batch reactors were constructed of 0.5″ OD × 0.035″ wall thickness × 6″ long Hastelloy C-276 tubing (Maine Valve and Fitting Co., Bangor, ME). A sand bath (Model SBL-2D, Techne Co., Princeton, NJ) was used to heat up the reactors to the target reaction temperatures of 140–190 °C. A solid concentration of 5% was employed in all pretreatments, and the following pretreatment conditions were applied: 140 °C with 1% sulfuric acid for 40 min, 160 °C with 0.5% sulfuric acid for 16, 18, 20, 22, 24 min, and 190 °C water-only for 10, 12, 14, 16, 18 min. For surfactant pretreatments, 3% (w/w) Tween-80 or other surfactants were added prior to pretreatment based on dry biomass weight. Control samples were prepared in parallel at the same solid loadings but without addition of surfactants. All samples were soaked in the reaction tubes overnight to assure sufficient penetration of liquid into the corn stover solids.

2.3. Sugar analysis

Liquid samples were filtered through 0.2 μm nylon filter vials (Alltech Associates Inc., Deerfield, IL), pipetted into 500 μl polyethylene HPLC vials (Alltech Associates Inc., Deerfield, IL), and kept refrigerated at 4 °C until analyzed. Liquid samples together with calibration sugar standards were run on a Waters Alliance HPLC system (Model 2695, Waters Corporation, Milford, MA) employing an Aminex HPX-87P column (Bio-Rad Laboratories, Hercules, CA) and a refractive index detector (Waters 2414). Concentrations of monomeric glucose and xylose were calculated based on calibration sugar standards.

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis of washed pretreated solids was performed according to NREL Laboratory Analytical Procedure LAP 009 at a solids loading of 2% (w/v) in 125 ml Erlenmeyer flasks to which was added 0.05 M acetate buffer (pH 4.8). To prevent possible microorganism contamination, 400 μg of 10 mg/ml tetracycline antibiotic in 70% ethanol and 300 μg of 10 mg/ml cyclohexamide in DI water were added to the hydrolysis broth before adding enzymes. The flasks were placed in a thermostated water bath shaker at 48 ± 3 °C at a rotating speed of 200 rpm (NREL, 1996). The enzyme loadings were 10 and 60 FPU/g glucan in the pretreated solids (corresponding to about 21.5 and 129 mg protein/g glucan) supplemented with β-glucosidase at a loading of 20 and 120 CBU/g glucan (CBU to FPU activity ratio of 2:1). Substrate blanks without enzyme and enzyme blanks without substrate were tested in parallel with other samples. Samples taken after 4, 24, 48, 72, and 96 h of hydrolysis were analyzed with an HPLC to follow the reaction course and determine final yields.

2.5. Determination of protein adsorption

Adsorption experiments were carried out at 4 °C to prevent hydrolysis of substrate using a concentration of 1% solid substrate in a total volume of 1.1 ml hydrolysis broth contained in 1.5 ml Eppendorf® Lobind microcentrifuge tubes (protein loss <3%). Protein (cellulase and β-glucosidase) was added to bring the final protein concentration to about 100 mg/g biomass. Then, the samples were rotated slowly at 4 °C in a refrigerator for 4 h followed by centrifuging (Eppendorf Centrifuge 5415D) at 4 °C at a maximum speed of 16.1 rpm for a minimum of 10 min. The resulting solid and liquid fractions were each weighed into a 2 ml tin capsule and sealed, and the nitrogen content of these samples was determined by an Elemental Analyzer (FLASH 1112 CHNOS Analyzer, CE ELANTECH, Lakewood, NJ) using aspartic acid as a standard. Protein adsorption could then be determined based on a mass balance for nitrogen (Yang et al., 2006).

2.6. Hydrophobicity test

The hydrophobicity of each sample was determined using a VCA Optima Contact Angle and Surface Analysis system. Biomass

samples were ground to smaller than 150 mesh particle size and pressed at a pressure of 4000 psi to form small pellets. The VCA Optima was used with a syringe size of 100 μL. Each drop of water was set to 2 μL using a medium drop speed and the hydrophobicity of each sample was characterized by degree of contact angle, with a larger contact angle meaning a greater hydrophobicity. Because of the heterogeneous nature of corn stover, all samples were prepared in triplicates, with averages and standard deviations calculated (Deng and Abazeri, 1998).

2.7. Determination of lignin content and lignin removal

The total acid-insoluble lignin in the raw corn stover before pretreatment and the solids after pretreatment with or without

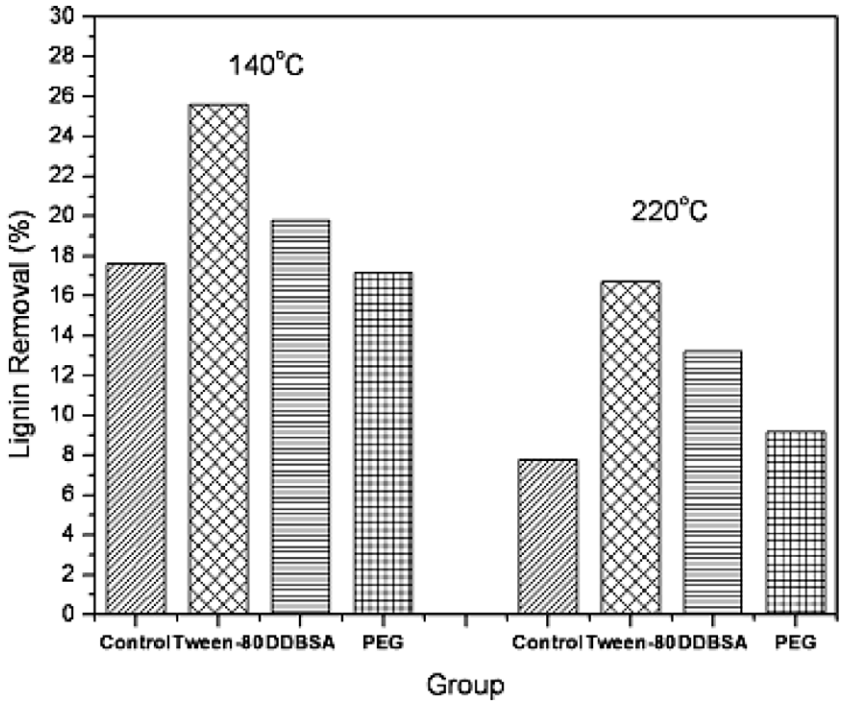

Fig. 1. Lignin removal from corn stover by different surfactants when used for 40 min with 1% sulfuric acid at 140 °C (left four bars) and with just water for 30 min at 220 °C (right four bars).

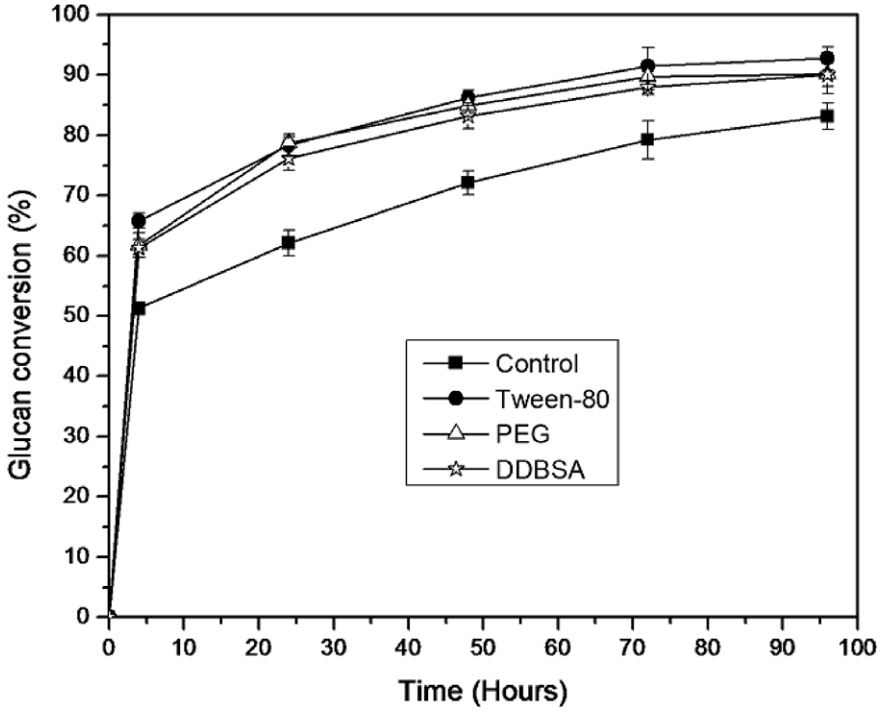

Fig. 2a. Glucan conversion at high enzyme loadings for solids produced by pretreatment of 5 wt% corn stover concentrations with surfactants and 1% sulfuric acid at 140 °C for 40 min. Enzymatic hydrolysis conditions: 50 °C, pH 4.8, 2% solids loading for 96 h total. Enzyme loading = 60 FPU/g glucan and 120 CBU/g glucan.

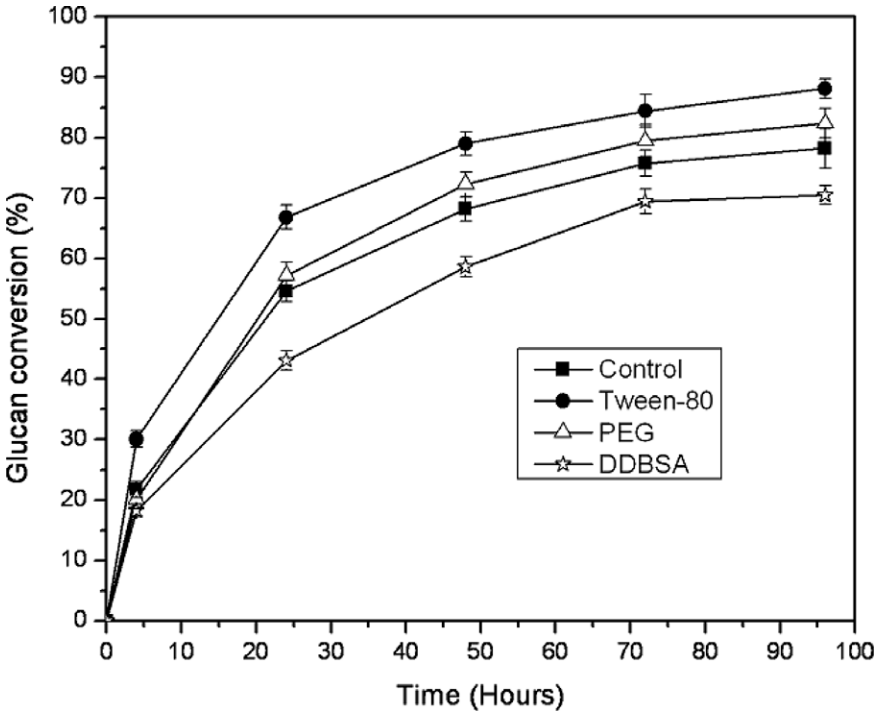

Fig. 2b. Glucan conversion at lower enzyme loadings for solids produced by pretreatment of 5 wt% corn stover concentrations with surfactants and 1% sulfuric acid at 140 °C for 40 min. Enzymatic hydrolysis condition: 50 °C, pH 4.8, 2% solids loading for 96 h total. Enzyme loading = 10 FPU/g glucan and 20 CBU/g glucan.

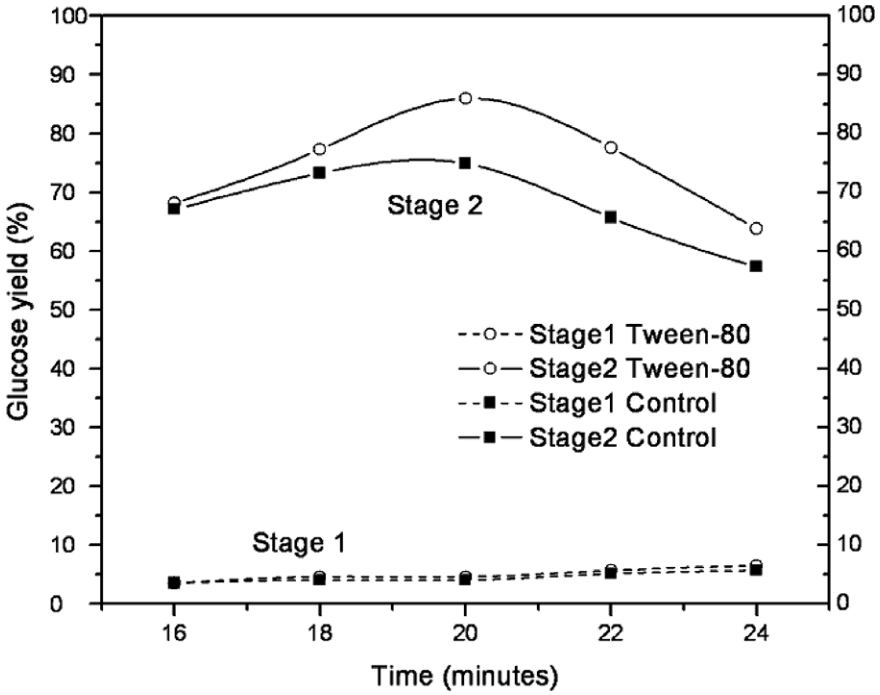

Fig. 3a. Comparison of glucose yields from dilute acid pretreatment (Stage 1) and enzymatic hydrolysis (Stage 2) with or without Tween-80 treatment. Pretreatment conditions: 160 °C with 0.5% sulfuric acid for 16–24 min. Enzyme loading = 10 FPU/g glucan and 20 CBU/g glucan. Glucose yields were calculated based on potential glucose in raw corn stover (0.37 g glucan/g of raw corn stover).

addition of surfactants was determined by the Klason-lignin method according to NREL Laboratory Analytical Procedure #003 (NREL, 1996). Lignin removal was calculated as follows:

$$\text{Lignin removal}\ (\%) = (\text{The amount of total lignin in raw biomass} - \text{The amount of total lignin in pretreated biomass/g})/\text{The total amount of lignin in raw biomass} \times 100\%.$$

3. Results

3.1. Lignin removal by surfactants

The effects of Tween-80, DDBSA, and PEG surfactants on lignin removal were evaluated for corn stover that was soaked in surfactant solution overnight prior to dilute acid (140 °C, 1% sulfuric acid

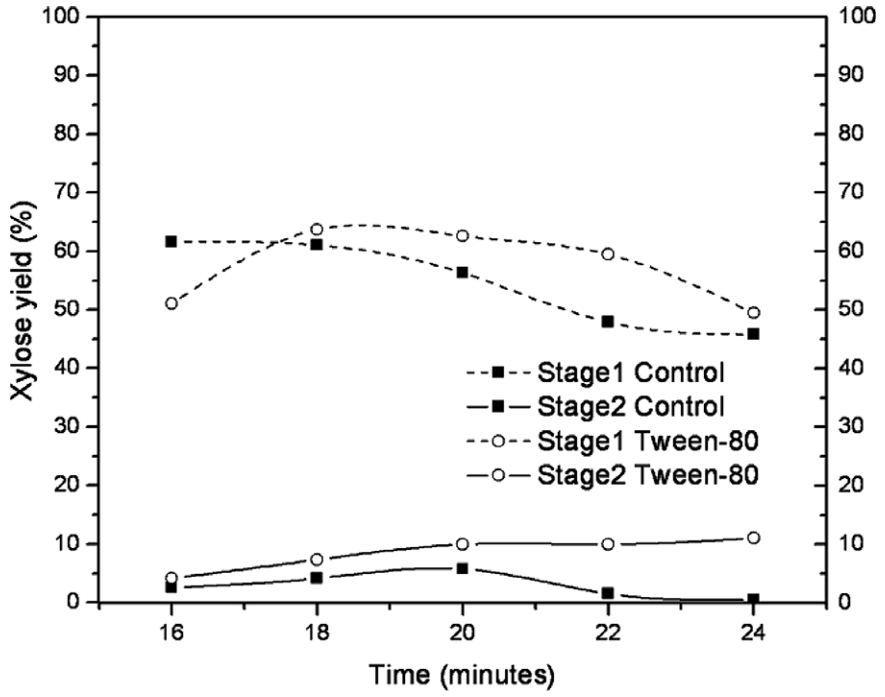

Fig. 3b. Comparison of xylose yields from dilute acid pretreatment (Stage 1) and enzymatic hydrolysis (Stage 2) with or without Tween-80 treatment. Pretreatment conditions: 160 °C with 0.5% sulfuric acid for 16–24 min. Enzyme loading = 10 FPU/g glucan and 20 CBU/g glucan. Xylose yields were calculated based on potential xylose in raw corn stover (0.19 g xylan/g of raw corn stover).

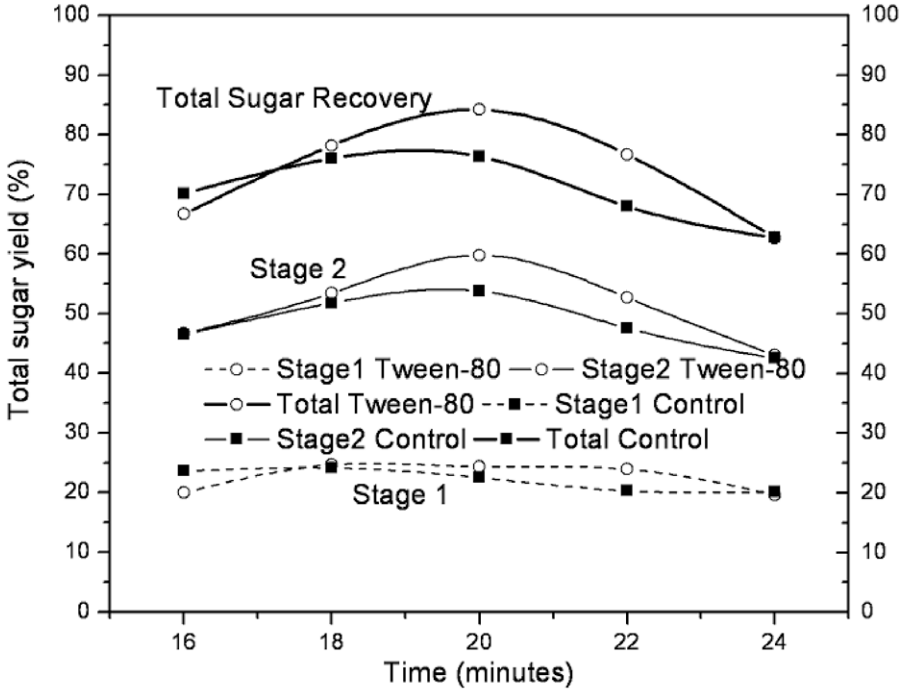

Fig. 3c. Comparison of total sugar (glucose plus xylose) yields from dilute acid pretreatment (Stage 1) and enzymatic hydrolysis (Stage 2) with or without Tween-80 addition. Pretreatment conditions: 160 °C with 0.5% sulfuric acid for 16–24 min. Enzyme loading = 10 FPU/g glucan and 20 CBU/g glucan. Total sugar yields were calculated based on potential glucose plus xylose in raw corn stover (0.56 g total sugars/g of raw corn stover).

for 40 min) or water-only (220 °C, 30 min) pretreatments. As shown in Fig. 1, lignin removal was about 17.0% and 7.8% for dilute acid pretreatment and water-only pretreatment controls, respectively, without addition of surfactants. By comparison, Tween-80 treatment increased lignin removal to 25.6% and 16.7% for dilute acid and water-only pretreatments, respectively. DDBSA treatment increased these levels to 19.8% and 13.2%, respectively. PEG showed less effect on lignin removal, resulting in equal or slightly greater lignin removal than that for the controls. Thus, for both dilute acid and water-only pretreatment, addition of Tween-80 gave

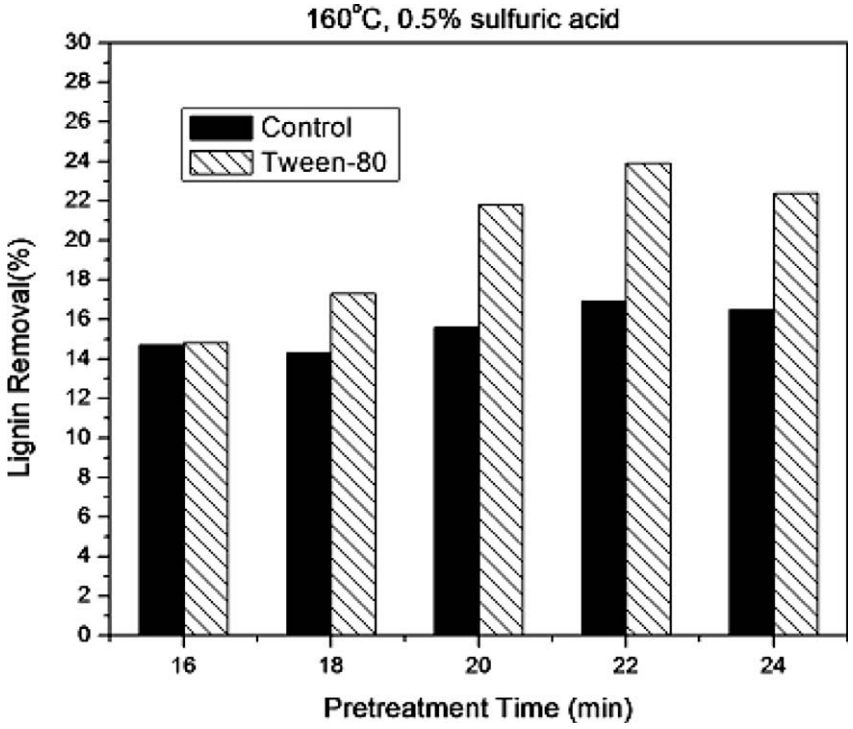

Fig. 3d. Lignin removal from corn stover with and without Tween-80 addition prior to pretreatment at 160 °C with 0.5% sulfuric acid for 16–24 min.

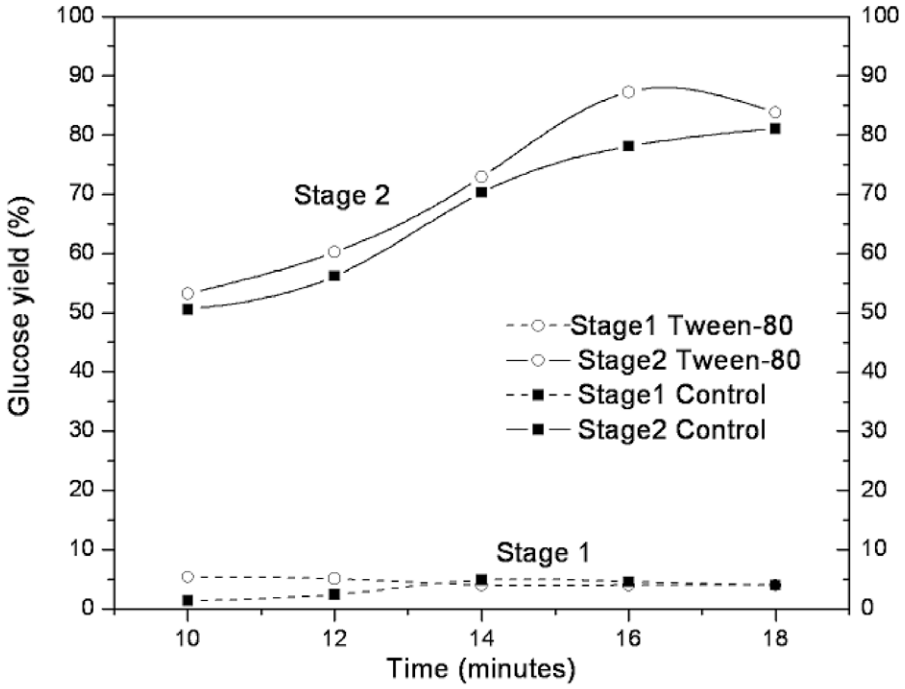

Fig. 4a. Comparison of glucose yields for water-only pretreatment at 190 °C (Stage 1) and enzymatic hydrolysis (Stage 2) with and without use of Tween-80. Enzyme loading = 10 FPU/g glucan and 20 CBU/g glucan. Glucose yields were calculated based on potential glucose in raw corn stover based on original glucan content (0.37 g glucan/g of raw corn stover).

the greatest lignin removal, enhancing it by 51.8% and 114% for dilute acid and water-only pretreatments, respectively.

3.2. Effects of surfactant pretreatment on enzymatic hydrolysis

To compare the effects of surfactants on sugar release, enzymatic hydrolysis was performed at enzyme loadings ranging from 10 to 60 FPU/g glucan for solids that had been pretreated at 140 °C with 1% sulfuric acid for 40 min with and without addition of surfactants. At a high enzyme loading of 60 FPU/g glucan supplemented with 120 CBU/g glucan, addition of Tween-80, PEG, and DDBSA prior to pretreatment improved glucan conversion to 90–93% following 96 h of enzymatic hydrolysis compared to 83.1% for the control (Fig. 2a). Differences in sugar yields from

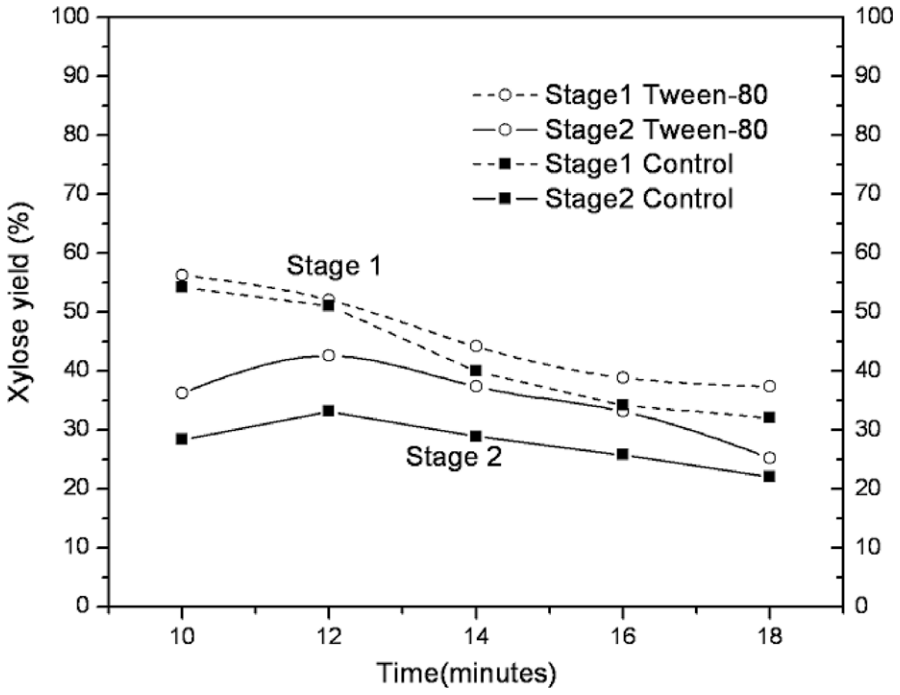

Fig. 4b. Comparison of xylose yields for water-only pretreatment at 190 °C (Stage 1) and enzymatic hydrolysis (Stage 2) with and without use of Tween-80. Enzyme loading = 10 FPU/g glucan and 20 CBU/g glucan. Xylose yields were calculated based on potential xylose in raw corn stover based on original xylose content (0.19 g xylan/g of raw corn stover).

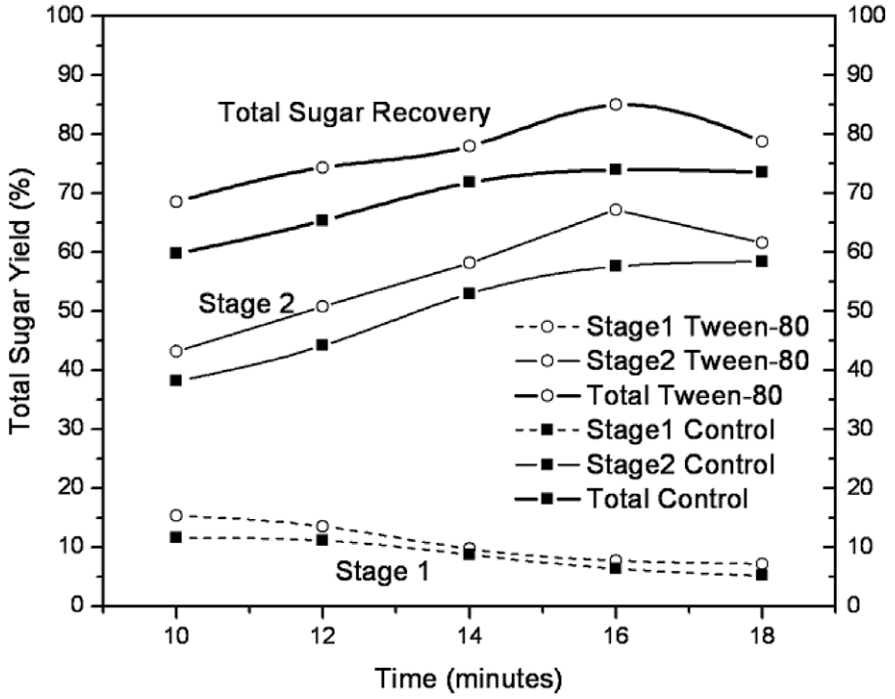

Fig. 4c. Comparison of total sugar (glucose plus xylose) yields for water-only pretreatment at 190 °C (Stage 1) and enzymatic hydrolysis (Stage 2) with and without use of Tween-80. Enzyme loading = 10 FPU/g glucan and 20 CBU/g glucan. Total sugar yields were calculated based on potential glucose plus xylose in raw corn stover based on original glucan plus xylan content (0.56 g total sugar/g of raw corn stover).

enzymatic hydrolysis of solids prepared with different surfactants were more obvious at a lower enzyme loading of 10 FPU/g glucan supplemented with 20 CBU/g glucan, as shown in Fig. 2b. In this case, Tween-80 treatment enhanced glucan conversion to 88.1% at 96 h compared to 78.2% for the control, while addition of PEG showed only a slightly higher cellulose conversion. DDBSA addition actually reduced glucan conversion compared to the control at lower enzyme loading of 10 FPU/g glucan supplemented with 20 CBU/g glucan. The enhancement in glucan hydrolysis at lower enzyme loadings for solids pretreated with Tween-80 indicated

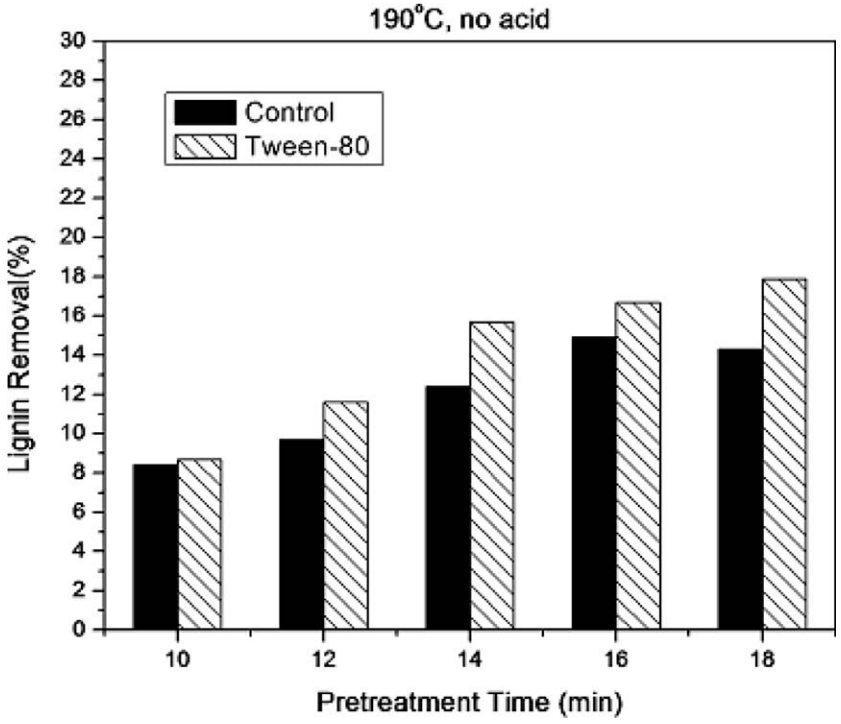

Fig. 4d. Lignin removal from corn stover with or without Tween-80 addition for pretreatment at 190 °C with just water for 10–18 min.

that surfactants could reduce enzyme use during hydrolysis, a possibly dramatic benefit in light of the high price of enzymes and comparatively low price of Tween-80 (Wingren et al., 2005; Wyman, 2007). Moreover, further optimization of Tween-80 pretreatment conditions may lower Tween-80 loadings to achieve similar benefits.

3.3. Effect of Tween-80 on sugar yields

Based on the above results, Tween-80 was selected to further investigate the effect of surfactant pretreatment on glucose and xylose yields over the course of bioconversion. Corn stover was impregnated with Tween-80 and then pretreated at a solids loading of 5% with 0.5% sulfuric acid at 160 °C or with just water at 190 °C over times ranging from 16 to 24 min and 10– 18 min, respectively, to span the time where the highest total sugar yields were expected. Figs. 3 summarizes glucose (Fig. 3a), xylose (Fig. 3b), total sugar (Fig. 3c) yields and lignin removal (Fig. 3d) for dilute acid pretreatment at 160 °C (Stage 1) with and without Tween-80 addition followed by enzymatic hydrolysis (Stage 2), at enzyme loadings of 10 FPU and 20 CBU/g glucan. Although Tween-80 did not significantly improve Stage 1 glucose yields when used with dilute acid or just water, Stage 2 glucose yields with Tween-80 pretreatment were higher than the control, especially within the time span of 20–22 min for pretreatment at 160 °C with 0.5% acid (Fig. 3a). Furthermore, addition of Tween-80 improved xylose yields in Stage 1 compared to the control, especially for pretreatment times greater than 20 min (Fig. 3b). For Stage 2, the control exhibited a decreasing trend in xylose recovery while addition of Tween-80 gave a maximum recovery at 18 min of pretreatment and greater yields than the maximum for the control between 18 and 24 min. Overall glucose and xylose yields for pretreatment with Tween-80 for 20 min were 59.8% and 24.3% (based on total sugar), respectively, and there was a 10.2% improvement over yields for pretreatment with 0.5% acid at 160 °C without Tween-80. Fig. 3c shows that the maximum total glucose plus xylose yields for Stages 1 and 2 combined was 85% at 20 min when Tween-80 was added but only 76% without adding Tween-80 for pretreatment with 0.5% acid at 160 °C. Furthermore, addition of Tween-80 resulted in higher total sugar yields from Stage 2 for dilute acid pretreatment but only slightly increased Stage 1 total sugar yields compared to the control. Lignin removal was higher for Tween-80 pretreated samples than for the controls, with the maximum gain being for pretreatment for 22 min. Moreover, lignin removal by Tween-80 became greater as pretreatment was prolonged (Fig. 3d).

For pretreatment with just water at 190 °C, Tween-80 had little effect on Stage 1 glucose yields but increased Stage 2 glucose yields significantly, as shown in Figs. 4. Furthermore, the highest Stage 2 glucose yield with Tween-80 pretreatment was 90% at 16 min of pretreatment, a 10% improvement over yields of the control (Fig. 4a). Fig. 4b shows that Tween-80 pretreatment improved xylose yields in both Stages 1 and 2 and resulted in an overall xylose yield of 73%, 13% higher than that of control. The fact that Stage 1 xylose yields did not drop much until pretreatment times were more than 12 min suggests that Tween-80 reduced xylose degradation during water-only pretreatment. Fig. 4c shows that Tween-80 increased total sugar yields from Stage 2 more than from Stage 1. Overall, the maximum total sugar yield for Tween-80 pretreatment with just water at 190 °C was 86% at 16 min, 12% greater than for water-only pretreatment without Tween-80. Fig. 4d shows that impregnation of corn stover with Tween-80 increased lignin removal for water-only pretreatment at190 °C, but the difference between Tween-80 samples and control samples was not as distinct as that for pretreatment with 0.5% sulfuric acid at 160 °C.

3.4. Comparison of the effect of Tween-80 for dilute acid and water-only pretreatments

Total sugar yields for dilute acid and water-only pretreatments at optimized conditions are summarized in Table 1. For both dilute acid and water-only pretreatment at all three tested temperatures, Tween-80 treatment improved total sugar yields in pretreatment only slightly but increased enzymatic hydrolysis yields much

Table 1
Summary of glucose, xylose, and total glucose plus xylose yields for pretreatments at different conditions with and without addition of Tween-80.

Temp. (°C)	Pretreatment time (min)	Acid (% w/w)	Glucose yield[a] (%)		Xylose yield[a] (%)		Total sugar yield[b] (%)	
			Control	T-80	Control	T-80	Control	T-80
140	40	1.0	82	90	75	86	79	89
160	20	0.5	80	89	65	73	76	85
190	16	0	83	93	60	73	74	86

[a] Total glucose or xylose yields from pretreatment and enzymatic hydrolysis stages. Enzymatic hydrolysis conditions: 50 °C, pH 4.8, solid loading = 2%, enzyme loading = 10 FPU/g glucan and 20 CBU/g glucan.
[b] Total sugar yields were calculated based on potential glucose plus xylose in raw corn stover based on original glucan plus xylan content (0.56 g total sugar/g of raw corn stover).

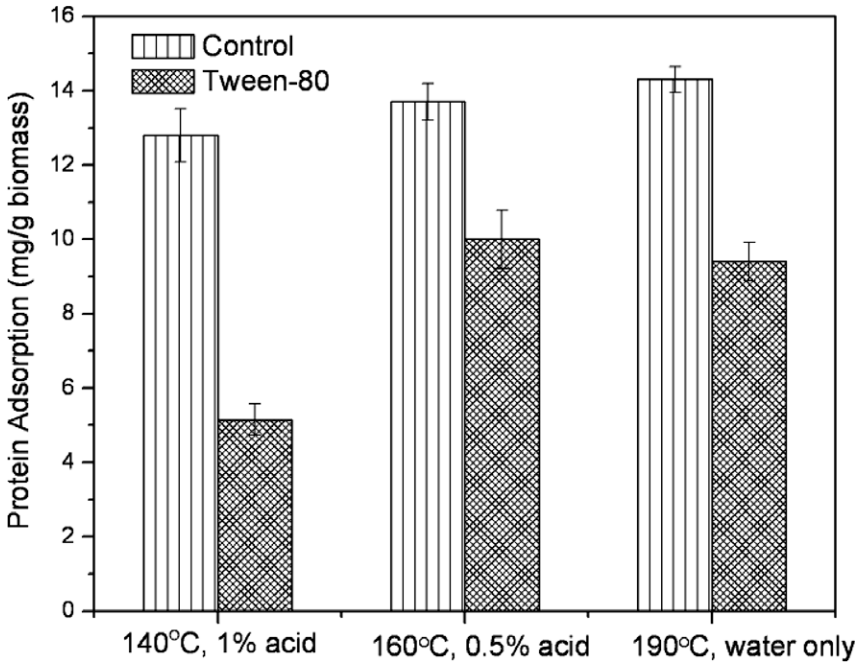

Fig. 5. Protein adsorption on biomass pretreated with and without Tween-80 at 140 °C with 1% sulfuric acid for 40 min, 160 °C with 0.5% sulfuric acid for 20 min, and 190 °C with just water for 16 min.

more. Overall, results showed that Tween-80 could increase overall glucose and xylose yields from combined pretreatment and enzymatic hydrolysis by 9–12%. Furthermore, the maximum glucose, xylose, and total sugar yields were comparable for both dilute acid and water-only pretreatment under different conditions when Tween-80 was used in pretreatment. These results suggest that adding surfactants to pretreatment could significantly reduce and possibly even eliminate acid use to achieve comparable sugar yields (Table 1).

3.5. Effect of Tween-80 on hydrophobicity and enzyme adsorption

Cellulase protein adsorption was measured for samples pretreated with just water or dilute acid at 140, 160, and 190 °C with and without addition of Tween-80 to further determine whether surfactant pretreatment made the substrate more accessible to enzymes. As shown in Fig. 5, the amount of cellulase protein adsorbed on the solids decreased by 60%, 28%, and 35% when corn stover was pretreated with Tween-80 at 140, 160, and 190 °C, respectively. These results are also consistent with enzymatic hydrolysis results that showed solids pretreated at 140 °C exhibited higher lignin removal and the best enzymatic hydrolysis performance. Hydrophobicity tests of 140 °C pretreated samples indicated that the contact angle for samples pretreated without surfactants decreased by only 2.9% compared to raw corn stover while Tween-80 pretreatment reduced the contact angle by more than 13%, as shown in Fig. 6. Thus, these results suggested that pretreatment with Tween-80 appeared to make the surface of the solids more hydrophilic.

4. Discussion

4.1. Effect of surfactants on lignin removal

Lignin is a large, cross-linked, racemic macromolecule with molecular weights in excess of 10,000 u (Boerjan et al., 2003) and is relatively hydrophobic and aromatic in nature (Freudenberg, 1968). With acid pretreatment at high temperature and pressure, lignin has been shown to condense and form so-called "lignin balls" on the pretreated biomass surface when the temperature was dropped after pretreatment (Donohoe et al., 2008; Shevchenko et al., 1999). These lignin balls were believed to adsorb enzymes during enzymatic hydrolysis and reduce enzyme mobility, thus reducing enzyme action (Berlin et al., 2006; Lu et al., 2002). Although relatively high cellulose conversion was achieved at high enzyme loadings with limited lignin removal by some thermochemical pretreatments, high yields could not be maintained at lower enzyme loadings without applying lignin-blocking techniques (Yang and Wyman Charles, 2006). Surfactants have both hydrophobic and hydrophilic properties that could decrease

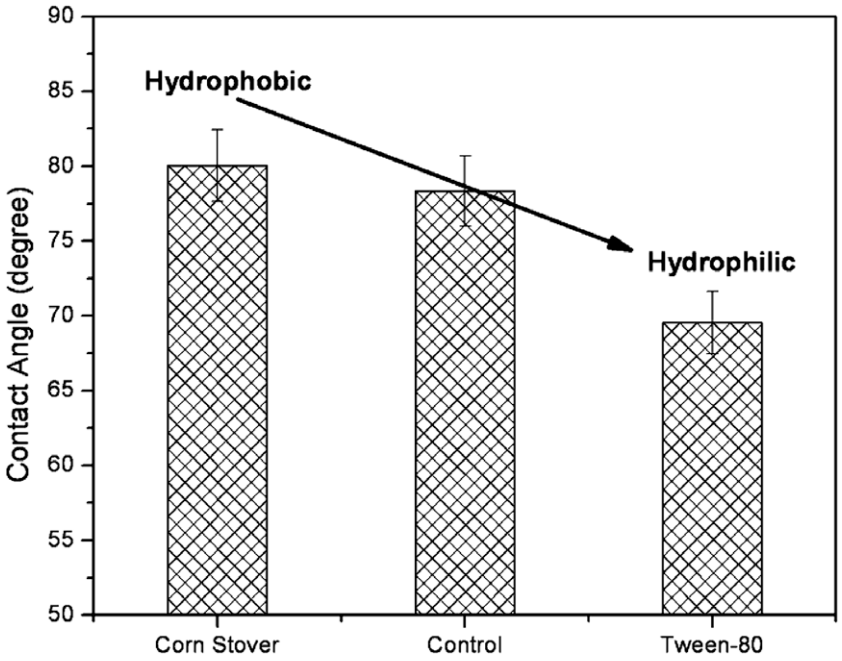

Fig. 6. Hydrophobicity of raw corn stover, corn stover solids produced by pretreatment for 40 min with 1% sulfuric acid at 140 °C, and corn stover solids produced by pretreatment for 40 min with 1% sulfuric acid plus 3% Tween-80 at 140 °C.

surface tension between two liquid phases and improve the removal of hydrophobic compounds (Escalante et al., 2005), which makes surfactants good candidates as pretreatment additives. It was reported that surfactants successively extract hydrophobic degradation products from lignin and hemicellulose, thereby enhancing lignin removal during pretreatment (Kurakake et al., 1994).

Our results showed that Tween-80, which has good surface activation properties, increased lignin removal by about 52% and 114% for batch dilute acid pretreatment (140 °C, 1% H_2SO_4) and water-only pretreatment (220 °C), respectively (Fig. 1). In addition to slightly increasing sugar yields in pretreatment, Tween-80 treatment improved both glucan and xylan yields of enzymatic hydrolysis after dilute acid or water-only pretreatment, particularly at a lower enzyme loading of 10 FPU/g glucan. These results indicate that pretreating corn stover with surfactants could remove more lignin from the solids and enhance enzymatic hydrolysis of cellulose, perhaps by forming emulsions that reduce lignin redeposition back on the biomass surface through interaction of surfactant with hydrophobic lignin during pretreatment, with the possible benefit that less lignin is left on the surface to non-productively adsorb or block enzyme during enzymatic hydrolysis.

4.2. Modification of biomass surface by surfactants

Because the performance of enzymatic hydrolysis of cellulose depends on physical and chemical characteristics of the substrate (Zhang and Lynd, 2004), structural or surface modifications introduced by surfactants during pretreatment could cause changes in the sequential enzymatic hydrolysis. Hydrophobicity tests suggested that Tween-80 pretreatment made the biomass surface much more hydrophilic, thereby making it easier for enzymes dissolved in water to access the surface. The higher lignin removal afforded by surfactants could contribute to such surface change. On the other hand, the special properties of surfactants also positively modified the solid surface resulting in improved enzymatic hydrolysis of pretreated biomass. Kim et al. (1997) studied the effect of Tween-20 on adsorption and kinetics of endoglucanase I (Endo I) and exoglucanase II (Exo II) with microcrystalline cellulose and showed that the adsorption affinity of Exo II on cellulose was weakened while desorption of Exo II from insoluble substrate was enhanced by this non-ionic surfactant. Via simulation with a kinetic model, the increase in hydrolytic performance of cellulose by Tween-20 was attributed to both activation of Exo II and partial defibrillation of cellulose (Kim et al., 1997). By measuring protein concentrations of native CBH1 and core CBH1 in the supernatant after mixing Tween-80 with ball milled cellulose (BMC), Tween-80 was found to decrease adsorption of both native CBH1 and core CBH1 onto BMC. SEM experiments revealed that Tween-80 caused cellulose filter paper to swell and enhanced surface cracks and filaments caused by native CBH I but not by core CBH I (Kim et al., 2006). It was also reported that ethylene oxide containing surfactants and polymers bonded with lignin by hydrophobic interactions and hydrogen bonding, reducing unproductive binding of enzyme (Borjesson et al., 2007). Thus, applying surfactants during pretreatment could modify the surface properties of the lignin remaining on the pretreated solids and improve cellulase effectiveness and accessibility to the surface.

It was reported that lignin in pretreated lignocellulosic biomass was responsible for a large portion of protein adsorption during enzymatic hydrolysis and blocking such non-productive adsorption of cellulases on lignin could enhance cellulose conversion (Willies, 2006; Yang and Wyman Charles, 2006). The adsorption of purified tritium labeled cellulases from *Trichoderma reesei,* CBH I (Cel7A) and EG II (Cel5A) and their catalytic domains was tested for steam pretreated softwood (SPS) and lignin (Palonen et al., 2004). Both CBH I and its catalytic domain exhibited a higher affinity for SPS than EG II or its catalytic domain, and significant amounts of CBH I and EG II also bound to isolated lignin. Surprisingly, the catalytic domain of EG II was able to absorb on alkaline isolated lignin with a high affinity, whereas the catalytic domain of CBH I did not adsorb on any of the lignin tested. These results indicated that the cellulose binding domain played a significant role in unspecific binding

of cellulases to lignin (Palonen et al., 2004). Cellulose adsorption experiments strongly supported the notion that surfactants prevent unproductive enzyme adsorption to the lignin surface (Kristensen et al., 2007). Our results suggest that less protein was adsorbed by surfactant pretreated solids even though the surface was more hydrophilic while the glucan and xylan yield was improved by surfactant pretreatment especially at lower enzyme loading. This could result from less lignin being available to non-productively bind with cellulase and/or modification of lignin made it less attractive to enzymes while the effective cellulases adsorption was increased leading to higher cellulose conversion and xylan yield during enzymatic hydrolysis.

Especially at lower enzyme loading, when enzyme to substrate ratio was low, surfactant pretreatment seemed to be more important to reaching high sugar yields. X-ray photoelectron spectroscopy (XPS) of adsorption of bovine serum albumin and Celluclast, a commercial cellulase from *Trichoderma reesei*, on particulate and fibrous celluloses showed that Tween-80 reduced enzyme adsorption and improved reaction rates at low concentrations (Gama and Mota, 1997). Our results showed that reduced cellulase adsorption on pretreated lignocellulosic biomass with surfactant treatment had a positive effect on glucose and xylose yields during enzymatic hydrolysis.

5. Conclusions

Surfactant pretreatment shows potential as a novel pretreatment method that increases both glucose and xylose yields in pretreatment and subsequent enzymatic hydrolysis compared to pretreatment without their addition. Greater removal of lignin by many of the surfactants could result in less non-productive binding of enzyme to lignin and also reduce obstacles to cellulase action. In addition, the greater hydrophobicity of the surface resulting from Tween-80 treatment, possibly due to greater lignin removal, should facilitate enzyme access to cellulose. The observation that enzyme adsorption is reduced for pretreatment with surfactants appears to be inconsistent with greater hydrophobicity making surface more accessible to enzymes but could result from changes in and removal of lignin, reducing non-productive binding of enzyme to lignin.

Acknowledgements

We gratefully acknowledge funding from Bourns College of Engineering, UC Riverside. We are thankful to Center for Environmental Research and Technology (CE-CERT) for making equipments and facilities important to this work available.

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