ORIGINAL PAPER

Cellulose solvent-based pretreatment for corn stover and avicel: concentrated phosphoric acid versus ionic liquid [BMIM]Cl

Noppadon Sathitsuksanoh · Zhiguang Zhu · Y.-H. Percival Zhang

Abstract Since cellulose accessibility has become more recognized as the major substrate characteristic limiting hydrolysis rates and glucan digestibilities, cellulose solvent-based lignocellulose pretreatments have gained attention. In this study, we employed cellulose solvent- and organic solvent-based lignocellulose fractionation using two cellulose solvents: concentrated phosphoric acid [$\sim$85 % (w/w) H_3PO_4] and an ionic liquid Butyl-3-methylimidazolium chloride ([BMIM]Cl). Enzymatic glucan digestibilities of concentrated phosphoric acid- and [BMIM]Cl-pretreated corn stover were 96 and 55 % after 72 h at five filter paper units of cellulase per gram of glucan, respectively. Regenerated amorphous cellulose by concentrated phosphoric acid and [BMIM]Cl had digestibilities of 100 and 92 %, respectively. Our results suggested that differences in enzymatic glucan digestibilities of concentrated phosphoric acid- and [BMIM]Cl-pretreated corn stover were attributed to combinatory factors. These results provide insights into mechanisms of cellulose solvent-based pretreatment and effects of residual cellulose solvents and lignin on enzymatic cellulose hydrolysis.

Keywords Biofuels · Biomass pretreatment · Cellulase inhibition · Cellulose accessibility to cellulase · Cellulose solvent and organic solvent-based lignocellulose fractionation (COSLIF) · Enzymatic cellulose hydrolysis · Ionic liquid

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Introduction

The issues of climate change associated with processing of fossil fuels and concerns pertaining to depleting fossil fuels have driven the search for sustainable and economically viable renewable energy options. Most current liquid transportation fuels are derived from crude oil. The production of cellulosic ethanol and advanced biofuels from the most abundant, low-cost, and non-food lignocellulosic biomass is anticipated to replace a significant fraction of liquid fossil fuel consumption in the future. But the largest obstacle to commercial biorefineries is cost-effective release of fermentable soluble sugars from lignocellulosic biomass, such as agricultural wastes, bioenergy crops, and woody biomass (Lynd et al. 2008; Zhang 2008). Lignocellulosic biomass is mainly composed of cellulose, hemicellulose, and lignin intertwined

together, forming a recalcitrant matrix. Biological saccharification of biomass usually involves two steps: pretreatment and enzymatic hydrolysis. Pretreatment converts recalcitrant biomass to reactive (solid) cellulosic intermediate, and cellulases (mainly) hydrolyze pretreated biomass to soluble sugars.

Maize or corn (*Zea mays* L. spp.) is one of the most important agricultural crops in the world. In 2009, nearly one-third of corn kernels in the USA were used to produce ~10.6 billion gallons of ethanol, which was blended with gasoline as a transportation fuel. Since corn kernels are mainly used as food and animal feed, production of biofuels from corn kernels negatively impacts food price and supply. Corn stover, agricultural wastes after harvesting corn kernels, is a potential biofuels feedstock and is not fully utilized. Currently only a small fraction of corn stover is used for animal feeding, barn bedding, and heating fuel. Therefore, employing corn stover would greatly increase biofuels production potential in the future without affecting food markets.

To extract soluble sugars from corn stover, it is necessary to overcome its recalcitrance to biological saccharification. Recalcitrance of lignocellulosic biomass is attributed to numerous factors: low substrate accessibility, high degree of polymerization (DP) of cellulose, presence of lignin and hemicellulose, high crystallinity, particle size, and porosity (Himmel et al. 2007). Most of these factors are correlated with substrate accessibility, which is suggested to be the most important substrate parameter impacting hydrolysis rate. Ball milling and cellulose dissolution in cellulose solvents followed by regeneration in anti-solvents can greatly increase cellulose accessibility before enzymatic hydrolysis. Although ball milling may be among the most efficient biomass pretreatment, it consumes so much energy that employing ball milling is not pragmatic for biorefineries (Chang et al. 1981; Ryu and Lee 1982). Alternatively, dissolution of cellulose in cellulose solvents followed by regeneration in anti-solvents can increase cellulose accessibility by disrupting highly-ordered hydrogen bonds in crystalline cellulose fibers (Ladisch et al. 1978; Swatloski et al. 2002). The resulting amorphous cellulose has much larger surface accessibility than that before pretreatment (Zhu et al. 2009).

Cellulose solvent-based biomass pretreatment was first developed by Ladisch et al. (Ladisch et al. 1978). They utilized Cadoxen, an alkali solution of CdO in aqueous ethylenediamine, to dissolve cellulose and biomass. The resulting regenerated cellulose from pure cellulose is hydrolyzed quickly by cellulase with 100 % cellulose digestibility, while enzymatic glucan digestibilities of pretreated biomass vary from modest to high, depending on biomass type. But toxicity and corrosiveness of cadoxen along with costly recycling technology prevents its commercialization. As such, many green cellulose solvent systems have been developed to pretreat cellulose and biomass, such as N-Methylmorpholine-N-oxide (NMMO) (Kuo and Lee 2009b), NaOH/urea (Zhao et al. 2008), and ionic liquids (Dadi et al. 2006).

Zhang and his coworkers developed a cellulose solvent- and organic solvent-based lignocellulose fractionation (COSLIF) by using a cellulose solvent followed by an organic solvent (e.g., acetone or ethanol) under modest reaction conditions (e.g., ~50 °C) to pretreat biomass and also fractionate biomass components for co-product utilization (Zhang et al. 2007). Zhang et al. (2006a) employed concentrated phosphoric acid as a cellulose solvent and found that phosphoric acid concentration beyond critical value permitted a phase transition from cellulose swelling to cellulose dissolution. Cellulose regenerated from dissolution in concentrated phosphoric acid possesses high reactivity to cellulase. Moreover, regenerated cellulose by concentrated phosphoric acid appears to be amorphous (Sathitsuksanoh et al. 2011). Consequently, regenerated cellulose by concentrated phosphoric acid is referred to as regenerated amorphous cellulose ($RAC_{H_3PO_4}$). The use of concentrated phosphoric acid as a cellulose solvent in COSLIF has been applied to a number of feedstocks, such as corn stover, switchgrass, poplar, and douglas fir (Zhang et al. 2007; Zhu et al. 2009). Although concentrated phosphoric acid-pretreated materials yield high sugar yields in short enzymatic saccharification times, a large amount of concentrated phosphoric acid usage needs to be optimized.

Similarly, another type of cellulose solvents, ionic liquids, can dissolve cellulose (Swatloski et al. 2002). A number of ionic liquids have been synthesized and employed to pretreat pure cellulose (Dadi et al. 2006; Kuo and Lee 2009a; Yang et al. 2010; Zhao et al. 2009b) and lignocellulosic biomass (Fu et al. 2010; Lee et al. 2009; Liu and Chen 2006; Nguyen et al. 2010; Singh et al. 2009; Zhao et al. 2009a). Ionic liquids, such as, 1-ally-3-methylimidazolium chloride (AMIM[Cl]),

1-ethyl-3-methylimidazolium acetate (EMIM[OAc]), 1-butyl-3-methylimidazolium chloride ([BMIM]Cl), and 1-ethyl-3-methylimidazolium diethyl-phosphate (EMIM[DEP]) have gained increasing interest as cellulose solvents in the pretreatment step (Li et al. 2009; Zavrel et al. 2009). The dissolved cellulose in ionic liquids can be regenerated with an addition of antisolvents, such as water, methanol, and ethanol. The ionic liquid-pretreated biomass exhibit reduced crystallinity, which enhances enzymatic glucan digestibility. Enzymatic hydrolysis of ionic liquid-pretreated biomass appears to depend on many factors, such as type of lignocellulose, type of ionic liquids, size of lignocellulose particles, lignocellulose loading, water contents in lignocellulose and ionic liquid, as well as dissolution conditions (Lee et al. 2009; Sun et al. 2009).

The use of cellulose solvents, such as concentrated phosphoric acid and ionic liquids, for lignocellulose pretreatment has shown great promise. By exploiting different solubilities of different lignocellulosic components in different organic solvents, lignocellulose can be fractionated for production of not only biofuels but also value-added by-products. But development of cellulose solvent-based pretreatment is still in the early stage; very little is known about how cellulose solvents interact with enzymes. Understanding dissolution mechanisms of lignocellulose by cellulose solvents would greatly enhance potential usage of cellulose solvents, optimize overall conversion process parameters, and lower usage amount of cellulose solvents and costly enzymes. In this study, we employed COSLIF using-concentrated phosphoric acid or [BMIM]Cl–to pretreat corn stover and microcrystalline cellulose (Avicel). Pretreated materials were hydrolyzed by cellulase enzymes under a low enzyme loading. The influences of dissolution mechanisms, cellulose solvents remaining, and presence of lignin on enzymatic saccharification were investigated.

Materials and methods

Chemicals and materials

All chemicals were reagent grade and purchased from Sigma–Aldrich (St. Louis, MO, USA), unless otherwise noted. Phosphoric acid (85 % w/w) and ethanol (95 % v/v) were purchased from Fisher Scientific (Houston, TX, USA). 1-*n*-Butyl-3-methylimidazolium chloride ([BMIM]Cl, 96 %) was purchased from Alfa Aesar (Ward Hill, MA, USA). The *Trichoderma reesei* cellulase (Novozyme® 50013) and *β*-glucosidase (Novozyme® 50010) were gifted by Novozymes North America (Franklinton, NC, USA). They had activities of 84 filter paper units (FPUs) of cellulase per mL and 270 units of *β*-glucosidase per mL. Microcrystalline cellulose (Avicel PH105) was purchased from FMC (Philadelphia, PA, USA). Lignin was isolated from sugar cane bagasse through Kraft pulping and NaOH treatment at 170 °C. Corn stover was procured from the National Renewable Energy Laboratory (Boulder, CO, USA). The naturally-dried corn stover was milled into small particles by a Pallmann counter-rotating knife ring flaker (Clifton, NJ) to the nominal sizes of 40–60 mesh (250–400 μm). Both Avicel and milled corn stover were stored in a sealed container at 4 °C. All feedstocks were dried in the convection oven at 105 °C over night prior to pretreatment to eliminate the effect of moisture content.

COSLIF using concentrated phosphoric acid as a cellulose solvent

Regenerated amorphous cellulose via concentrated phosphoric acid ($RAC_{H_3PO_4}$) was prepared from Avicel through cellulose dissolution in 85 % (w/w) phosphoric acid at 2 % (w/v) solid loading, followed by regeneration in water as described elsewhere (Zhang et al. 2006a). The concentrated phosphoric acid-pretreated corn stover ($CS_{H_3PO_4}$) was prepared as described previously (Zhu et al. 2009) with some modification. In short, one gram of corn stover was mixed with 8 mL of 85 % (w/w) H_3PO_4 at 50 °C, 1 atm for 30 min. The corn stover/phosphoric acid slurry was stopped by adding 20 mL of 95 % (v/v) ethanol and then mixed well. Solid–liquid separation was conducted in a swing bucket centrifuge at 4,500 rpm at room temperature for 10 min. After the supernatant was discarded, the pellets were suspended in 40 mL of 95 % (v/v) ethanol. After centrifugation, the solid pellets were washed by 80 mL of deionized water. After centrifugation, the remaining solid pellets were neutralized with 2 M sodium carbonate.

COSLIF using [BMIM]Cl as a cellulose solvent

Regenerated amorphous cellulose made by using [BMIM]Cl ($RAC_{[BMIM]Cl}$) was prepared through

Avicel dissolution in [BMIM]Cl. The dissolution condition was adopted from Zhao et al. (Zhao et al. 2009a). In short, one gram of Avicel was mixed with 20 g of [BMIM]Cl at a solid loading of ~ 5 % (w/w) on a block heater at 105–110 °C for 15 min. The hydrogel-like solution was allowed to cool, and 20 mL of water was added to precipitate the solid. After centrifugation, the supernatant was discarded. An additional 400 mL of water was used to wash residual [BMIM]Cl from the solid pellets. The [BMIM]Cl-pretreated corn stover ($CS_{[BMIM]Cl}$) was conducted by mixing 1 g of corn stover with 20 g of [BMIM]Cl at 105–110 °C for 30 min. The mixture was allowed to cool, and 20 mL of 95 % (v/v) ethanol was added to precipitate the solid. After centrifugation, the supernatant was discarded. The solid pellets were suspended by additional 160 mL of 95 % (v/v) ethanol. After centrifugation, the pellets were washed by 320 mL of deionized water to remove residual [BMIM]Cl from the solid pellets. The resulting solid pellets, denoted as $CS_{[BMIM]Cl}$, were used in the enzymatic hydrolysis experiments. It should be noted that Avicel and corn stover were added step-wise to lessen the over-heating and aggregation during dissolution.

Carbohydrate and lignin assays

The structural carbohydrate composition of corn stover was determined by the standard NREL biomass protocol (Sluiter et al. 2006). Monomeric sugars were measured by a Shimadzu HPLC with a Bio-Rad Aminex HPX-87H column (Richmond, CA, USA) equipped with refractive index (RI) detector. The concentrations of glucose and xylose were measured in enzymatic hydrolysate, whereby galactose and mannose were co-eluted with xylose. The column was operated with five mM H_2SO_4 as a mobile phase at 60 °C and a flow rate of 0.6 mL/min. Intact CS contained approximately 37.1 ± 1.8 wt.% glucan, 18.1 ± 0.6 % xylan, 1.9 ± 0.1 wt.% galactan, 3.4 ± 0.4 wt.% arabinan, and 19.0 ± 0.1 wt.% lignin.

Enzymatic hydrolysis

The concentrated phosphoric acid- and [BMIM]Cl-pretreated samples were diluted to 10 g of glucan per liter in a 50 mM sodium citrate buffer (pH 4.8) supplemented with 0.1 % (w/v) NaN_3. Concentrated phosphoric acid- and [BMIM]Cl-pretreated samples were completely suspended in the reaction media to ensure that the cellulose particle surface was accessible to the cellulases, thereby optimizing cellulase adsorption and activity. All hydrolysis experiments were carried out in a rotary shaker at 250 rpm at 50 °C. The enzyme loadings were five FPUs/g of glucan and 10 units of β-glucosidase per gram of glucan, otherwise noted.

Other assays

Total substrate accessibility to cellulase (TSAC), cellulose accessibility to cellulase (CAC), and non-cellulose accessibility to cellulase (NCAC) were determined based on the maximum adsorption capacity of the TGC protein containing a green fluorescence protein and a cellulose-binding module (Zhu et al. 2009). The recombinant TGC fusion protein was produced in *Escherichia coli* BL21 (pNT02) and purified by affinity adsorption followed by ethylene glycol washing. The scanning electron microscopic (SEM) images of the pure cellulosic and lignocellulosic materials were procured from a Zeiss-DSM940 (Carl Zeiss, Okerkochen, Germany). All samples were sputter-coated with gold prior to imaging.

Results and discussion

Corn stover was pretreated by COSLIF using concentrated phosphoric acid or [BMIM]Cl. Intact corn stover showed plant fibril structure under SEM (Fig. 1b). After two cellulose solvent pretreatments, pretreated corn stover samples did not have any fibril structure (Fig. 1c, d). Concentrated phosphoric acid- and [BMIM]Cl-pretreated corn stover samples were hydrolyzed by 5 FPU of cellulase and 10 units of β-glucosidase per gram of glucan (Fig. 1a). Corn stover pretreated by concentrated phosphoric acid ($CS_{H_3PO_4}$) was hydrolyzed fast and glucan digestibilities were 83 % after 24 and 93 % after 72 h. In contrast, the hydrolysis rates of corn stover pretreated by [BMIM]Cl ($CS_{[BMIM]Cl}$) were slower and only 55 % glucan digestibility was reached at after 72 h. Incomplete hydrolysis of pretreated corn stover might be due to combination of factors, such as residual cellulose solvents and the presence of lignin.
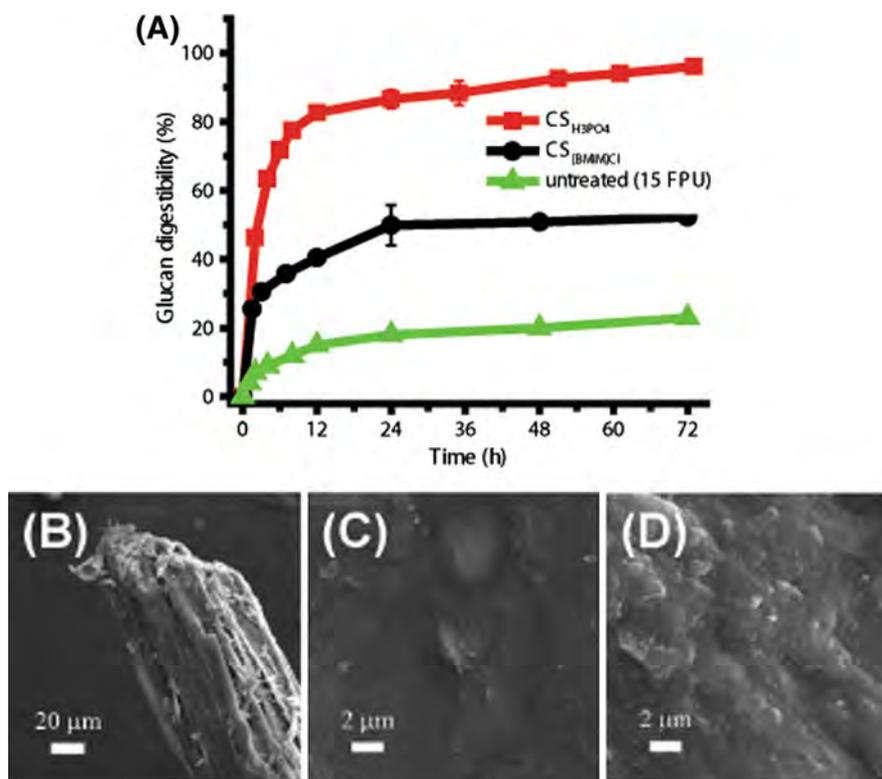
Fig. 1 Enzymatic hydrolysis profiles of intact corn stover and corn stover samples pretreated by concentrated phosphoric acid and [BMIM]Cl at an enzyme loading of 5 FPUs of cellulase per gram of glucan (**a**); SEM micrographs of intact corn stover (**b**), after COSLIF using concentrated phosphoric acid (**c**), and after COSLIF using [BMIM]Cl (**d**)

Due to the factors associated with the lignin in corn stover, it is beneficial to first investigate the isolated interaction of pure cellulose, cellulose solvents, and cellulase. Avicel (microcrystalline cellulose), a pure cellulosic material containing crystalline and amorphous cellulose, can be dissolved completely in both concentrated phosphoric acid and [BMIM]Cl (Fig. 2b, c). After cellulose dissolution, cellulose solutions appeared transparent, indicating that both cellulose solvents completely dissolved Avicel. Regenerated amorphous cellulose samples by concentrated phosphoric acid and [BMIM]Cl ($RAC_{H_3PO_4}$ and $RAC_{[BMIM]Cl}$) were hydrolyzed by 5 FPUs of cellulase and 10 units of β-glucosidase per gram of glucan (Fig. 2a). Initial hydrolysis rates of $RAC_{H_3PO_4}$ and $RAC_{[BMIM]Cl}$ (from 0 to 6 h) were almost the same, but the overall hydrolysis rates of $RAC_{[BMIM]Cl}$ were slower than those of $RAC_{H_3PO_4}$ and had a glucan digestibility of $\sim$90 % after 72 h. Complete hydrolysis was achieved for $RAC_{H_3PO_4}$ after 6 h. Higher glucan digestibilities and faster hydrolysis rates

were obtained on $RAC_{H_3PO_4}$ compared to that of $RAC_{[BMIM]Cl}$, in agreement with the previous report (Kuo and Lee 2009a). The slower overall hydrolysis rates of $RAC_{[BMIM]Cl}$ might be due to a decreased stability of cellulases in the presence of residual [BMIM]Cl. Moreover, it was noted that cellulose was dissolved in concentrated phosphoric acid at $\sim$0 °C. At this temperature, spontaneous hydrolysis of cellulose was minimized so that RAC_{COSLIF} had the same DP as that of Avicel (Zhang and Lynd 2005). At evaluated temperatures, however, cellulose dissolution was accompanied with DP decrease (data not shown).

One major drawback of cellulose solvent-based pretreatment is removal of residual cellulose solvents. It would be impractical to completely wash residual cellulose solvents out, as it requires a large amount of water. Measuring residual cellulose solvents in the pretreated solids is difficult. $RAC_{H_3PO_4}$ was hydrolyzed completely in a short time, suggesting a slight or no negative effect of residual H_3PO_4 on cellulase activities. With regards to ionic liquid-pretreated materials,

Fig. 2 Enzymatic cellulose hydrolysis profiles of Avicel, RAC$_{H_3PO_4}$, and RAC$_{[BMIM]Cl}$ (**a**); illustrations of Avicel dissolution in 85 % (w/w) H$_3$PO$_4$ (**b**) and [BMIM]Cl (**c**)
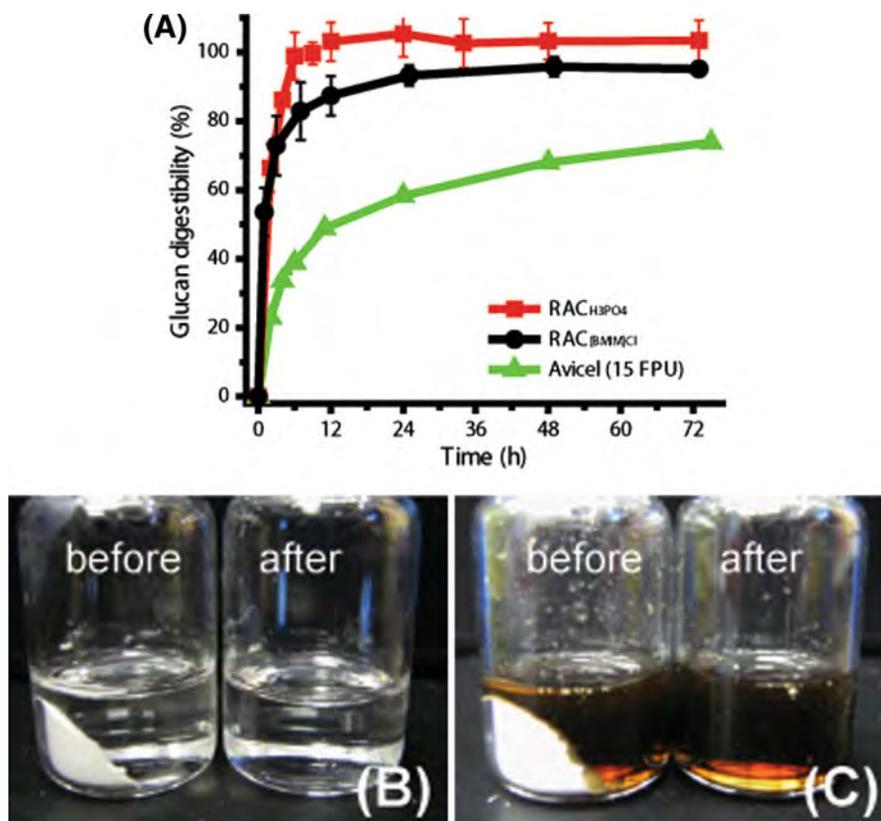

~10–15 % (v/v) of residual ionic liquid always remains in the reaction media (Engel et al. 2010). Consequently, [BMIM]Cl effects on cellulase activities from 1 to 10 g [BMIM]Cl/L were investigated on pure cellulose of different structures: microcrystalline cellulose (Avicel) and RAC$_{H_3PO_4}$ (Fig. 3). The presence of [BMIM]Cl concentrations from 1 to 10 g/L slightly decreased hydrolysis rates of RAC$_{H_3PO_4}$, but such a decrease was not statistically significant (Fig. 3a). In contrast, the presence of [BMIM]Cl had significant negative effects on enzymatic Avicel hydrolysis (Fig. 3b). Without [BMIM]Cl, enzymatic hydrolysis of Avicel had glucan digestibility of 68 % after 72 h. In the presence of 10 g/L [BMIM]Cl, the glucan digestibility was decreased to 40 %. The normalized instantaneous hydrolysis rates of RAC$_{H_3PO_4}$ and Avicel were shown in Fig. 3c, d, respectively. These decreases of instantaneous hydrolysis rates of RAC and Avicel over time might be due to decreased substrate reactivity, product inhibition, and enzyme deactivation. For RAC$_{H_3PO_4}$, different levels of [BMIM]Cl did not cause significant

decreases in hydrolysis rates (Fig. 3c), while high levels of ionic liquids resulted in significant decreases in hydrolysis rates of Avicel (Fig. 3d). Such large differences pertaining to the effect of residual [BMIM]Cl on cellulase activities on Avicel and RAC$_{H_3PO_4}$ can be explained by two major factors: (1) RAC$_{H_3PO_4}$ is a homogeneous large surface substrate while Avicel is a heterogeneous low surface substrate (Zhang et al. 2006b); and (2) different modes of action of cellulase components (endoglucanse and exoglucanase) had different sensitivity to [BMIM]Cl (Engel et al. 2010).

We hypothesized that lignin might play a synergistically negative role in lowered substrate reactivities. Negative effects of residual lignin on enzymatic cellulose hydrolysis may be attributed to (1) cellulase adsorption by lignin (Ooshima et al. 1986; Zhu et al. 2009) and (2) blockage of lignin on the surface of cellulose so that cellulase cannot access cellulose (Kumar and Wyman 2009; Pan 2008; Zhang et al. 2007). Therefore, we investigated the effects of lignin addition on enzymatic hydrolysis of pretreated

Fig. 3 Enzymatic hydrolysis profiles of RAC$_{H_3PO_4}$ (**a**) and Avicel (**b**) in the presence of increasing [BMIM]Cl as well as their instantaneous hydrolysis rates on RAC$_{H_3PO_4}$ (**c**) and Avicel (**d**)
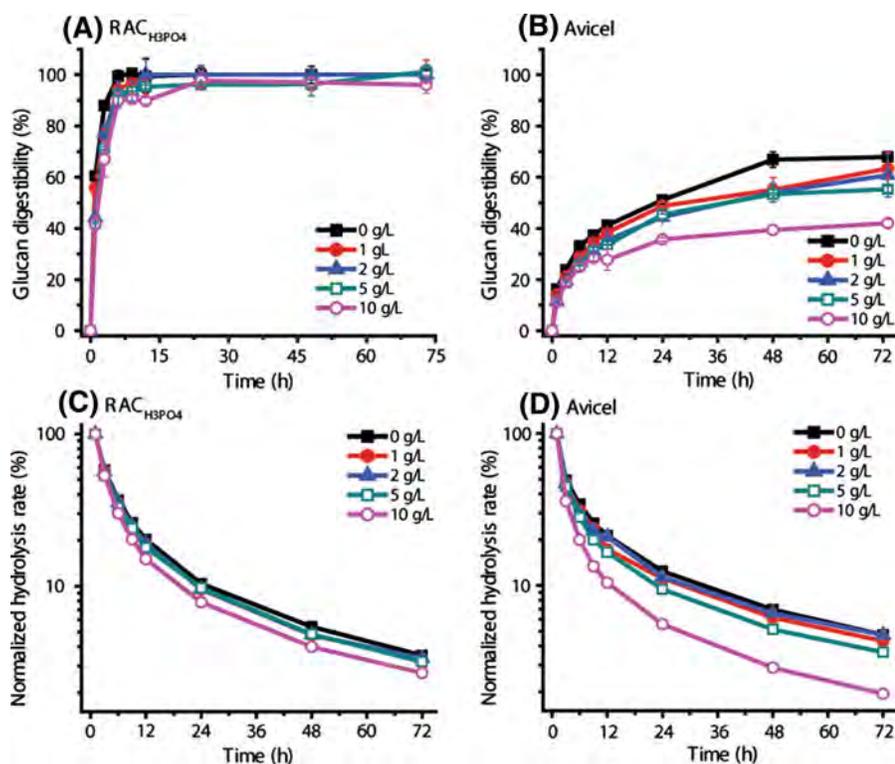
cellulosic materials. When isolated lignin was mixed with Avicel, RAC$_{H_3PO_4}$, and RAC$_{[BMIM]Cl}$ in a similar ratio present in untreated corn stover, the enzymatic hydrolysis profiles were examined (Fig. 4). It should be noted that currently there is no available lignin isolation method that does not modify lignin, so isolated lignin is commonly used to observe its influence on enzymatic hydrolysis (Nakagame et al. 2010a, b; Pan 2008). Direct addition of lignin clearly decreased glucan digestibility regardless of cellulosic substrates: Avicel, RAC$_{H_3PO_4}$ and RAC$_{[BMIM]Cl}$ (Fig. 4). Another possibility pertaining to lignin's negative effects may be due to changes of lignin property after the pretreatment. But this possibility was eliminated by the control experiments of added concentrated phosphoric acid-pretreated lignin (lignin$_{H3PO4}$) and [BMIM]Cl-pretreated lignin (lignin$_{[BMIM]Cl}$). The lignin pretreated by cellulose solvents had slightly weaker negative impacts on enzymatic digestibility than those cases of direct lignin addition (Fig. 4).

It was hypothesized that dissolved lignin during pretreatment can redeposit and redistribute on the surface of cellulose, which can block cellulose accessibility to cellulase (Donohoe et al. 2008; Selig et al. 2007; Singh et al. 2009). To test this hypothesis, we mixed isolated lignin and Avicel, followed by cellulose solvent pretreatments, obtaining two substrates of (Avicel + lignin)$_{H_3PO_4}$ and (Avicel + lignin)$_{[BMIM]Cl}$. Such pretreated cellulosic materials in the presence and absence of lignin were hydrolyzed by cellulases (Fig. 5). There were no significant differences in hydrolysis rates and glucan digestibilities between (Avicel + lignin)$_{H_3PO_4}$ and (Avicel + lignin)$_{[BMIM]Cl}$ as compared to respective RAC$_{H_3PO_4}$ + lignin$_{H_3PO_4}$ and RAC$_{[BMIM]Cl}$ + lignin$_{[BMIM]Cl}$, respectively. These results suggested that there was no significant effect due to lignin redistribution. One possible reason was both concentrated phosphoric acid and [BMIM]Cl in pretreatment enhanced cellulose accessibility to cellulase, which played a greater role in enzymatic hydrolysis performance than any specific change in the adsorption of the lignin to the enzymes (Nakagame et al. 2010a; Nakagame et al. 2010b).

Both concentrated phosphoric acid and [BMIM]Cl can dissolve cellulose and biomass well (Fig. 2), regenerated cellulosic materials appeared to have low crystallinities (Kuo and Lee 2009a; Sathitsuksanoh
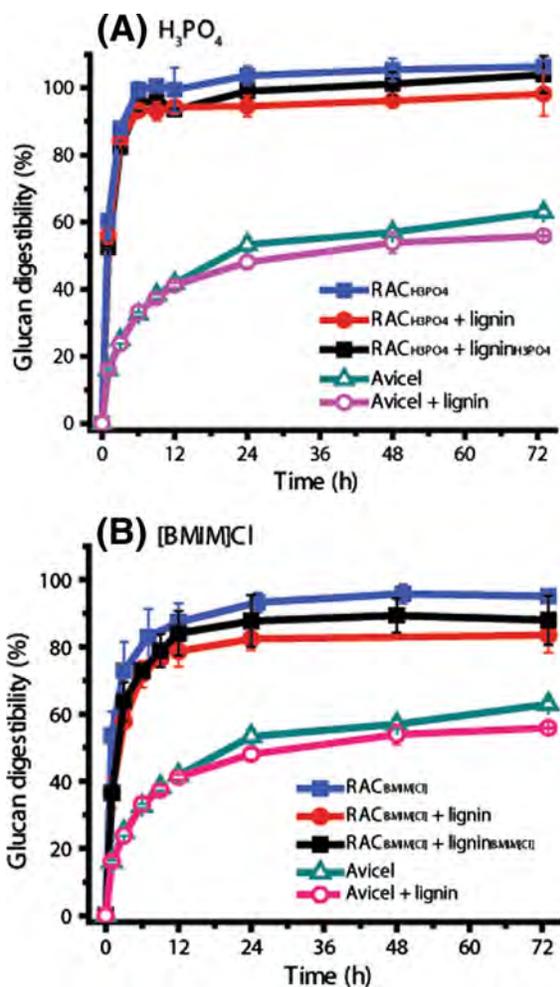
Fig. 4 Effects of isolated lignin and pretreated isolated lignin on enzymatic hydrolysis of $RAC_{H_3PO_4}$ (**a**) and $RAC_{[BMIM]Cl}$ (**b**)

Fig. 5 Effects of lignin redistribution on the surface of RAC pretreated by concentrated phosphoric acid (**a**) and [BMIM]Cl (**b**)

et al. 2011). Intact lignocellulosic biomass had a low cellulose accessibility to cellulase (CAC) of 0.42 ± 0.01 m^2/g cellulose, based on the adsorption of a fusion protein, thioredoxin-GFP-cellulose binding module (TGC). After pretreatments, $CS_{H_3PO_4}$ had the highest CAC value of 11.6 ± 0.8 m^2/g biomass, as compared to those pretreated by [BMIM]Cl and dilute sulfuric acid (DA) pretreatment (Table 1). At the same enzyme loading of 5 FPU/g glucan, enzymatic glucan digestibilities in a decreasing order were 96 % (concentrated phosphoric acid), 60 % (DA) (Zhu et al. 2009), and 55 % ([BMIM]Cl). This decreasing digestibility trend complied with the CAC values (Table 1), suggesting that CAC values were highly likely related to enzymatic glucan digestibilities.
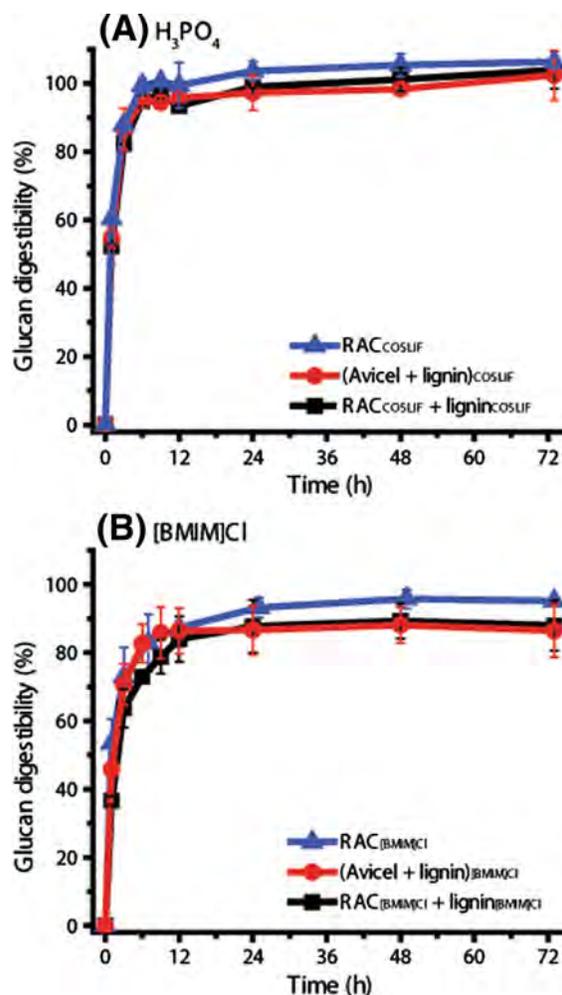
The hydrolysis rates and final digestibilities for pretreated cellulose and lignocellulosic biomass by using cellulose solvents varied greatly in the literature, depending on selection of cellulose solvent, type of biomass, and pretreatment conditions. The strategic illustration of utilization of cellulose solvents for pretreatment and saccharification is shown in Fig. 6. Solid black lines represent the preferred biomass saccharification pathway–biomass pretreatment followed by enzymatic hydrolysis. Zhang et al. (2007) suggested that COSLIF using concentrated phosphoric acid as a cellulose solvent operating at higher temperatures (>50 °C) resulted in a loss in solid weight of regenerated cellulose due to strong acid hydrolysis and/or thermal degradation of sugars.

Table 1 Total surface accessibility to celulase (TSAC), cellulose accessibility to cellulase (CAC) non-cellulose accessibility of cellulase (NCAC), and glucan digestibility after 72 h under 5 FPU of cellulase and 10 units of β-glucosidase per gram of glucan of corn stover (CS), and CS pretreated by H_3PO_4, [BMIM]Cl, and dilute acid (DA)

Materials	TSAC (m^2/g biomass)	CAC (m^2/g biomass)	NCAC (m^2/g biomass)	Glucan digestibility (%)	References
Corn stover (CS)	1.13 ± 0.01	0.42 ± 0.01	0.71 ± 0.01	23	This study
$CS_{H_3PO_4}$	14.4 ± 1.1	11.6 ± 0.8	2.9 ± 0.2	96	This study
$CS_{[BMIM]Cl}$	5.8 ± 0.3	5.0 ± 0.2	0.73 ± 0.09	55	This study
CS_{DA}	7.7 ± 0.6	5.9 ± 0.3	1.8 ± 0.6	60	Zhu et al. (2009)
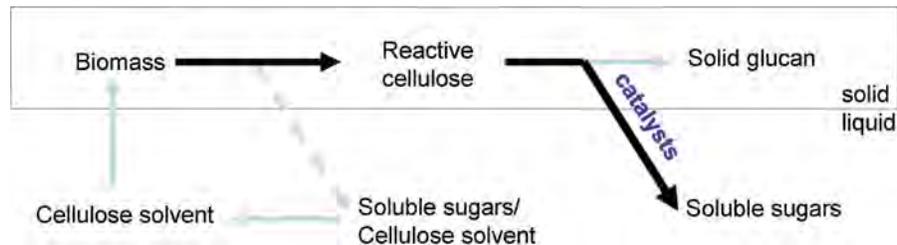
Fig. 6 Biomass saccharification strategies based on cellulose solvent followed by hydrolysis by cellulase involving the separation and recycling of cellulose solvent. The *solid black line* represents the preferred way of biomass saccharification so to avoid excessive separation of soluble sugars/cellulose solvent

Fu et al. (2010) found that when the cellulose dissolution temperature in ionic liquids increased from 90 to 150 °C, the solid weight of regenerated cellulose decreased greatly. This finding suggested that a significant fraction of cellulose was hydrolyzed at the elevated temperatures during washing step with anti-solvents. Although biomass pretreated by ionic liquids had much better enzymatic digestibility at high temperatures, net glucose yields from enzymatic hydrolysis at high temperature were lower than those at low temperatures (as shown in the present study). Consequently, development of ionic liquids that can dissolve cellulose at low temperatures (<100 °C) would be of technical and economic importance. The elevated temperature operation would present great technical and economic challenges in separation of a large amount of soluble sugars from cellulose solvents and efficient recycle of cellulose solvents (Mora Pale et al. 2011; Zhang 2008). Since it was vital to retain most cellulose as a solid substrate for the following enzymatic or acid hydrolysis, it was not recommended to dissolve biomass in cellulose solvents at high temperatures. This was to avoid auto-hydrolysis, high energy input from high temperature biomass pretreatment, high overall loss of sugars via thermal degradation, and high cost associated with separation of soluble sugars and cellulose solvents, making biorefinering processes more costly.

The use of cellulose solvents, specifically concentrated phosphoric acid and ionic liquids, for pretreatment of lignocellulose in the future industrial processes in biorefineries holds much promise, including environmental friendly, high yield of sugars over short saccharification times, and possible integrated processes for production of value-added products (Li et al. 2010; Volynets and Dahman 2011; Zhang 2008). However, the large amount of cellulose solvents usage and recycle of the cellulose solvents are major technical and economic challenges for the rapid commercialization in biorefineries (Chundawat et al. 2011). In the case of concentrated phosphoric acid, phosphoric acid is highly water soluble, and it can be easily washed from pretreated materials. Moreover, residual phosphoric acid from pretreatment and enzymatic hydrolysis in the solid material may be used as a medium nutrient for the following microbial fermentation. Furthermore, it has been proposed that the fermentation broth containing unrecovered phosphate after fermentation and distillation may be used as a fertilizer for nearby dedicated bioenergy crop

plantations. Ionic liquids with hydrophilic anions–being non volatile, non-flammable, recyclable and designer friendly–have been increasingly recognized as green cellulose solvents for dissolution and pretreatment of cellulose and lignocelulose. The choice of ionic liquids in biological saccharification of lignocellulose imposes a trade-off between biomass dissolution and enzymatic activity (Murugesan and Linhardt 2005). Some authors have reported decreased enzyme activity in the presence of ionic liquids (Docherty and Kulpa 2005; Turner et al. 2003; 2004). Datta et al. (2010) reported that a commercial endoglucanase from *Tricoderma viridie* lost its activity in the presence of small concentration of EMIM[OAc]. Complete removal of ionic liquids is not pragmatic, as it requires a large amount of water and complex recycling systems (Engel et al. 2010). Consequently, Datta et al. (2010) suggested leaving residual ionic liquids in the reaction media and developed ionic liquid-tolerant hydrophillic cellulases. Other authors employ acid-catalyzed hydrolysis of ionic liquid-pretreated cellulose (Dee and Bell 2011; Kim et al. 2010).

Currently there are no perfect cellulose solvents for pretreatment; however, cellulose solvent-based lignocellulose pretreatments offer many strategic advantages to the development of integrated biorefineries—from high sugar yields in short saccharification times to fractionation of biomass components for possible integrated co-products utilization processes. The ideal cellulose solvent for pretreatment in biorefineries should have numerous features: (1) dissolving cellulose at low temperatures, (2) dissolving wet cellulose (i.e., no biomass drying step required), (3) less costly or high recyclable, (4) less volatile for easy recycling, (5) thermostable and chemostable, (6) nontoxic to the subsequent enzymatic hydrolysis and microbial fermentation, (7) high cellulose dissolution capacity (>10 % wt. cellulose/vol), and (8) fast diffusion rate in solid lignocellulose composite (Engel et al. 2010; Klein-Marcuschamer et al. 2011; Wu et al. 2011; Zhang 2008).

Conclusions

Corn stover samples pretreated by COSLIF using concentrated phosphoric acid and [BMIM]Cl exhibited glucan digestibilities of 96 and 55 % after 72 h at

5 FPUs of cellulase per gram of glucan, respectively. Our results suggested that an incomplete hydrolysis was attributed to combinatorial factors: (1) a tradeoff between disrupting crystalline cellulose in biomass and maintaining cellulose in the solid phase; (2) residual cellulose solvent in the pretreated biomass decreased cellulase activity; and (3) residual lignin on the cellulosic materials after pretreatments decreased cellulase hydrolysis performance. Although cellulose solvent-based biomass pretreatments are receiving more interest, many obstacles require attentions, such as validating cellulose solvent recycling technologies on large scales, decreasing cellulose solvent volume use, and conducting detailed techno-economic analysis.

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