Review

Received: 7 September 2012 Accepted: 13 September 2012 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jctb.3959

New lignocellulose pretreatments using cellulose solvents: a review

Noppadon Sathitsuksanoh,[a†] Anthe George[b,c] and Y-H Percival Zhang[a,d,e*]

Abstract

Non-food lignocellulosic biomass is the most abundant renewable bioresource as a collectable, transportable, and storable chemical energy that is far from fully utilized. The goal of biomass pretreatment is to improve the enzymatic digestibility of pretreated lignocellulosic biomass. Many substrate factors, such as substrate accessibility, lignin content, particle size and so on, contribute to its recalcitrance. Cellulose accessibility to hydrolytic enzymes is believed to be the most important substrate characteristic limiting enzymatic hydrolysis. Cellulose solvents effectively break linkages among cellulose, hemicellulose and lignin, and also dissolve highly-ordered hydrogen bonds in cellulose fibers accompanied with great increases in substrate accessibility. Here the history and recent advances in cellulose solvent-based biomass pretreatment are reviewed and perspectives provided for addressing remaining challenges. The use of cellulose solvents, new and existing, provides opportunities for emerging biorefineries to produce a few precursors (e.g. monosaccharides, oligosaccharides, and lignin) for the production of low-value biofuels and value-added biochemicals.

Keywords: biofuels; biomass pretreatment and fractionation; cellulose solvent; enzymatic cellulose hydrolysis; substrate accessibility

INTRODUCTION

The production of biofuels and value-added biochemicals from evenly-distributed non-food lignocellulosic biomass would decrease net greenhouse gas emissions by replacing the use of fossil fuels and would bring benefits to rural economy, national energy security, and the balance of trade.[1,2] Additionally, it would create a large number of new biomanufacturing jobs, which cannot be outsourced, because of the high transportation costs for lower energy density biomass feedstocks compared with crude oil, coal, and corn kernels.[1,3]

Lignocellulosic biomass, the most abundant renewable bioresource, is mainly composed of three major biopolymeric components: cellulose, hemicellulose, and lignin. The interwoven linkages among biopolymers result in a natural recalcitrant composite, and this is the largest technical and economic hurdle to cost-effectively releasing fermentable sugars for biorefineries.[1,4] Two major routes convert lignocellulose into biofuels and bio-products: thermochemical and biochemical conversions. Compared with the biochemical process, thermochemical conversion has fewer processing steps and a shorter processing time but requires more energy input, i.e. lower energy efficiency. Biochemical conversion features potentially high product yields, low energy consumption, and modest reaction conditions. Both thermochemical and biochemical processes are being extensively studied. Clearly, each process will have its specific applications by considering properties and prices of diverse biomass feedstocks and products that we want to produce. In this review, we will narrow down biochemical conversion by using cellulose solvents.

Biological saccharification of lignocellulosic biomass usually involves two sequential steps: (i) pretreatment, which increases substrate reactivity for hydrolytic enzymes; and (ii) enzymatic hydrolysis, which releases soluble sugars by hydrolytic enzymes. Pretreatment usually accounts for up to 40% of the total processing cost of bioconversion of lignocellulosic biomass.[5] Moreover, pretreatment influences downstream processing costs in detoxification, enzymatic hydrolysis rate, and enzyme use, as well as product concentration and purification.[6] Consequently, an efficient pretreatment technology that affords rapid and high-digestion enzymatic saccharification is of great importance for economically sustainable biorefineries.

In spite of intensive efforts to develop low-cost commercial-available fungal cellulase, cellulase remains costly for second-generation biorefineries. The study in 2012 by the Joint BioEnergy Institute suggests that the cost contribution of current fungal cellulase to cellulosic ethanol was at least $0.68 per gallon or potentially higher.[7,8] One of the key reasons for high enzyme cost per gallon of ethanol is high ratios of enzyme to substrate, e.g. approximately 20 mg protein needed per gram of cellulosic materials, at least one order of magnitude higher

* *Correspondence to: Y-H Percival Zhang, Biological Systems Engineering Department, 210-A Seitz Hall, Virginia Tech, Blacksburg, VA 24061, USA. E-mail: ypzhang@vt.edu*

† *Present address: Joint BioEnergy Institute, Deconstruction Division, Emeryville, CA, 94608, USA*

a *Biological Systems Engineering Department, 210-A Seitz Hall, Virginia Tech, Blacksburg, VA, 24061, USA*

b *Joint BioEnergy Institute, Deconstruction Division, Emeryville, CA, 94608, USA*

c *Sandia National Laboratory, Livermore, CA, USA*

d *Institute for Critical Technology and Applied Sciences (ICTAS) Virginia Tech, Blacksburg, VA, 24061, USA*

e *Gate Fuels Inc, Blacksburg, VA, 24060, USA*

than that for starch hydrolysis.[9,10] To drastically decrease cellulase use to 2–5 mg protein per gram of glucan, mass-specific activity of cellulase can be enhanced by several approaches: improvement in individual components by directed evolution[11,12] and rational design,[13] reconstitution of non-complexed cellulase cocktails,[14,15] construction of complexed cellulases (called synthetic cellulosomes),[16–19] and cellulosomes/cellulases displayed on the surface of microorganisms.[17,20,21] However, hydrolysis performances of various cellulolytic systems from individual cellulases, non-complexed cellulase mixtures, complexed cellulases (cellulosomes), and cell-surface cellulosomes are strongly associated with substrate reactivity of pretreated biomass,[15,17,18,22,23] resulting in great challenges in finding the best match between pretreatments and available/developing cellulolytic systems. Alternatively, decreasing mass ratio of cellulase to substrate could be achieved by increasing substrate reactivity by using cellulose solvent-based biomass pretreatment so that current fungal cellulase system can work more efficiently.

Here we briefly review the key root cause of biomass recalcitrance – low cellulose accessibility to cellulase (CAC) and its influence on enzymatic hydrolysis mechanisms, as well as recent advances in cellulose solvent-based biomass pretreatments, which greatly increase cellulose accessibility more than conventional biomass pretreatments, such as dilute acid pretreatment, steam explosion, hot water.

BIOMASS RECALCITRANCE IS MAINLY DUE TO LIMITED SUBSTRATE ACCESSIBILITY TO CELLULASE

The root causes of biomass recalcitrance is attributed to a number of factors, such as substrate accessibility, cellulose degree of polymerization (DP), crystallinity, particle size, porosity, as well as hemicellulose and lignin contents.[24–26] Among these factors, substrate accessibility has shown to be the most important substrate characteristic impacting efficient enzymatic cellulose hydrolysis at low enzyme loadings.[27–32]

Classic surface accessibility methods can be used for measuring cellulose accessibility, such as nitrogen adsorption-based Brunauer–Emmett–Teller (BET),[33–35] size exclusion chromatography,[36] vapor adsorption,[37] dye adsorption,[38] small angle X-ray scattering (SAXS).[35,39] However, they are not perfectly applied to enzymatic cellulose hydrolysis process because (i) enzymatic cellulose hydrolysis occurs on the surface of hydrated solid matter in the aqueous phase (i.e. dried cellulosic samples have completely different supramolecular structures from hydrated samples);[31,33,40] (ii) cellulases are large-size molecules with a size of approximately 5 nm, much larger than nitrogen and water;[29–31] and (iii) cellulase is preferentially adsorbed on the 110 face of cellulose fibers that cellulase can hydrolyze.[41] Small-size molecule adsorption methods, such as BET and vapor, could result in overestimation of CAC.[29] Cellulase-size exclusion chromatography can neither differentiate the effective cellulose surface for adsorption and hydrolysis nor account for the external surface[31,36,42] but this method could provide an approximate estimate of CAC.

A quantitative assay for determining CAC has been established based on adsorption of a non-hydrolytic fusion protein (TGC) containing a family 3 cellulose-binding module (CBM) and a green fluorescence protein (GFP) (Fig. 1).[29] This new approach could assess substrate accessibility related to enzymatic cellulose hydrolysis more accurately than traditional methods, such as size

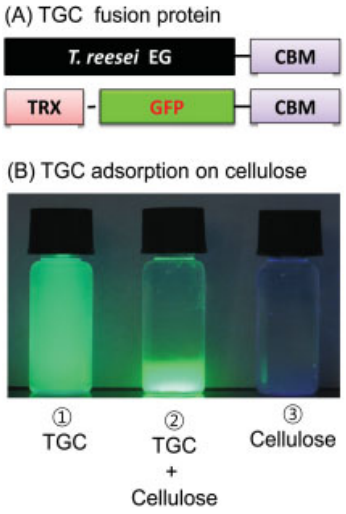


Figure 1. Schematic diagram of thioredoxin-GFP-CBM fusion protein (A). The TGC protein is similar in size to *T. reesei* EG1. Illustration of TGC (B1), TGC in cellulose solution (B2), and cellulose solution (B3) under UV excitation. TGC binds specifically on cellulose surface through CBM and fluoresces under UV excitation. This figure is modified from reference 29.

exclusion, Simon's staining technique,[43] and small angle X-ray scattering.[29] The TGC protein is similar in size to *Trichoderma reesei* EG1 (Fig. 1(A)). Under UV excitation, TGC protein fluoresces a green color (Fig. 1(B1)) while there is no color in the cellulose solution (Fig. 1(B3)). After TGC was mixed with a cellulose solution, TGC can bind on the surface of cellulose through its CBM, suggesting that TGC can specifically bind on cellulose (Fig. 1(B2)). TGC adsorption obeys the Langmuir isotherm and the CAC value can be calculated based on the maximum binding capacity of TGC in terms of $m^2\ g^{-1}$ of cellulose, where a molecule of TGC is estimated to occupy an area of 21 cellobiose lattice.[29] Zhu *et al.*[44] further applied this protein to pretreated biomass by quantitative differentiation of CAC and total substrate accessibility to cellulase (TSAC) (Fig. 2(A)). Lignin fraction can be blocked by using excess bovine serum albumin before TGC adsorption (Fig. 2(B)). Non-cellulose accessibility to cellulase (NCAC) can be calculated by taking the difference between TSAC and CAC.

Zhu and coworkers[31] compared cellulose accessibility measurements based on different-size solute exclusion and adsorption of cellulase and TGC on a set of hornified lignocellulosic substrates derived by drying the never dried pretreated sample. They found that the substrate enzymatic digestibilities of the hornified substrates were proportional to the measured cellulose accessibilities. More than 90% of the digestibility was contributed by the accessible pore surfaces of the hornified substrates, suggesting that the substrate external surface plays a minor role contributing to cellulose accessibility and digestibility.[31]

Although the belief that removing lignin can increase cellulose hydrolysis was widely accepted by most biomass pretreatment scientists, the results of Rollin *et al*. present a bigger picture for the relationship among cellulose accessibility, lignin removal, and cellulose digestibility[28] (Fig. 3). For conventional biomass pretreatments, such as dilute acid and steam explosion, which modestly increase substrate accessibility to cellulase mainly via the removal of hemicelluloses,[33,45–48] removing lignin clearly increased enzymatic hydrolysis digestibility (Fig. 3). However, when substrate accessibility is increased greatly by using cellulose solvents, such as cellulose solvent and organic solvent lignocellulose fractionation

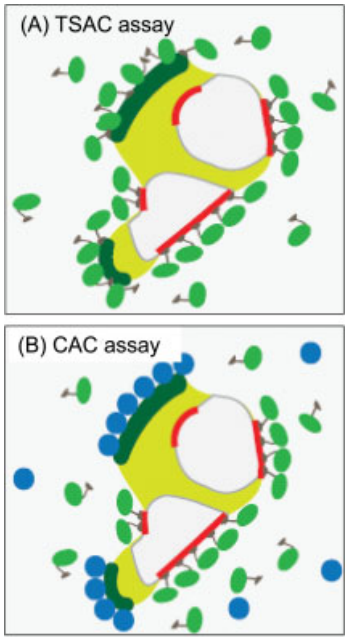


Figure 2. Illustrations of adsorption mechanism of TGC. To determine total substrate accessibility to cellulase (TSAC), TGC equilibration is conducted without BSA (A). When BSA blocking is used prior to TGC equilibration, cellulose accessibility to cellulase (CAC) can be determined (B). Cellulose (110) planes susceptible to cellulase binding are highlighted in red. This figure is reprinted from reference 28.

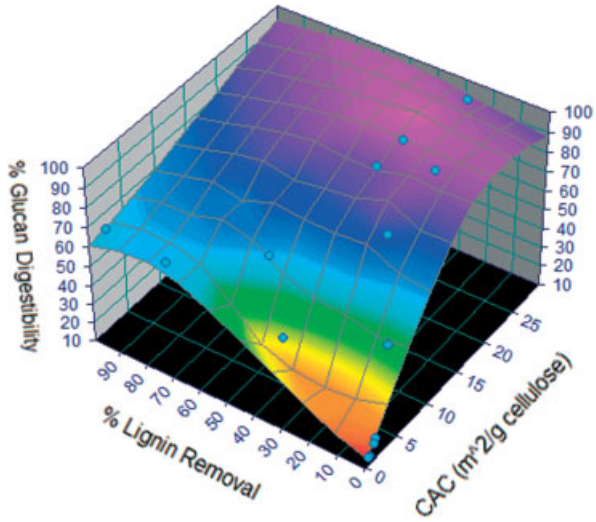


Figure 3. Digestibility as a function of delignification and CAC. This figure is reprinted from reference 28.

(COSLIF), removing lignin has a limited benefit to enhance the digestibility of high-accessibility pretreated biomass (Fig. 3). The above result clearly suggests that increasing substrate accessibility may be more important than removing lignin. The bottom line is whether removing lignin is important or not depends on whether we can increase substrate accessibility significantly.

The study of enzymatic hydrolysis for low-accessibility microcrystalline cellulose (Avicel) and high-accessibility regenerated amorphous cellulose (RAC) clearly presents different hydrolysis mechanisms for a non-complexed cellulase mixture (Fig. 4). Avicel is a typical heterogeneous substrate; its glucan chains are aligned in the same direction, and highly ordered hydrogen bonds among adjacent sugar chains result in low surface accessibility to cellulose.[15] In contrast, RAC is a homogeneous amorphous cellulose, whose highly ordered hydrogen bonds in the cellulose chains are disrupted through cellulose dissolution in concentrated phosphoric acid and regeneration in water,[40,49] its surface area is at least 20 times higher than that of Avicel based on the adsorption of TGC.[15,50] Avicel hydrolysis by the cellulase mixture was a typical peeling or layer-by-layer hydrolysis process (Fig. 4(A)). The ends of β-glucosidic bond on the surface of Avicel generated by adsorbed endoglucanase cannot be hydrolyzed by exoglucanase until endoglucanase moves elsewhere and exoglucanase moved to the reducing ends. For low-accessibility Avicel, the ends of cellulose chains are limited to exoglucanase.[18,32,51,52] High-accessibility RAC allows most endoglucanase to efficiently and rapidly hydrolyze substrate, resulting in a rapid decrease in DP at the beginning when limited liquefaction occurs[53] (Fig. 4(B)). As a result, the reducing and non-reducing ends of RAC are in excess to exoglucanase. Therefore, each cellulase component in the non-complexed cellulase mixture works independently so that synergy between exoglucanase and endoglucanase is not vital to complete hydrolysis of RAC.[15,18]

In a word, effectively increasing biomass accessibility via cellulose/biomass dissolution in cellulose solvents very effectively overcomes biomass recalcitrance to hydrolytic enzymes at low enzyme loadings. Therefore, the use of cellulose solvents from biomass pretreatment could be very promising in future biorefineries.

CELLULOSE SOLVENTS AND THEIR APPLICATIONS IN BIOMASS SACCHARIFICATION

Cellulose solvent-based lignocellulose pretreatments have gained more and more attention because they can break biomass recalcitrant structure by increasing cellulose accessibility more effectively than traditional biomass pretreatments (e.g. steam explosion,[54] AFEX,[22] soaking in aqueous ammonia (SAA),[28,55] dilute acid pretreatment,[44] organosolv[56]). As a result, hydrolysis rate and digestibility of pretreated biomass are increased and enzyme use decreased.[57–59] Also, cellulose solvent-based pretreatments may be regarded as a biomass-independent pretreatment.[59] As shown in Fig. 5, the fibril structure of switchgrass was completed disrupted by concentrated phosphoric acid and an ionic liquid [C_2mim][OAc].

Crystallinity index (CrI) of switchgrass before and after cellulose solvent-based pretreatment can be determined by X-ray diffraction (XRD) and cross polarization/magic angle spinning (CP/MAS) ^{13}C nuclear magnetic resonance (NMR).[26,60] CrI values vary greatly depending on measurement techniques, calculation approaches, and sample drying conditions, suggesting that the effects of CrI data obtained from dried samples on enzymatic hydrolysis of hydrated cellulosic materials should be interpreted with caution.[40] The CrI values of COSLIF- and [C_2mim][OAc]-pretreated swichgrass determined by XRD are 3.2 and 2.6%, respectively, compared with 67.0% of non-pretreated swichgrass (Table 3). The 20.9- and 25.8-fold reductions in CrI values of COSLIF- and [C_2mim][OAc]-pretreated switchgrass are accompanied with significant enhancement of enzymatic glucan digestibility (> 90%) in 24 h (data not shown). Here we would like to urge that decreasing CrI of biomass is not a root cause for enhanced digestibility and this inverse correlation between CrI and digestibility is sometimes

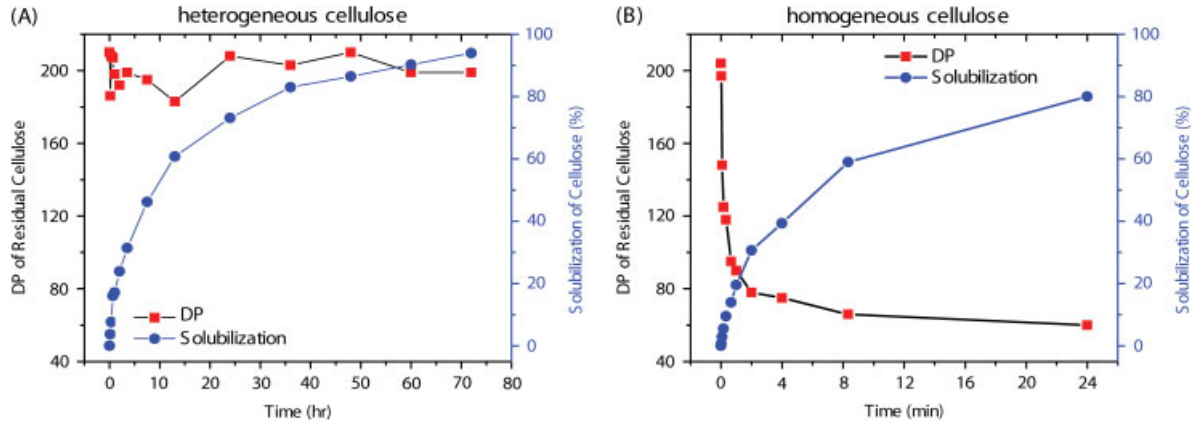


Figure 4. Profiles of enzymatic hydrolysis of Avicel (A) and regenerated amorphous cellulose (RAC) (B). Enzymatic cellulose hydrolysis was carried out at 50°C using a 50 mmol L^{-1} citric acid buffer (pH 4.8) in a rotary shaker at 200 rpm. For Avicel, hydrolysis was carried out at 10 g L^{-1} Avicel with an enzyme loading of 15 FPU Spezyme cellulose g^{-1} Avicel, supplemented with 60 IU cellobiase g^{-1} Avicel. For RAC, hydrolysis was carried out at 5 g L^{-1} RAC with an enzyme loading of 0.5 FPU g^{-1} RAC. This figure is modified from reference 53.

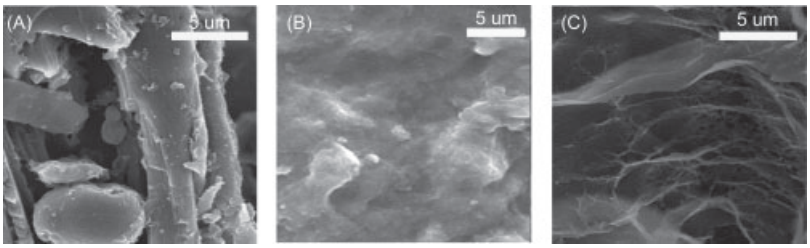


Figure 5. SEM micrographs of intact switchgrass (A), COSLIF-pretreated switchgrass (B), and [C_2mim][OAc]-pretreated switchgrass (C). This figure is modified from references 40 and 76.

due to a coincident relation between substrate accessibility and CrI. A good exception is that ammonia-pretreated biomass has both increases in CrI value and enzymatic digestibility.[26,61]

A history of applications of cellulose solvents in biomass hydrolysis and pretreatment may be categorized into three generations:

1 First generation: one step biomass dissolution and hydrolysis.
2 Second generation: biomass dissolution followed by enzymatic hydrolysis.
3 Third generation: lignocellulose fractionation.

First generation

Concentrated acids (e.g. sulfuric acid, hydrochloric acid, and nitric acid) have long been known as good cellulose solvents as well as hydrolysis agents (Pereira *et al*., 1988).[62] Their hydrolysis ability associated with sugar degradation could be minimized by decreasing cellulose dissolution temperature. High cellulose conversion yields are usually reported, such as the Bergius process,[62] but not for hemicelluloses.[63] Another advantage of using concentrated acids is that it is biomass-independent, and can be applied to a wide range of feedstocks (herbaceous, hardwood, and softwood)[35] Currently several companies, such as BlueFire Renewables (USA) and Virdia (USA), employ concentrated acid-based biomass saccharification technologies. However, these approaches have three major technical and economic hurdles: (i) soluble acid/soluble sugar separation, (ii) acid recovery, and (iii) acid re-concentration (Table 1).[64] To address such challenges, biomass pretreatment followed by enzymatic hydrolysis becomes an alternative approach, because it retains most cellulose as a solid substrate so that solid substrate is easily separated from the cellulose solvent.[65] Limited hydrolysis in cellulose solvents can avoid the degradation of labile sugars (e.g. hemicellulose)[66] but requires costly cellulase input (Table 1).

Second generation

Overcoming lignocellulose recalcitrance by using non-hydrolytic cellulose solvents followed by enzymatic hydrolysis was first proposed by Ladisch and Tsao in 1978.[67] After searching for a number of cellulose solvents, Cadoxen, an alkali solution of CdO in aqueous ethylenediamine, was found to dissolve dry biomass. The resulting cellulose regenerated from pure cellulose can be hydrolyzed quickly in high yields by cellulose,[67] but glucan digestibility was modest for pretreated biomass. Because Cadoxen is corrosive and toxic, a trace amount of the solvent in the pretreated biomass could inhibit subsequent hydrolysis and fermentation steps. Consequently, this technology's world patent was given up by their inventors long before its patent expiration date.

Third generation: lignocellulose fractionation

Considering a very narrow margin between sugars (e.g. approximately 30 US cents per kg of sugars) and feedstocks (e.g. $60–100 per ton of biomass, containing approximately 600 kg of sugars), it is economically important to fractionate natural composite biomass for its co-utilization.[1] The use of cellulose solvents along with other solvents that can dissolve different lignocellulose components enables the fractionation of lignocellulosic components under modest reaction conditions.[1,68] A few cellulose solvent-based strategies are being developed, such as concentrated phosphoric acid (85% (w/w)), ionic liquids, NMMO, NaOH/urea, and DMAc/LiCl.

Table 1. Two main approaches for saccharification of lignocellulose

Approaches	Advantages	Disadvantages
Acid saccharification (i.e. H_2SO_4, HNO_3 and HCl)		
	nearly theoretical yield of cellulose	separation of sugars and acids
	Good for all lignocellulose	acid recovery
	elevated temperatures for diluted acid	modest yield of hemicellulose
	low temperatures for concentrated acids	high investment cost for corrosion-resistant equipmentacid re-concentration
Enzymatic cellulose hydrolysis after pretreatment		
	mild reaction for enzymatic hydrolysis	pretreatment required
		high cellulase cost and long reaction time
		low or modest yields of cellulose and hemicellulose

CONCENTRATED PHOSPHORIC ACID AS A CELLULOSE SOLVENT

Cellulose solvent- and organic solvent-based lignocellulose fractionation (COSLIF) was developed to fractionate lignocellulose using a combination of concentrated phosphoric acid as a cellulose solvent and an organic solvent (e.g. acetone or ethanol) under modest reaction conditions.[68] The key ideas of COSLIF are (i) removal of partial lignin and hemicellulose (i.e. eliminating the major obstacles to hydrolysis and allowing cellulase to access the substrate more efficiently),[6,69] (ii) de-crystallization of cellulose fibers (i.e. providing better cellulose accessibility to cellulase),[32,49] and (iii) modest reaction conditions (i.e. a decrease in sugar degradation, less inhibitor formation, lower utility consumption, and less capital investment).[69] Some studies have shown that concentrated phosphoric acid can completely dissolve cellulose fibers, resulting in effective disruption of highly ordered hydrogen bonding network of crystalline cellulose[40,70] and drastic increases in CAC.[28,44]

COSLIF has been demonstrated to efficiently pretreat a wide range of feedstocks, such as bamboo,[57] bermudagrass,[71] common reed,[58,71] corn stover,[44] gamagrass,[72] giant reed,[73] elephant grass,[73] sugarcane,[73] hemp hurd,[69] Miscanthus,[59] poplar,[59] switchgrass.[40] Different species of untreated biomass feedstocks show a large variation in their glucan digestibilities at 15 filter paper units (FPUs) of cellulase per gram of glucan, reflecting their different recalcitrant degrees (Fig. 6). However, all of the COSLIF-pretreated biomass feedstocks have similar high digestibilities (>87%) after 72 h at an enzyme loading of 5 FPUs of cellulase per gram of glucan (Fig. 6). Therefore, COSLIF could be regarded as a feedstock-independent pretreatment. Because of the high cost of current fungal cellulase (i.e. approximately 100 US cents) per gallon of cellulosic ethanol, 3–5-fold reduction in cellulase use means up to 80 cents saving per gallon of ethanol produced.[57] The COSLIF technology is being tested in a pilot plant by Optafuel in southern Virginia (USA).

Figure 7 shows a correlation between CAC values of numerous feedstocks before and after pretreatment and enzymatic glucan digestibility. Untreated biomass feedstocks with different carbohydrate and lignin contents[66] have low CAC values, resulting in low enzymatic glucan digestibility (lower than 20%). An exception is bagasse possibly because it was prepared through leaching, drying, followed by milling that may disrupt biomass fiber more efficiently than other untreated feedstocks through simple particle size reduction. Note: energy-intensive milling is a very efficient biomass pretreatment for increasing substrate accessibility but is too costly.[34] After pretreatments, such as dilute acid, SAA, and lime, pretreated biomass samples have enhanced CAC values, accompanied by enhanced glucan digestibility. This correlation between CAC and digestibility suggests that increasing substrate accessibility for most pretreatments is important for achieving enhanced enzymatic glucan digestibility. When CAC values are higher than a critical value of 8 $m^2\ g^{-1}$ biomass, very high glucan digestibilities were obtained. In these cases, digestibilities were independent of CAC values, suggesting further enhancement of CAC higher than the critical value was not important. Although COSLIF very effectively overcomes lignocellulose recalcitrance, a large volume of cellulose solvents and organic solvents are employed so that process modification and optimization must be conducted to make the whole process economically attractive.

IONIC LIQUIDS (ILs) AS CELLULOSE SOLVENTS

ILs are organic salts that are liquids at low temperatures. Many ILs are liquid even at room temperature. Because of their low volatility, they are often regarded as a green solvent in organic synthesis. A combination of various cations and anions gives a great possibility to design ILs meeting different needs. After intensive study, it is found that ILs having imidazolium or pyridinium cations paired with Cl^-, $CF_3SO_3^-$, $CF_3CO_2^-$, $CH_3CO_2^-$, $HCOO^-$, $R_2PO_4^-$ anions are able to dissolve cellulose fibers through strong hydrogen bond basicity. The dissolution of lignocellulose in ILs disrupts the primary bonds among cellulose, hemicellulose and lignin, yielding more substrate accessibility to hydrolytic enzymes.[74] With suitable choice of anti-solvents (e.g. water, acetone, and alcohol), up to 80% lignin and hemicellulose can be fractionated.[75–77] A few ILs that have been employed for biomass pretreatment and fractionation are shown in Table 2. More details on the use of ionic liquid in biomass can be found elsewhere.[78–83] Comparative studies among IL pretreatment, dilute acid, and ammonia fiber explosion[76,84] show that [C_2mim][OAc]-pretreated biomass was hydrolyzed more rapidly and higher glucan digestibility was obtained.[80,82]

The choice of ionic liquids imposes a trade-off between biomass dissolution and biological hydrolysis.[85] Most ionic liquids are toxic to hydrolytic enzymes.[86–88] Datta *et al*.[89] found that a commercial endoglucanase from *Tricoderma viridie* lost its activity in the presence of low concentration [C_2mim][OAc]. However, complete removal of ILs is nearly economically infeasible because it requires the consumption of a large amount of water or anti-solvent, complete mixing, and complex recycling systems.[90] Consequently, some researchers have developed a more stable cellulase cocktail in the presence of ILs.[91,92]

A small amount of catalyst may be added in IL-based pretreatment for better fractionation or conversions. Diedericks

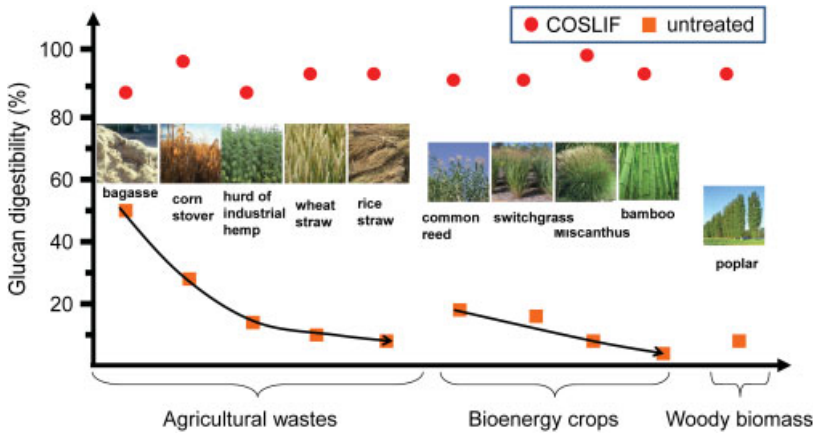


Figure 6. COSLIF is a biomass-independent technology. Different types of feedstocks – agricultural wastes, bioenergy crops, and woody biomass – have different degrees of recalcitrance. After COSLIF, pretreated biomass has high glucan digestibility at low enzyme loading. This figure is modified from reference 59.

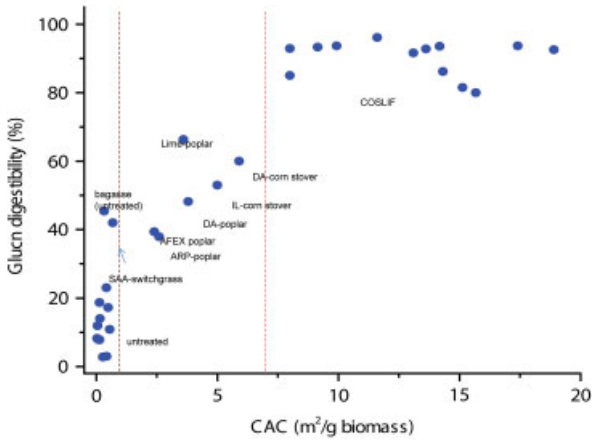


Figure 7. Correlation between CAC and glucan digestibility at 72 h from various pretreated substrates.

et al. investigated the use of 1-butyl-3-methylimidazolium methylsulfate ([BMiM]$MeSO_4$) plus an acid catalyst (i.e. H_2SO_4) on sugarcane bagasse.[93] The use of an acid catalyst contributed to a more digestible solid and a higher degree of delignification. However, the [BMiM]$MeSO_4$-H_2SO_4 combination failed to produce a fully digestible solid and a maximum cellulose digestibility of 77% (w/w) was obtained at the optimum pretreatment condition of 125°C for 2 h. Furthermore, up to half of the lignin content could be extracted during pretreatment and nearly complete removal of xylan.

Another biomass fractionation is based on 1-butyl-3-methylimidazolium chloride followed by precipitation in acetone/water (9:1, v/v) and extraction with 3% NaOH solution.[94] The ionic liquid was easily recycled after concentration and treatment with acetonitrile. Bagasse was fractionated using this method to 36.8% cellulose, 26.0% hemicelluloses, and 10.5% lignin, accounting for 47.2 and 33.9% of the original polysaccharides and 54.6% of the original lignin, respectively.

Enzymatic hydrolysis of pretreated biomass is preferred at high solid loading because it decreases capital investment and avoids energy-intensive sugar re-concentration.[95] A recent study shows an increase in biomass loading up to 50 wt% in [C_2mim][OAc] without compromising the sugar yields and enzymatic hydrolysis rates.[96] Up to four or five time recycled ILs maintain their ability to dissolve biomass.[97–100] Moreover, recycled ILs containing high level solubilized lignin can be separated as a raw material in the production of polymeric materials and liquid hydrocarbons.[1,98,101]

N-METHYL-MORPHOLINE-*N*-OXIDE (NMMO)

NMMO is used industrially in the Lyocell process to produce cellulose fibers from dissolving pulp.[102] In it, NMMO dissolves cellulose fibers due to its high polarity N–O bond, which breaks the hydrogen bond network of the cellulose and forms new hydrogen bonds with the solute. Since NMMO is a strong oxidant, an antioxidant, such as propyl gallate is added in the Lyocell proess to stabilize the cellulose/NMMO mixture.[103,104] Since lignin has been shown to be a radial scavenger and antioxidant, lignocellulose can be pretreated in NMMO directly. Recent studies have shown the potential of NMMO for pretreating pure cellulose,[105] sugarcane bagasse,[106] spruce,[107] oak,[107] rice straw,[108] and poplar.[108] Shafiei *et al*.[107] used 85% (w/w) NMMO to pretreat oak and spruce at 90–130°C and ambient pressure for 1–3 h. They found that NMMO-pretreated oak and spruce yielded enzymatic glucan digestibilities of 64.6% and 83.5%, respectively.

UREA/NaOH

The NaOH/urea solutions were found to dissolve cellulose at a subzero temperature for the homogeneous synthesis of cellulose derivatives.[106,109–111] Recently, the NaOH/urea solution was applied to pretreat lignocellulose. Spruce pretreated by NaOH/urea showed slight removal of cellulose, hemicellulose, and lignin while a significant increase in enzymatic glucan digestibility was obtained.[106] However, it may be too costly to prepare pre-chilled NaOH/urea and recycle this solution, especially in the case of biomass pretreatment that is used to produce low-value biocommodities. For example, NaOH-based pulping used to cause serious water pollution in China, and has been abandoned. Note: pulp is several times more valuable than ethanol.

N,*N*-DIMETHYLACETAMIDE (DMAc)/LiCl

DMAc/LiCl solution can dissolve cellulose[112] because hydrogen bonding of the hydroxyl protons of cellulose with the chloride ions allows the solvent to penetrate into cellulose fibers. DMAc/LiCl

Table 2. Selected ionic liquids (RTILs) used in biomass pretreatment

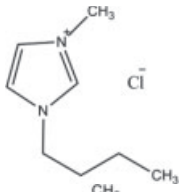
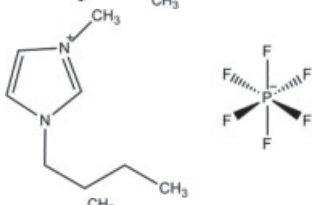
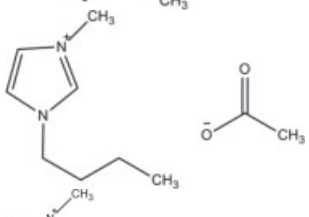
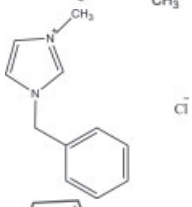
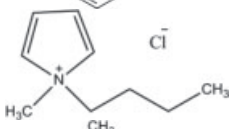
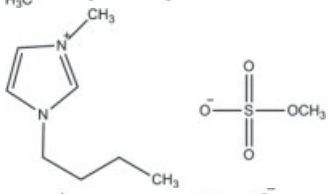
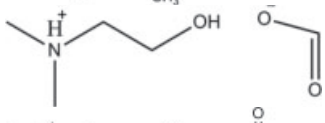
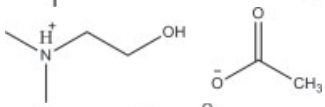
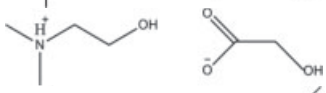
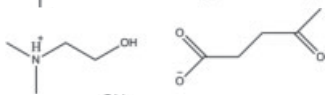
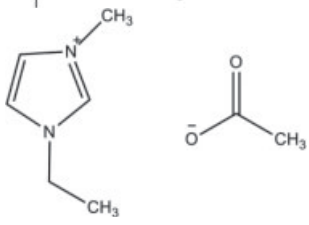
Chemical name	Structure of ILs	Ref
1-butyl-3-methylimidazolium chloride		118–123
1-butyl-3-methylimidazolium hexafluorophosphate		120
1-butyl-3-methylimidazolium acetate		123
1-benzyl-3-methylimidazolium chloride		98
1-butyl-1-methylpyrrolidinium chloride		120
1-butyl-3-methylimidazolium methylsulfate		124
N,*N*-dimethylethanolammonium formate		121
N,*N*-dimethylethanolammonium acetate		120,121
N,*N*-dimethylethanolammonium glycolate		121
N,*N*-dimethylethanolammonium succinate		121
1-ethyl-3-methylimidazolium acetate		98,99,119,121,123,125,126

Table 2. Conitnued

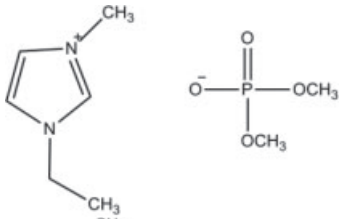
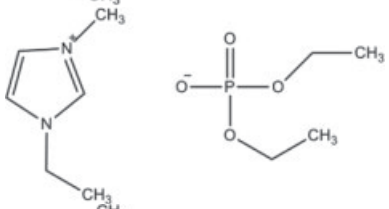
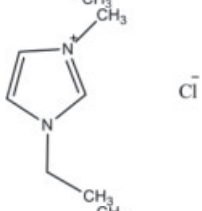
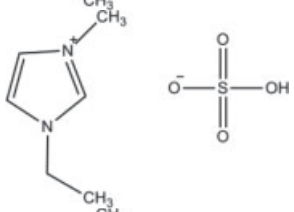
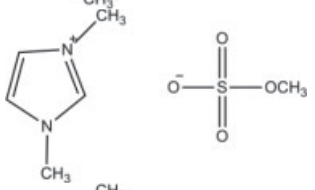
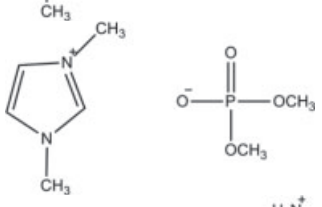
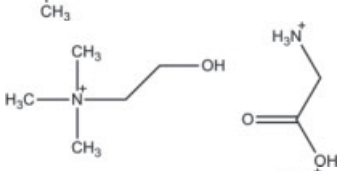
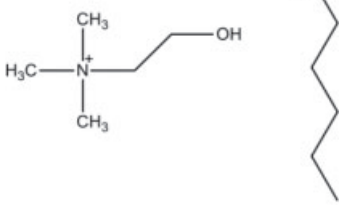
Chemical name	Structure of ILs	Ref
1-ethyl-3-methylimidazolium dimethyl phosphate		120
1-ethyl-3-methylimidazolium diethyl phosphate		97,120,127
1-ethyl-3-methylimidazolium chloride		119,128
1-ethyl-3-methylimidazolium hydrogen sulfate		119
1,3-dimethylimidazolium methyl sulfate		98,120
1,3-dimethylimidazolium dimethyl phosphate		120
Cholinium glycine		129
Cholinium lysine		130

Table 3. Changes in CrI values of COSLIF- and IL-pretreated switchgrass

	CrI (%)					
	XRD			ssNMR		
Materials	Peak height	Peak deconvolution	Amorphous subtraction	C_4 peak separation	Amorphous subtraction	Ref.
Intact switchgrass	67.0	59.4	60.9	38.9	33.6	40
COSLIF-pretreated swichgrass	3.2	14.0	ND	17.6	19.1	40
IL-pretreated switchgrass	2.6	-	-	-	-	76

ND: Not detectable

is suitable for processing and derivatizing pure cellulose. Recently, Wang *et al*. conducted a comparative study using different cellulose solvents – LiOH/urea, LiCl/DMAc, concentrated phosphoric acid, 1-butyl-3-methylimidazolium chloride, and NMMO.[108] Except for the cellulosic sample regenerated from LiCl/DMAc system, all the other treated samples exhibited lower cellulose crystallinity and degree of polymerization (DP), and consequently, exhibited a significant enhancement of enzymatic hydrolysis kinetic. The regenerated cellulose from concentrated phosphoric acid almost completely consisted of cellulose II, and achieved the highest saccharification yield.[108]

PERSPECTIVES: CHALLENGES AND OPPORTUNITIES

Cellulose solvent-based lignocellulose fractionation has many advantages, such as high glucan digestibility at low enzyme loading, fast hydrolysis rate, and potential revenues from separated co-products (e.g. hemicellulose, lignin). The ideal cellulose solvent should have numerous features: (i) dissolving cellulose at modest temperature (i.e. low energy input and less sugar degradation); (ii) dissolving wet cellulose (i.e. no biomass drying step required); (iii) highly recyclable; (iv) nonvolatile or highly volatile for easy recycling; (v) thermostable and chemostable for nearly an unlimited number recycling; (vi) nontoxic to the sequential steps of enzymatic hydrolysis and microbial fermentation; (vii) high cellulose dissolution capacity (>10% wt. cellulose/vol); and (viii) fast diffusion rate in solid lignocellulose composite.

Although cellulose solvent-based pretreatment has shown great promise, several challenges remain because of the production of low-value biocommodities, such as low ratios of biomass to cellulose solvent, high processing cost for efficient recycling of cellulose solvents, and high capital investment.[99,113] Therefore, further studies of cellulose solvent-based pretreatment should focus on:

1 discovering new cellulose solvents meeting the above criteria;
2 decreasing cellulose solvent use per biomass;[114]
3 validating cellulose solvent recycling on a relatively large scale and for a long time;[99]
4 examining chemostability and thermostability of cellulose solvents;
5 assessing potential environmental impact of lost cellulose solvents during the recycling;[115,116]
6 developing new approaches for cellulose solvent recycling;[99]
7 developing enzymes and microorganisms tolerant to the solvents if they are toxic;[91,92,117] and
8 co-utilizing fractionated lignocellulose components and developing value-added chemicals from fractionated lignocellulose components.[1,6,78,79]

The demands for renewable low-cost sugars fractionated from non-food lignocellulose biomass as a new oil are driving the development of better ways to cost-effectively overcome biomass recalcitrance. The use of cellulose solvents, both old and new, would open up opportunities for emerging biorefineries.

ACKNOWLEDGEMENTS

This work was supported mainly by the DOE BioEnergy Science Center (BESC) to YPZ. BESC is the US Department of Energy Bioenergy Research Centers supported by the Office of Biological and Environmental Research in the DOE Office of Science. This work (YPZ) is also partially supported by the USDA Bioprocessing and Biodesign Center.

REFERENCES

1 Zhang Y-HP, What is vital (and not vital) to advance economically-competitive biofuels production? *Process Biochem* **46**:2091–2110 (2011).
2 Lynd LR, Weimer PJ, van Zyl WH and Pretorius IS, Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol Mol Biol Rev* **66**:506–577 (2002).
3 Judd JD, Sarin SC and Cundiff JS, Design, modeling, and analysis of a feedstock logistics system. *Bioresource Technol* **103**:209–218 (2012).
4 Lynd LR, Laser MS, Bransby D, Dale BE, Davison B, Hamilton R, Himmel M, Keller M, McMillan JD, Sheehan J and Wyman CE, How biotech can transform biofuels. *Nat Biotechnol* **26**: 169–172 (2008).
5 Wyman CE, Biomass ethanol: technical progress, opportunities, and commercial challenges. *Annu Rev Energy Environ* **24**:189–226 (1999).
6 Zhang Y-HP, Reviving the carbohydrate economy via multi-product biorefineries. *J Ind Microbiol Biotechnol* **35**:367–375 (2008).
7 Klein-Marcuschamer D, Oleskowicz-Popiel P, Simmons BA and Blanch HW, The challenge of enzyme cost in the production of lignocellulosic biofuels. *Biotechnol Bioeng* **109**:1083–1087 (2012).
8 Blanch HW, Bioprocessing for biofuels. *Curr Opin Biotechnol* 390–395 (2012). Volume number??
9 Zhang Y-HP, Production of biocommodities and bioelectricity by cell-free synthetic enzymatic pathway biotransformations: challenges and opportunities. *Biotechnol Bioeng* **105**:663–677 (2010).
10 Zhu Z, Sathitsuksanoh N and Zhang Y-HP, Direct quantitative determination of adsorbed cellulase on lignocellulosic biomass with its application to study cellulase desorption for potential recycling. *Analyst* **134**:2267–2272 (2009).
11 Zhang X-Z and Zhang Y-HP, Simple, fast and high-efficiency transformation system for directed evolution of cellulase in *Bacillus subtilis*. *Microbiol Biotechnol* **4**:98–105 (2011).
12 Anbar M, Gul O, Lamed R, Sezerman UO and Bayer EA, Improved thermostability of *Clostridium thermocellum* Endoglucanase Cel8A by using consensus-guided mutagenesis. *Appl Environ Microbiol* **78**:3458–3464 (2012).
13 Li Y, Irwin DC and Wilson DB, Increased crystalline cellulose activity via combinations of amino acid changes in the family 9 catalytic domain and family 3c cellulose binding module of *Thermobifida fusca* Cel9A. *Appl Environ Microbiol* **76**:2582–2588 (2010).

14 Rosgaard L, Pedersen S, Langston J, Akerhielm D, Cherry JR and Meyer AS, Evaluation of minimal *Trichoderma reesei* cellulase mixtures on differently pretreated barley straw substrates. *Biotechnol Prog* **23**:1270–1276 (2007).
15 Liao HH, Zhang XZ, Rollin JA and Zhang Y-HP, A minimal set of bacterial cellulases for consolidated bioprocessing of lignocellulose. *Biotechnol J* **6**:1409–1418 (2011).
16 Moraïs S, Barak Y, Caspi J, Hadar Y, Lamed R, Shoham Y, Wilson DB and Bayer EA, Cellulase-xylanase synergy in designer cellulosomes for enhanced degradation of a complex cellulosic substrate. *mBio* **1**:e00285–00210 (2010).
17 You C, Zhang X-Z, Sathitsuksanoh N, Lynd LR and Zhang Y-HP, Enhanced microbial cellulose utilization of recalcitrant cellulose by an *ex vivo* cellulosome-microbe complex. *Appl Environ Microbiol* **78**:1437–1444 (2012).
18 You C, Zhang X-Z and Zhang YHP, Mini-scaffoldin enhanced mini-cellulosome hydrolysis performance on low-accessibility cellulose (Avicel) more than on high-accessibility amorphous cellulose. *Biochem Eng J* **63**:57–65 (2012).
19 Gefen G, Anbar M, Morag E, Lamed R and Bayer EA, Enhanced cellulose degradation by targeted integration of a cohesin-fused β-glucosidase into the *Clostridium thermocellum* cellulosome. *Proc Nat Acad Sci USA* **109**:10298–10303 (2012).
20 Lu Y, Zhang Y-HP and Lynd LR, Enzyme-microbe synergy during cellulose hydrolysis by *Clostridium thermocellum*. *Proc Nat Acad Sci USA* **103**:16165–16169 (2006).
21 Wen F, Sun J and Zhao H, Yeast surface display of trifunctional minicellulosomes for simultaneous saccharification and fermentation of cellulose to ethanol. *Appl Environ Microbiol* **76**:1251–1260 (2010).
22 Shao X, Jin M, Guseva A, Liu C, Balan V, Hogsett D, Dale BE and Lynd L, Conversion for Avicel and AFEX pretreated corn stover by *Clostridium thermocellum* and simultaneous saccharification and fermentation: insights into microbial conversion of pretreated cellulosic biomass. *Bioresource Technol* **102**:8040–8045 (2011).
23 Jin M, Balan V, Gunawan C and Dale BE, Consolidated bioprocessing (CBP) performance of *Clostridium phytofermentans* on AFEX-treated corn stover for ethanol production. *Biotechnol Bioeng* **108**:1290–1297 (2011).
24 Himmel ME, Ding S-Y, Johnson DK, Adney WS, Nimlos MR, Brady JW and Foust TD, Biomass recalcitrance: engineering plants and enzymes for biofuels production. *Science* **315**:804–807 (2007).
25 Chandra R, Bura R, Mabee W, Berlin A, Pan X and Saddler J, Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocellulosics? *Adv Biochem Eng Biotechnol* **108**:67–93 (2007).
26 Zhang Y-HP and Lynd LR, Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplexed cellulase systems. *Biotechnol Bioeng* **88**:797–824 (2004).
27 Arantes V and Saddler J, Access to cellulose limits the efficiency of enzymatic hydrolysis: the role of amorphogenesis. *Biotechnol Biofuels* **3** (2010). Page range??
28 Rollin JA, Zhu Z, Sathisuksanoh N and Zhang Y-HP, Increasing cellulose accessibility is more important than removing lignin: a comparison of cellulose solvent-based lignocellulose fractionation and soaking in aqueous ammonia. *Biotechnol Bioeng* **108**:22–30 (2011).
29 Hong J, Ye X and Zhang Y-HP, Quantitative determination of cellulose accessibility to cellulase based on adsorption of a nonhydrolytic fusion protein containing CBM and GFP with its applications. *Langmuir* **23**:12535–12540 (2007).
30 Luterbacher JS, Parlange J-Y and Walker LP, A pore-hindered diffusion and reaction model can help explain the importance of pore size distribution in enzymatic hydrolysis of biomass. *Biotechnol Bioeng*: n/a-n/a (2012P). Latest info required
31 Wang QQ, He Z, Zhu Z, Zhang Y-HP, Ni Y, Luo XL and Zhu JY, Evaluations of cellulose accessibilities of lignocelluloses by solute exclusion and protein adsorption techniques. *Biotechnol Bioeng* **109**:381–389 (2012).
32 Zhang Y-HP and Lynd LR, A functionally-based model for hydrolysis of cellulose by fungal cellulase. *Biotechnol Bioeng* **94**:888–898 (2006).
33 Fan LT, Lee Y-H and Beardmore DR, The influence of major structural features of cellulose on rate of enzymatic hydrolysis. *Biotechnol Bioeng* **23**:419–424 (1981).
34 Ryu DDY and Lee SB, Effect of compression milling on cellulose structure and on enzymatic hydrolysis kinetics. *Biotechnol Bioeng* **24**:1047–1067 (1982).
35 Krassig HA, *Cellulose: Structure, Accessibility, and Reactivity*. Gordon and Breach Science Publishers, Yverdon, Switzerland (1993).
36 Grethlein HE, The effect of pore size distribution on the rate of enzymatic hydrolysis of cellulose substrates. *Biotechnol Technol* **3**:155–160. (1985). Please confirm/correct
37 Zografi G, Kontny MJ, Yang AYS and Brenner GS, Surface area and water vapor sorption of macrocrystalline cellulose. *Int J Pharm* **18**:99–116 (1984).
38 Inglesby M and Zeronian S, The accessibility of cellulose as determined by dye adsorption. *Cellulose* **3**:165–181 (1996).
39 Nakano M, Sugita A, Matsuoka H and Handa T, Small-angle X-ray scattering and 13C NMR investigation on the internal structure of ''Cubosomes''. *Langmuir* **17**:3917–3922 (2001).
40 Sathitsuksanoh N, Zhu ZG, Wi S and Zhang Y-HP, Cellulose solvent-based biomass pretreatment breaks highly ordered hydrogen bonds in cellulose fibers of switchgrass. *Biotechnol Bioeng* **108**:521–529 (2011).
41 Dagel DJ, Liu Y-S, Zhong L, Luo Y, Himmel ME, Xu Q, Zeng Y, Ding S-Y and Smith S, *In situ* imaging of single carbohydrate-binding modules on cellulose microfibrils. *J Phys Chem B* **115**:635–641 (2010).
42 Stone JE, Scallan AM, Donefer E and Ahlgren E, Digestibility as a simple function of a molecule of a similar size to a cellulase enzyme. *Adv Chem Ser* **95**:219–241 (1969).
43 Esteghlalian A, Bilodeau M, Mansfield S and Saddler J, Do enzymatic hydrolyzability and Simons' stain reflect the changes in the accessibility of lignocellulosic substrates to cellulase enzymes? *Biotechnol Prog* **17**:1049–1054 (2001).
44 Zhu Z, Sathitsuksanoh N, Vinzant T, Schell DJ, McMillan JD and Zhang Y-HP, Comparative study of corn stover pretreated by dilute acid and cellulose solvent-based lignocellulose fractionation: enzymatic hydrolysis, supramolecular structure, and substrate accessibility. *Biotechnol Bioeng* **103**:715–724 (2009).
45 Chang VS and Holtzapple MT, Fundamental factors affecting biomass enzymatic reactivity. *Appl Biochem Biotechnol* **84–86**:5–37 (2000).
46 Liu C and Wyman CE, The effect of flow rate of compressed hot water on xylan, lignin, and total mass removal from corn stover. *Ind Eng Chem Res* **42**:5409–5416 (2003).
47 Yang B, Boussaid A, Mansfield SD, Gregg DJ and Saddler JN, Fast and efficient alkaline peroxide treatment to enhance the enzymatic digestibility of steam-exploded softwood substrates. *Biotechnol Bioeng* **77**:678–684 (2002).
48 Ishizawa CI, Jeoh T, Adney WS, Himmel ME, Johnson DK and Davis MF, Can delignification decrease cellulose digestibility in acid pretreated corn stover? *Cellulose* **16**:677–686 (2009).
49 Zhang Y-HP, Cui J, Lynd LR and Kuang LR, A transition from cellulose swelling to cellulose dissolution by o-phosphoric acid: evidence from enzymatic hydrolysis and supramolecular structure. *Biomacromolecules* **7**:644–648 (2006).
50 Hong J, Ye X, Wang Y and Zhang Y-HP, Bioseparation of recombinant cellulose binding module-protein by affinity adsorption on an ultra-high-capacity cellulosic adsorbent. *Anal Chim Acta* **621**:193–199 (2008).
51 Kurašin M and Väljamäe P, Processivity of cellobiohydrolases is limited by the substrate. *J Biol Chem* **286**:169–177 (2011).
52 Levine SE, Fox JM, Blanch HW and Clark DS, A mechanistic model of the enzymatic hydrolysis of cellulose. *Biotechnol Bioeng* **107**:37–51 (2010).
53 Zhang Y-HP and Lynd LR, Determination of the number-average degree of polymerization of cellodextrins and cellulose with application to enzymatic hydrolysis. *Biomacromolecules* **6**:1510–1515 (2005).
54 Bura R, Mansfield SD, Saddler JN and Bothast RJ, SO_2-catalyzed steam explosion of corn fiber for ethanol production. *Appl Biochem Biotechnol* **98**/100: 59–72 (2002).
55 Kim TH and Lee YY, Fractionation of corn stover by hot-water and aqueous ammonia treatment. *Bioresource Technol* **97**:224–232 (2006).
56 Pan X, Xie D, Yu RW and Saddler JN, The bioconversion of mountain pine beetle-killed lodgepole pine to fuel ethanol using the organosolv process. *Biotechnol Bioeng* **101**:39–48 (2008).
57 Sathitsuksanoh N, Zhu Z, Ho T-J, Bai M-D and Zhang Y-HP, Bamboo saccharification through cellulose solvent-based biomass

pretreatment followed by enzymatic hydrolysis at ultra-low cellulase loadings *Bioresource Technol* **101**:4926–4929 (2010).
58 Sathitsuksanoh N, Zhu Z, Templeton N, Rollin J, Harvey S and Zhang Y-HP, Saccharification of a potential bioenergy crop, *Phragmites australis* (common reed), by lignocellulose fractionation followed by enzymatic hydrolysis at decreased cellulase loadings. *Ind Eng Chem Res* **48**:6441–6447 (2009).
59 Sathitsuksanoh N, Zhu Z and Zhang Y-HP, Cellulose solvent- and organic solvent-based lignocellulose fractionation enabled efficient sugar release from a variety of lignocellulosic feedstocks. *Bioresource Technol* **117**:228–233 (2012).
60 Park S, Baker J, Himmel M, Parilla P and Johnson D, Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. *Biotechnol Biofuels* **3**:10 (2010).
61 Kim TH, Kim JS, Sunwoo C and Lee YY, Pretreatment of corn stover by aqueous ammonia. *Bioresource Technol* **90**:39–47 (2003).
62 Pereira AN, Mobedshahi M and Ladisch MR, Preparation of cellodextrins. *Methods Enzymol* **160**:26–43 (1988).
63 Bergius F, Conversion of wood to carbohydrates. *Ind Eng Chem* **29**:247–253 (1937).
64 Mäki-Arvela Pi, Salmi T, Holmbom B, Willför S and Murzin DY, Synthesis of sugars by hydrolysis of hemicelluloses - a review. *Chem Rev* **111**:5638–5666 (2011).
65 Fengel D and Wegener G, *Wood: Chemistry, Ultrastructure, Reactions*. Walter de Gruyter and Co, Berlin (1984).
66 Sathisuksanoh N, Zhu ZG and Zhang Y-HP, Cellulose solvent-based pretreatment for corn stover and Avicel: concentrated phosphoric acid versus ionic liquid [BMIM]Cl. *Cellulose* **19**:1161–1172 (2012).
67 Moxley G and Zhang Y-HP, More accurate determination of acid-labile carbohydrate composition in lignocellulose by modified quantitative saccharification. *Energy Fuels* **21**:3684–3688 (2007).
68 Ladisch MR, Ladisch CM and Tsao GT, Cellulose to sugars: new path gives quantitative yield. *Science* **201**:743–745 (1978).
69 Zhang Y-HP, Ding S-Y, Mielenz JR, Elander R, Laser M, Himmel M, McMillan JD and Lynd LR, Fractionating recalcitrant lignocellulose at modest reaction conditions. *Biotechnol Bioeng* **97**:214–223 (2007).
70 Moxley GM, Zhu Z and Zhang Y-HP, Efficient sugar release by the cellulose solvent based lignocellulose fractionation technology and enzymatic cellulose hydrolysis *J Agric Food Chem* **56**:7885–7890 (2008).
71 Conte P, Maccotta A, De Pasquale C, Bubici S and Alonzo G, Dissolution mechanism of crystalline cellulose in H3PO4 as assessed by high-field NMR spectroscopy and fast field cycling NMR relaxometry. *J Agric Food Chem* **57**:8748–8752 (2009).
72 Li H, Kim N-J, Jiang M, Kang JW and Chang HN, Simultaneous saccharification and fermentation of lignocellulosic residues pretreated with phosphoric acid–acetone for bioethanol production. *Bioresource Technol* **100**:3245–3251 (2009).
73 Ge X, Green VS, Zhang N, Sivakumar G and Xu J, Eastern gamagrass as an alternative cellulosic feedstock for bioethanol production. *Process Biochem* **47**:335–339 (2012).
74 Ge X, Burner DM, Xu J, Phillips GC and Sivakumar G, Bioethanol production from dedicated energy crops and residues in Arkansas, *USA. Biotechnol J* **6**:66–73 (2011).
75 Sun N, Rahman M, Qin Y, Maxim M, Rodríguez H and Rogers R, Complete dissolution and partial delignification of wood in the ionic liquid 1-ethyl-3-methylimidazolium acetate. *Green Chem* **11**:646–655 (2009).
76 Li W, Sun N, Stoner B, Jiang X, Lu X and Rogers RD, Rapid dissolution of lignocellulosic biomass in ionic liquids using temperatures above the glass transition of lignin. *Green Chem* **13**:2038–2047 (2011).
77 Li C, Knierim B, Manisseri C, Arora R, Scheller HV, Auer M, Vogel KP, Simmons BA and Singh S, Comparison of dilute acid and ionic liquid pretreatment of switchgrass: biomass recalcitrance, delignification and enzymatic saccharification. *Bioresource Technol* **101**:4900–4906 (2010).
78 Labbé N, Kline LM, Moens L, Kim K, Kim PC and Hayes DG, Activation of lignocellulosic biomass by ionic liquid for biorefinery fractionation. *Bioresource Technol* **104**:701–707 (2012).
79 Vancov T, Alston AS, Brown T and McIntosh S, Use of ionic liquids in converting lignocellulosic material to biofuels. *Renew Energy* **45**:1–6 (2012).
80 Blanch HW, Simmons BA and Klein-Marcuschamer D, Biomass deconstruction to sugars. *Biotechnol J* **6**:1086–1102 (2011).
81 Tadesse H and Luque R, Advances on biomass pretreatment using ionic liquids: an overview. *Energy Environ Sci* **4**:3913–3929 (2011).
82 Mora-Pale M, Meli L, Doherty TV, Linhardt RJ and Dordick JS, Room temperature ionic liquids as emerging solvents for the pretreatment of lignocellulosic biomass. *Biotechnol Bioeng* **108**:1229–1245 (2011).
83 Liu C-Z, Wang F, Stiles AR and Guo C, Ionic liquids for biofuel production: opportunities and challenges. *Appl Energy* **92**:406–414 (2012).
84 Langan P, Gnanakaran S, Rector KD, Pawley N, Fox DT, Cho DW and Hammel KE, Exploring new strategies for cellulosic biofuels production. *Energy Environ Sci* **4**:3820–3833 (2011).
85 Li C, Cheng G, Balan V, Kent MS, Ong M, Chundawat SPS, daCosta Sousa L, Melnichenko YB, Dale BE and Simmons BA, Influence of physico-chemical changes on enzymatic digestibility of ionic liquid and AFEX pretreated corn stover. *Bioresource Technol* **102**:6928–6936 (2011).
86 Murugesan S and Linhardt RJ, Ionic liquids in carbohydrate chemistry - current trends and future directions. *Curr Org Syn* **2**:437–451 (2005).
87 Docherty KM and Kulpa CF, Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids. *Green Chem* **7**:185–189 (2005).
88 Turner MB, Spear SK, Huddleston JG, Holbrey JD and Rogers RD, Ionic liquid salt-induced inactivation and unfolding of cellulase from Trichoderma reesei. *Green Chem* **5**:443–447 (2003).
89 Turner MB, Spear SK, Holbrey JD and Rogers RD, Production of bioactive cellulose films reconstituted from ionic liquids. *Biomacromolecules* **5**:1379–1384 (2004).
90 Datta S, Holmes B, Park JI, Chen Z, Dibble DC, Hadi M, Blanch HW, Simmons BA and Sapra R, Ionic liquid tolerant hyperthermophilic cellulases for biomass pretreatment and hydrolysis. *Green Chem* **12**:338–345 (2010).
91 Engel P, Mladenov R, Wulfhorst H, Jäger G and Spiess AC, Point by point analysis: how ionic liquid affects the enzymatic hydrolysis of native and modified cellulose. *Green Chem* **12**:1959–1966 (2010).
92 Bose S, Barnes CA and Petrich JW, Enhanced stability and activity of cellulase in an ionic liquid and the effect of pretreatment on cellulose hydrolysis. *Biotechnol Bioeng* **109**:434–443 (2012).
93 Park JI, Steen EJ, Burd H, Evans SS, Redding-Johnson AM, Batth T, Benke PI, D'Haeseleer P, Sun N, Sale KL, Keasling JD, Lee TS, Petzold CJ, Mukhopadhyay A, Singer SW, Simmons BA and Gladden JM, A thermophilic ionic liquid-tolerant cellulase cocktail for the production of cellulosic biofuels. *PLoS One* **7**: (2012). Latest info required
94 Diedericks D, van Rensburg E, Garcia-Aparicio MD and Gorgens JF, Enhancing the enzymatic digestibility of sugarcane bagasse through the application of an ionic liquid in combination with an acid catalyst. *Biotechnol Prog* **28**:76–84 (2012).
95 Lan W, Liu CF and Sun RG, Fractionation of bagasse into cellulose, hemicelluloses, and lignin with ionic liquid treatment followed by alkaline extraction. *J Agr Food Chem* **59**:8691–8701 (2011).
96 Chundawat SPS, Bellesia G, Uppugundla N, da Costa Sousa L, Gao D, Cheh AM, Agarwal UP, Bianchetti CM, Phillips GN, Langan P, Balan V, Gnanakaran S and Dale BE, Restructuring the crystalline cellulose hydrogen bond network enhances its depolymerization rate. *J Am Chem Soc* **133**:11163–11174 (2011).
97 Wu H, Mora Pale M, Miao J, Doherty TV, Linhardt RJ and Dordick JS, Facile pretreatment of lignocellulosic biomass at high loadings in room temperature ionic liquids. *Biotechnol Bioeng* **108**:2865–2875 (2011).
98 Li Q, He Y, Xian M, Jun G, Xu X, Yang J and Li L, Improving enzymatic hydrolysis of wheat straw using ionic liquid 1-ethyl-3-methyl imidazolium diethyl phosphate pretreatment. *Bioresource Technol* **100**:3570–3575 (2009).
99 Lee SH, Doherty TV, Linhardt RJ and Dordick JS, Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis. *Biotechnol Bioeng* **102**:1368–1376 (2009).
100 Shill K, Padmanabhan S, Xin Q, Prausnitz JM, Clark DS and Blanch HW, Ionic liquid pretreatment of cellulosic biomass: enzymatic hydrolysis and ionic liquid recycle. *Biotechnol Bioeng* **108**:511–520 (2011).
101 Auxenfans T, Buchoux S, Djellab K, Avondo C, Husson E and Sarazin C, Mild pretreatment and enzymatic saccharification of cellulose

with recycled ionic liquids towards one-batch process. *Carbohydr Polym* **90**:805–813 (2012).
102 Tan SSY, MacFarlane DR, Upfal J, Edye LA, Doherty WOS, Patti AF, Pringle JM and Scott JL, Extraction of lignin from lignocellulose at atmospheric pressure using alkylbenzenesulfonate ionic liquid. *Green Chem* **11**:339–345 (2009).
103 Fink HP, Weigel P, Purz H and Ganster J, Structure formation of regenerated cellulose materials from NMMO-solutions. *Prog Polym Sci* **26**:1473–1524 (2001).
104 Rosenau T, Potthast A, Adorjan I, Hofinger A, Sixta H, Firgo H and Kosma P, Cellulose solutions in N-methylmorpholine-N-oxide (NMMO)–degradation processes and stabilizers. *Cellulose* **9**:283–291 (2002).
105 Rosenau T, Potthast A, Sixta H and Kosma P, The chemistry of side reactions and byproduct formation in the system NMMO/cellulose (Lyocell process). *Prog Polym Sci* **26**:1763–1837 (2001).
106 Khodaverdi M, Jeihanipour A, Karimi K and Taherzadeh MJ, Kinetic modeling of rapid enzymatic hydrolysis of crystalline cellulose after pretreatment by NMMO. *J Ind Microbiol Biotechnol* **39**:429–438 (2012).
107 Kuo C-H and Lee C-K, Enhancement of enzymatic saccharification of cellulose by cellulose dissolution pretreatments. *Carbohydr Polym* **77**:41–46 (2009).
108 Shafiei M, Karimi K and Taherzadeh MJ, Pretreatment of spruce and oak by N-methylmorpholine-N-oxide (NMMO) for efficient conversion of their cellulose to ethanol. *Bioresource Technol* **101**:4914–4918 (2010).
109 Wang K, Yang HY, Xu F and Sun RC, Structural comparison and enhanced enzymatic hydrolysis of the cellulosic preparation from Populus tomentosa Carr. by different cellulose-soluble solvent systems. *Bioresource Technol* **102**:4524–4529 (2011).
110 Ruan D, Zhang L, Zhou J, Jin H and Chen H, Structure and properties of novel fibers spun from cellulose in NaOH/thiourea aqueous solution. *Macromol Biosci* **4**:1105–1112 (2004).
111 Wang Y, Zhao Y and Deng Y, Effect of enzymatic treatment on cotton fiber dissolution in NaOH/urea solution at cold temperature. *Carbohydr Polym* **72**:178–184 (2008).
112 Cai J, Zhang L, Zhou J, Li H, Chen H and Jin H, Novel fibers prepared from cellulose in NaOH/urea aqueous solution. *Macromol Rapid Commun* **25**:1558–1562 (2004).
113 Striegel AM, Theory and applications of DMAc/LiCl in the analysis of polysacharrides. *Carbohydr Polym* **34**:267–274 (1997).
114 Stark A, Ionic liquids in the biorefinery: a critical assessment of their potential. *Energy Environ Sci* **4**:19–32 (2011).
115 Wu H, Mora-Pale M, Miao JJ, Doherty TV, Linhardt RJ and Dordick JS, Facile pretreatment of lignocellulosic biomass at high loadings in room temperature ionic liquids. *Biotechnol Bioeng* **108**:2865–2875 (2011).
116 Kim T-W, Chokhawala HA, Hess M, Dana CM, Baer Z, Sczyrba A, Rubin EM, Blanch HW and Clark DS, High-throughput in vitro glycoside hydrolase (HIGH) screening for enzyme discovery. *Angew Chem Int Ed* **50**:11215–11218 (2011).
117 Klein-Marcuschamer D, Simmons BA and Blanch HW, Techno-economic analysis of a lignocellulosic ethanol biorefinery with ionic liquid pre-treatment. *Biofuels Bioprod Biorefin* **5**:562–569 (2011).
118 Singer SW, Reddy AP, Gladden JM, Guo H, Hazen TC, Simmons BA and VanderGheynst JS, Enrichment, isolation and characterization of fungi tolerant to 1-ethyl-3-methylimidazolium acetate. *J Appl Microbiol* **110**:1023–1031 (2011).
119 Dadi AP, Varanasi S and Schall CA, Enhancement of cellulose saccharification kinetics using an ionic liquid pretreatment step. *Biotechnol Bioeng* **95**:904–910 (2006).
120 Nguyen TD, Kim KR, Han SJ, Cho HY, Kim JW, Park SM, Park JC and Sim SJ, Pretreatment of rice straw with ammonia and ionic liquid for lignocellulose conversion to fermentable sugars. *Bioresource Technol* **101**:7432–7438 (2010).
121 Li Q, Jiang X, He Y, Li L, Xian M and Yang J, Evaluation of the biocompatibile ionic liquid 1-methyl-3-methylimidazolium dimethylphosphite pretreatment of corn cob for improved saccharification. *Appl Microbiol Biotechnol* **87**:117–126 (2010).
122 Fu D, Mazza G and Tamaki Y, Lignin extraction from straw by ionic liquids and enzymatic hydrolysis of the cellulosic residues. *J Agric Food Chem* **58**:2915–2922 (2010).
123 Kim SJ, Dwiatmoko AA, Choi JW, Suh YW, Suh DJ and Oh M, Cellulose pretreatment with 1-n-butyl-3-methylimidazolium chloride for solid acid-catalyzed hydrolysis. *Bioresource Technol* **101**:8273–8279 (2010).
124 Zhao H, Baker G and Cowins J, Fast enzymatic saccharification of switchgrass after pretreatment with ionic liquids. *Biotechnol Prog* **26**:127–133 (2009).
125 Brandt A, Ray MJ, To TQ, Leak DJ, Murphy RJ and Welton T, Ionic liquid pretreatment of lignocellulosic biomass with ionic liquid-water mixtures. *Green Chem* **13**:2489–2499 (2011).
126 Samayam IP and Schall CA, Saccharification of ionic liquid pretreated biomass with commercial enzyme mixtures. *Bioresource Technol* **101**:3561–3566 (2010).
127 Wang Y, Radosevich M, Hayes D and Labbé N, Compatible ionic liquid-cellulases system for hydrolysis of lignocellulosic biomass. *Biotechnol Bioeng* **108**:1042–1048 (2011).
128 Kamiya N, Matsushita Y, Hanaki M, Nakashima K, Narita M, Goto M and Takahashi H, Enzymatic in situ saccharification of cellulose in aqueous-ionic liquid media. *Biotechnol Lett* **30**:1037–1040 (2008).
129 Binder J and Raines R, Fermentable sugars by chemical hydrolysis of biomass. *Proc Nat Acad Sci USA* **107**:4516–4521 (2010).
130 Liu QP, Hou XD, Li N and M.H. Z, Ionic liquids from renewable biomaterials: synthesis, characterization and application in the pretreatment of biomass. *Green Chem* **14**:304–307 (2012).
131 Hou XD, Smith TJ, Li N and Zong MH, Novel renewable ionic liquids as highly effective solvents for pretreatment of rice straw biomass by selective removal of lignin. *Biotechnol Bioeng* **109**:2484–2493 (2012).

