

Co-solvent Pretreatment Reduces Costly Enzyme Requirements for High Sugar and Ethanol Yields from Lignocellulosic Biomass

Thanh Yen Nguyen,^[a, b] Charles M. Cai,^[a, c, d] Rajeev Kumar,^[a, d] and Charles E. Wyman^{*[a, b, c, d]}

We introduce a new pretreatment called co-solvent-enhanced lignocellulosic fractionation (CELf) to reduce enzyme costs dramatically for high sugar yields from hemicellulose and cellulose, which is essential for the low-cost conversion of biomass to fuels. CELf employs THF miscible with aqueous dilute acid to obtain up to 95% theoretical yield of glucose, xylose, and arabinose from corn stover even if coupled with enzymatic hydrolysis at only 2 mg_{enzyme} g_{glucan}⁻¹. The unusually high saccharification with such low enzyme loadings can be attributed to

a very high lignin removal, which is supported by compositional analysis, fractal kinetic modeling, and SEM imaging. Subsequently, nearly pure lignin product can be precipitated by the evaporation of volatile THF for recovery and recycling. Simultaneous saccharification and fermentation of CELf-pretreated solids with low enzyme loadings and *Saccharomyces cerevisiae* produced twice as much ethanol as that from dilute-acid-pretreated solids if both were optimized for corn stover.

Introduction

Lignocellulosic biomass, in the form of agricultural, herbage, and woody residues and energy crops, promises to provide a sufficient sustainable resource to address global energy demands and reduce dependence on petroleum-based liquid fuels.^[1] Its low cost is also attractive with lignocellulosic biomass at \$60 dryton⁻¹, which is equivalent in unit energy cost to oil at approximately \$20 barrel⁻¹.^[2] Natural gas production from fossil resources can serve to reduce petroleum consumption in the near future, however, it is a finite resource, and its consumption contributes to overall atmospheric greenhouse

gas (GHG) emissions. In contrast, bioenergy from lignocellulosic biomass is sustainable in the long term, and the GHGs produced from burning biofuels originate from atmospheric CO₂ sequestered by photosynthesis and are reabsorbed by growing new plants to replace those harvested. In light of this, realistic and scalable technologies are particularly needed to capture the energy in lignocellulosic biomass as aromatic, hydrocarbon, and alcohol transportation fuels because the transportation sector currently consumes approximately two-thirds of the world's petroleum production.^[3]

Lignocellulosic biomass is composed of hemicellulose, cellulose, and lignin in a heterogeneous matrix that is recalcitrant; it is structurally durable and resistant to microbial or enzymatic breakdown.^[4] This recalcitrance is the major economic obstacle to the conversion of biomass to sugars or other reactive intermediates with high yields at low enough costs for widespread use.^[1a,3b] Biomass pretreatment is essential to overcome the recalcitrance of most biomass materials to downstream biological and chemical processes. Chemical and physical pretreatment methods include heat, acid, and/or chemicals, usually in aqueous-based reactions.^[4a,5] In these two-stage operations, thermochemical pretreatment (Stage 1) opens up the biomass structure for subsequent enzymatic saccharification (Stage 2) by a mixture of enzymes ("cocktails") that release sugars from the pretreated solids.^[5a,6] Although modern saccharification enzymes are highly stable and selective, their effectiveness is largely influenced by the efficacy of the pretreatment stage in which 1) incomplete removal/relocation of hemicellulose and/or lignin impedes enzyme function, which necessitates higher enzyme loadings that increase costs significantly, and 2) ineffective alteration of the polysaccharides that remain in the solids from pretreatment can reduce enzyme access to binding sites, which limits potential sugar yields.^[7] Thus, it is desirable

[a] T. Y. Nguyen,⁺ Dr. C. M. Cai,⁺ Dr. R. Kumar, Prof. C. E. Wyman
Center for Environmental Research and Technology (CE-CERT)
Bourns College of Engineering
University of California
Riverside, 1084 Columbia Avenue, Riverside, California 92507 (USA)
Fax: (+1) 951-781-5790
E-mail: cewyman@engr.ucr.edu

[b] T. Y. Nguyen,⁺ Prof. C. E. Wyman
Department of Bioengineering
Bourns College of Engineering
University of California
Riverside, 217 Materials Science & Engineering
900 University Ave., Riverside, CA 92507 (USA)

[c] Dr. C. M. Cai,⁺ Prof. C. E. Wyman
Department of Chemical and Environmental Engineering
Bourns College of Engineering
University of California
Riverside, 446 Winston Chung Hall
900 University Ave., Riverside, CA 92507 (United States)

[d] Dr. C. M. Cai,⁺ Dr. R. Kumar, Prof. C. E. Wyman
BioEnergy Science Center (BESC)
Oak Ridge National Laboratory (ORNL)
Oak Ridge, TN (USA)

[⁺] These authors contributed equally to this work.

Supporting Information for this article is available on the WWW under <http://dx.doi.org/10.1002/cssc.201403045>.

for an effective pretreatment to remove hemicellulose and lignin to improve the accessibility of the pretreated solids to enzymes so that high total sugar yields from both hemicellulose and cellulose can be achieved with low enzyme loadings.^[7a,8]

Currently, leading pretreatment strategies that are attractive for commercial use include, but are not limited to, processes based on hydrothermal, dilute acid (DA), solvent (such as ethanol-organosolv or cellulose solvent and organic solvent based lignocellulose fractionation; COSLIF), ammonia (ammonia fiber expansion (AFEX), ammonia recycled percolation (ARP), and soaking in aqueous ammonia), and alkali treatment.^[6,9] Although each approach has its advantages, incremental implementation costs must be compensated by significant improvements in total sugar recovery and the ability to achieve high yields of fuels in downstream bioconversion processes at low enzyme loadings. We present here a new solvent-based pretreatment strategy called co-solvent-enhanced lignocellulosic fractionation (CELf) that employs aqueous THF solutions to enhance DA pretreatment significantly. DA pretreatment is a research and commercial pretreatment benchmark^[10] as evidenced by its wide adoption for pretreatment-related research and choice for techno-economic analyses by the National Renewable Energy Laboratory (NREL)^[11] as a relatively low-cost and effective pretreatment for corn stover and other feedstocks.^[12]

CELf employs THF because it is a unique polar aprotic solvent that is miscible with water over a wide range of conditions and concentrations and has the added benefit that it can be produced sustainably from biomass.^[13] In its miscible regime at high reaction severities, we showed previously that the reaction of lignocellulosic biomass with acidified aqueous THF solutions caused extensive lignin removal from biomass solids and catalyzed the solubilization of cellulose and hemicellulose to enhance the overall sugar dehydration product yields (furfural, 5-hydroxymethylfurfural, and levulinic acid) that could be used as fuel precursors for catalytic reactions.^[13,14] These results prompted us to hypothesize that less severe reaction conditions would solubilize most of the hemicellulose sugars with limited breakdown to dehydration products and remove most of the lignin to produce cellulose-rich solids that would lend themselves to nearly complete sugar release at low enzyme loadings. Thus, the study reported here was undertaken to optimize CELf as a pretreatment of corn stover as a model biomass feedstock, directly compare the resulting performance with that by DA pretreatment to define the unique advantages of CELf, and demonstrate its integration with simultaneous saccharification and fermentation (SSF) to produce valuable fuels and chemicals.

Results and Discussion

Optimization of corn stover pretreatment to maximize overall total sugar yields

This study focused on the fate of glucose, xylose, and arabinose during CELf and DA pretreatments in light of their dominance in corn stover composition. To present a fair compari-

son, we first optimized reaction conditions to achieve the highest total sugar yields (glucose, xylose, and arabinose) from CELf or DA pretreatment of corn stover (Stage 1) combined with subsequent enzymatic hydrolysis of the pretreated solids (Stage 2). Dilute sulfuric acid (0.5 wt%) was used for both CELf and DA pretreatments as it is an inexpensive strong acid that can be neutralized easily at dilute concentrations and is often the acid of choice. We selected a 1:1 THF/water volume mixture for all CELf reactions as it was found previously to be the lowest solvent concentration required for effective delignification of lignocellulosic biomass.^[13] Although higher solvent ratios up to 7:1 can further improve biomass solubilization, the formation of unwanted sugar dehydration products is also increased. For CELf, the lowest optimal reaction temperature of 150 °C was chosen to remain outside the known miscibility gap for THF/water mixtures between 71.8 and 137.1 °C.^[15] Short heat-up (<4 min) and cool-down (<1 min) times ensure that the reactants spend little time passing through the immiscible region to minimize the possibility of some impact on reaction kinetics. For optimization, Stage 2 enzymatic hydrolysis of pretreated corn stover solids was performed with the Accelerase 1500 enzyme cocktail (cellulase+ β -glucosidase) at an enzyme loading of 15 mg_{enzyme} g_{glucan}⁻¹ to yield glucose monomers. Enzyme loadings were based on the glucan composition of the corn stover before pretreatment for a fair comparison between the two pretreatments.^[5a]

The total sugar yields from combined pretreatment and enzymatic hydrolysis were determined over a range of pretreatment times to find the optimal reaction time for the CELf pretreatment of corn stover (Figure 1). Most of the xylose and arabinose was released in Stage 1, whereas the majority of the glucose was obtained in Stage 2, consistent with the typical production of sugars from DA pretreatment. However, in contrast to DA pretreatment trends in which Stage 2 glucose yields tend to increase continually with pretreatment time, Stage 2 glucose yields from the enzymatic hydrolysis of CELf-pretreated corn stover remained relatively constant over a range of pretreatment times (Figure 1A). As little degradation of glucose occurred under the CELf pretreatment conditions applied and enzymatic hydrolysis was effective over a wide range of pretreatment times, the total glucose yield from both stages was nearly 100% of the theoretical maximum that paralleled the trend for Stage 2. However, the Stage 1 xylose yield for CELf peaked at 89% for an approximately 25 min pretreatment, and further reaction reduced xylose yields as a result of degradation. As most of the xylose was released in Stage 1 and most of the xylose that remained in the pretreated solids was released in subsequent enzymatic hydrolysis, the total xylose yield paralleled the trend for Stage 1. Arabinose yields were fairly constant at approximately 80% of the theoretical yield over the course of the reaction times. As a result of these trends, the optimal reaction time of 25 min for the total combined sugars yield from CELf pretreatment shown in Figure 1C was largely dictated by the xylose yield from Stage 1 (Figure 1B). This outcome suggested that CELf pretreatment of corn stover could be optimized simply based on xylose recovery in the liquid hydrolyzate from pretreatment.

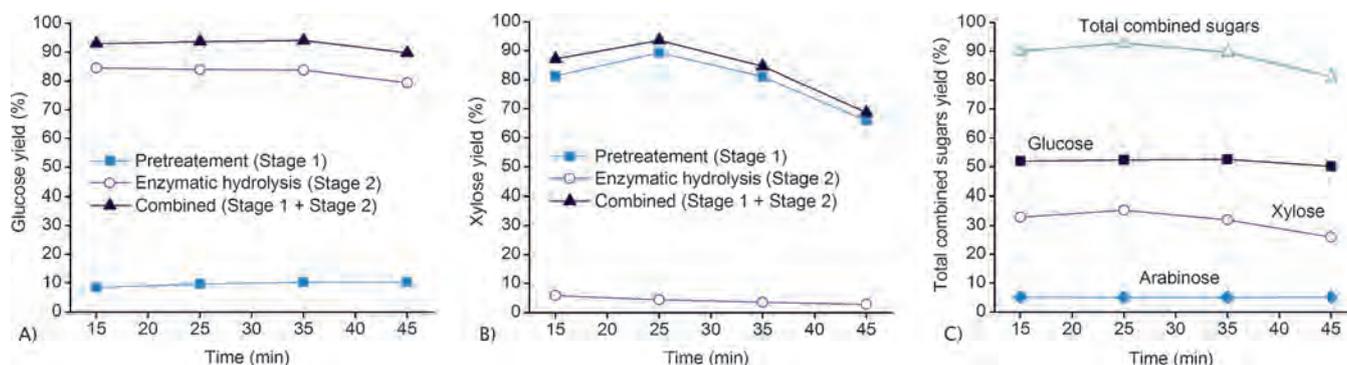


Figure 1. Optimization of pretreatment times for the CELF pretreatment of corn stover to maximize total sugar (glucose, xylose, and arabinose) yields from combined pretreatment (Stage 1) and enzymatic hydrolysis (Stage 2): A) glucose yields, B) xylose yields, and C) total combined glucose+xylose+arabinose yields. Reaction conditions: 5 wt% corn stover, 150 °C, 0.5% H₂SO₄, and 1:1 THF/water volume ratio. Stage 2 was performed using a 15 mg_{protein} g_{glucan}⁻¹ loading of Accellerase 1500 enzyme based on glucan in corn stover before pretreatment.

The optimal reaction conditions developed in this study for DA pretreatment of corn stover without THF were consistent with those established by previous work:^[16] a 20 min reaction at 160 °C with 0.5 wt% sulfuric acid. Maximization of the total sugar yields from combined pretreatment and enzymatic hydrolysis required tradeoffs as sugar yields from enzymatic hydrolysis increased continually with pretreatment time and xylose yields from pretreatment peaked well before the maximum glucose yield was obtained. This contrasts with CELF pretreatment in which xylose was not sacrificed in Stage 1 to realize high enough yields in Stage 2 to achieve the highest possible overall total sugar yields.^[16]

The compositions of solids from both DA and CELF pretreatments of corn stover under the optimal conditions for the highest overall total sugar yields are shown in Figure 2 based on 100 g of initial raw corn stover before pretreatment. These results show that although the fates of glucan and arabinan were similar for both pretreatments, CELF pretreatment improved the removal of xylan and lignin dramatically compared to DA pretreatment. The remaining component masses are 1.3 g xylan and 3.4 g lignin for the CELF-pretreated solids compared to 2.9 g xylan and 15.0 g lignin for the DA-pretreated solids based on 100 g of initial corn stover (Figure 2). The reduced solid mass from CELF pretreatment was largely because of extensive delignification, which is not possible with DA pretreatment, higher solubilization of other components such as ash, proteins, and extractives, and less pseudolignin formation caused by sugar degradation that may actually increase the measured K-lignin content in pretreated solids from the DA pretreatment.^[17] As observed previously with maple wood,^[13] THF is highly effective in the delignification of biomass and in this case produces a much more glucan-enriched material than that from DA pretreatment (75 vs. 52 wt% glucan, respectively). However, the total mass of glucan that remained in the solids was comparable after DA and CELF pretreatments despite a 10 °C reduction in temperature with CELF under optimal reaction conditions, which supports our early observation that THF can catalyze the hydrolysis of biomass sugars.^[13]

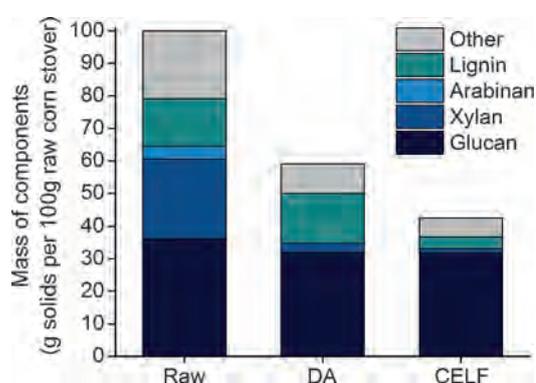


Figure 2. Tracking the mass of glucan, xylan, arabinan, lignin, and other compounds left in the solids produced by DA and CELF pretreatments at conditions optimized for the recovery of the highest total overall sugar yields. The values shown for DA and CELF are based on the content of each component in 100 g of corn stover before pretreatment. Reaction conditions: DA: 160 °C, 0.5% H₂SO₄, 20 min; CELF: 150 °C, 0.5% H₂SO₄, 25 min, 1:1 THF/water volume ratio.

Effect of reduced enzyme loadings on total sugar yields

As enzymes contribute approximately 20% of the total cost of the production of fermentable carbohydrates from lignocellulosic biomass,^[3b] one of the most important challenges in the development of effective pretreatment technologies is the reduction of the enzyme requirement for high sugar yields. Enzymes have been estimated to cost up to \$1.47 gallon_{ethanol}⁻¹ based on typical yields and conversions ($\approx 70\%$) for ethanol production from corn stover based on a benchmark DA pretreatment at a moderate loading of 20 mg_{enzyme} g_{glucan}⁻¹.^[18] Thus, as an example, the reduction of the enzyme loading by a factor of four could lower enzyme costs significantly to a more economical value of \$0.37 gallon_{ethanol}⁻¹. The results of the Stage 2 enzymatic hydrolysis of solids produced from optimized DA and CELF pretreatments of corn stover for loadings of 30, 15, 5, and 2 mg_{enzyme} g_{glucan}⁻¹ based on the raw glucan composition are shown in Figure 3. These results show that CELF pretreatment (Figure 3B) achieved higher total sugar yields at all enzyme loadings compared to DA pretreatment

(Figure 3 A). Additionally, the CELF-pretreated material realized nearly theoretical yields of glucose even at low loadings of 2 and 5 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$, albeit at longer incubation times (16 and 5 days, respectively). These profiles are in stark contrast to those for DA-pretreated corn stover solids that had lower rates of glucan release and incomplete hydrolysis at loadings of 15 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$ or less, likely caused by cellulose surface blockage by lignin and pseudolignin and/or enzyme inhibition/adsorption by lignin and/or pseudolignin.^[17a,19] The glucose yield profiles versus enzymatic hydrolysis incubation time also show that long-term glucose yields for solids from DA pretreatment decreased with each successively lower enzyme loading, whereas long-term yields reached nearly 100% for the CELF-pretreated material, although it required longer reaction times. Thus, CELF pretreatment reduced the recalcitrance of corn stover to such an extent that a 10-fold reduction in enzyme loading still achieved very high yields and could translate into a decrease of the enzyme cost to $\$0.15 \text{gallon}_{\text{ethanol}}^{-1}$, a saving of over $\$1.00 \text{gallon}_{\text{ethanol}}^{-1}$. Future techno-economic analysis is needed to evaluate the cost of implementing CELF pretreatment compared to DA pretreatment to demonstrate overall

cost savings. As described later, the extent of THF recovery would be crucial to plant economics.

The total combined sugar yields at loadings of 2, 5, and 15 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$ for solids from CELF and DA pretreatments under optimized pretreatment conditions expressed as the sum of glucose, xylose, and arabinose masses obtained from Stage 1+Stage 2 divided by the initial total mass of these three sugars and normalized based on 100 g total sugars in the initial material before pretreatment are shown in Figure 4.

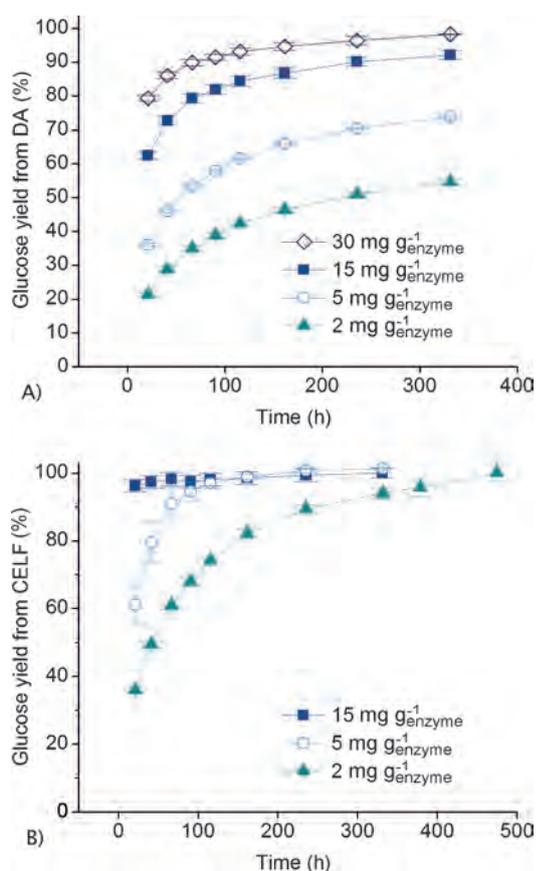


Figure 3. Comparison of glucose yields from the enzymatic hydrolysis of solids from optimized A) DA and B) CELF pretreatments of corn stover versus enzymatic hydrolysis time over a range of Accellerase 1500 enzyme loadings from 2–30 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$. Pretreatment reaction conditions were those that gave the highest total combined sugar yields at a loading of 15 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$: DA: 160 °C, 0.5% H_2SO_4 , 20 min; CELF: 150 °C, 0.5% H_2SO_4 , 25 min, 1:1 THF/water volume ratio.

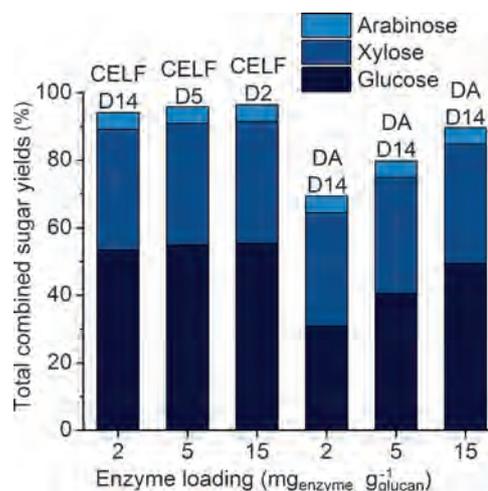


Figure 4. Overall Stage 1+Stage 2 yields of glucose, xylose, and arabinose from CELF- and DA-pretreated corn stover solids based on 100 g of total monomeric equivalent of the glucan, xylan, and arabinan content in untreated corn stover. The Stage 2 incubation time in days is shown at the top of each bar, e.g., D14 represents 14 days. 100% corresponds to the maximum amount of sugars that could be realized from the total glucan, xylan, and arabinan in corn stover.

Although the xylose (34–36%) and arabinose yields (5%) were comparable for both CELF and DA pretreatments as a result of the similar sugar release in Stage 1, total combined sugar yields were higher for CELF pretreatment than for DA pretreatment at all enzyme loadings because of the higher glucose release in Stage 2 from the CELF-pretreated solid. Notably, the overall sugar yields from CELF-pretreated solids reached approximately 95% even at the low enzyme loading of 2 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$, although it took 14 days to reach this level, whereas yields from DA-pretreated solids were only approximately 70% after 14 days at this low enzyme loading. The apparent rate of glucose release was also higher from CELF-pretreated solids, as illustrated by the recovery of over 95% of the total potential sugar in corn stover in only two days following CELF pretreatment compared to the 14 days needed for the DA-pretreated material to achieve an 85% total sugar yield at a loading of 15 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$. Even at the longer incubation times for the latter, the lower total combined sugar yields were mostly attributable to incomplete glucan hydrolysis in Stage 2, as xylose and arabinose recovery was comparable to that from CELF pretreatment. A mass balance that illustrates the fate of each sugar is shown in Supporting Information Figure S1).

Insights gained by a fractal model of enzymatic hydrolysis kinetics

A fractal kinetic model based on an empirical curve fitted to the enzymatic hydrolysis results presented provided additional insights into the cause of the sugar-yield plateau suffered by DA-pretreated corn stover compared to the highly reactive material produced by CELF (fractal model and experimental fit parameters are given in Table S1 and Figure S2, respectively). The fractal model used is based on classical first-order kinetics but replaces the rate constant k with a transient rate coefficient $k_t = kt^{-h}$ that decays over time with a fractal exponent h . The values of the transient rate parameter k_t can be interpreted in terms of both substrate features and enzyme loadings to suggest mechanistic differences in the enzymatic hydrolysis of CELF- and DA-pretreated solids. If k_t is plotted against the glucan conversion (Figure 5A), differences in the enzyme–substrate interactions can be seen. Specifically, in the higher glucan conversion regime of 60–100% in which the accessible substrate surface area was reduced significantly, CELF-pretreated corn stover exhibited higher k_t values that decreased more slowly with increasing conversion from 0.039–0.030 h^{-1} (Figure 5A) at the lower loading of 5 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$, compared to

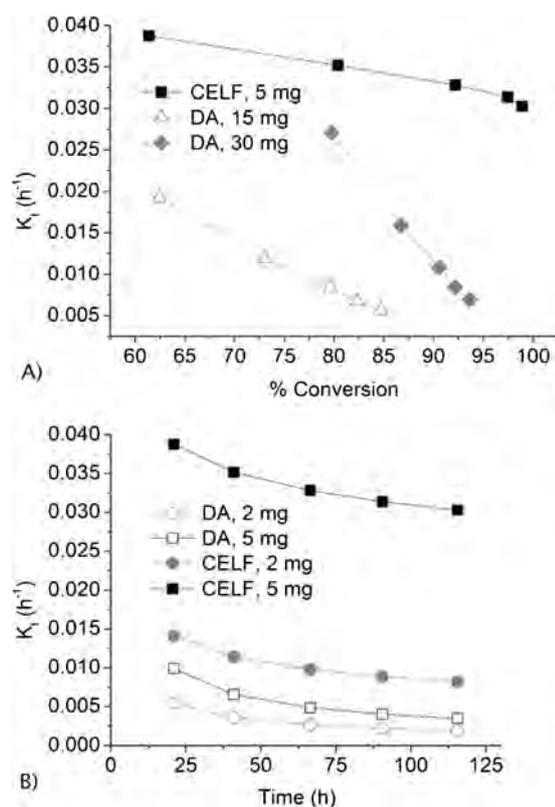


Figure 5. Comparison of the change in fractal kinetic rate coefficient with respect to: A) percent conversion in the higher glucan conversion regime of 60–100% in which the accessible substrate surface area had decreased significantly for CELF pretreatments at 5 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$ (CELF, 5 mg) and DA pretreatments at 15 and 30 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$ (DA, 15 and DA, 30 mg respectively) and B) enzymatic hydrolysis time for the DA and CELF pretreatments at low loadings of 2 and 5 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$ (denoted as DA/CELF, 2 mg and DA/CELF, 5 mg, respectively).

the larger decrease for DA-pretreated corn stover at higher loadings from 0.019–0.006 h^{-1} for 15 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$ and 0.027–0.007 h^{-1} for 30 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$. This large difference suggests that CELF pretreatment produces substrates that sustain a greater accessibility to enzymes over the course of hydrolysis compared to DA pretreatment, which results in the nearly complete digestion of CELF-pretreated corn stover solids.

From a different perspective, the change of reaction rate coefficient k_t with respect to hydrolysis time for both DA and CELF pretreatments is shown in Figure 5B. The temporal progression of k_t from the CELF-pretreated corn stover at 2 and 5 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$ loadings was consistently 3–4 times greater than that of DA-pretreated corn stover at corresponding enzyme loadings, which validates the superior enzymatic reactivity of CELF-pretreated corn stover. As DA and CELF removed similar levels of hemicellulose, these results can be attributed to the extensive removal of lignin during CELF pretreatment, which results in less cellulose surface blockage and enzyme inhibition than that of DA pretreatment. Wang et al.^[20] proposed that a smaller h value correlates with less lignin inhibition, and a lower h value was observed for CELF-pretreated corn stover (0.169 at 5 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$) in comparison to DA-pretreated material at the same enzyme loadings (0.607 at 5 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$; Table S1). Future work with CELF pretreatment will be coupled with fractal kinetic modeling to further investigate how delignification by CELF pretreatment affects substrate–enzyme interactions.

Changes resulting from lignin removal by CELF observed by SEM

An SEM comparison is shown in Figure 6 of the macro- and microstructure between raw corn stover and solids produced by DA and CELF pretreatments to aid us to understand mechanisms that could account for the extraordinary reactivity of CELF-pretreated corn stover with cellulolytic enzymes. As a result of the heterogeneous distribution of raw corn stover, particles with common surface features were imaged for comparison. The outer structure of raw corn stover appears porous with sheetlike pleats that surround the open vascular networks used for water and nutrient delivery to the plant (Figure 6A). If the corn stover was pretreated with DA, the macrostructure of the solids was changed by the destruction of the sheetlike walls to reveal the porous vascular network underneath (Figure 6B), which is likely a primary feature to improve accessibility for enzyme attack. The significant amount of lignin still present in solids following DA pretreatment apparently preserves the major features of raw corn stover. In contrast, CELF altered the macro- and microstructural features that are vital for the efficient deconstruction of lignocellulosic biomass significantly (Figure 6C),^[7a] which is likely caused by extensive lignin removal that collapses most of the superstructure so that neither the sheetlike walls nor the vascular channels can be distinguished easily. This drastic destruction and collapse shown in the dried cell-wall structure after CELF pretreatment may be responsible for the sustained reactivity of the substrate to enzymatic hy-

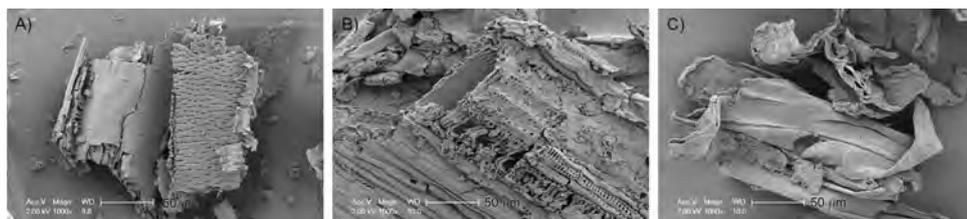


Figure 6. SEM images of solids at 1000X magnification from A) raw corn stover, B) DA-pretreated corn stover (160 °C, 0.5 wt% sulfuric acid, 20 min), and C) CELF-pretreated corn stover (150 °C, 0.5 wt% sulfuric acid, 25 min). Scale bar is shown.

drolysis. The visible “wrinkling” of the material after CELF pretreatment was likely because of the drying of the sample, which indicates that the material may be structurally weaker and become expanded readily if rehydrated by water, somewhat like a sponge. Our next steps include the application of ^{13}C P NMR spectroscopy and Simons’ staining and water retention to compare the relative effects of CELF and DA pretreatments on crystallinity and accessibility, respectively, as well as to record more extensive SEM images and particle size distributions to test these hypotheses.

Simultaneous saccharification and fermentation of pretreated corn stover to ethanol

We applied SSF to both CELF- and DA-pretreated solids to demonstrate the compatibility of CELF pretreatment with the achievement of high ethanol yields by fermentation. SSF combines the enzymatic hydrolysis of biomass to sugars with sugar fermentation to ethanol in a single step to reduce the end-product inhibition of enzymes by high soluble sugar concentrations and thereby increase ethanol yields and titers.^[10,21] In this study, SSF was applied to pretreated solids in 125 mL shake flasks at 4 wt% glucan loading; each flask contained 50 mL working volume and was fitted with a bubble trap and inoculated with the D₅A strain of *S. cerevisiae* yeast along with Accellerase 1500. The ethanol yields obtained if SSF was applied to solids produced by DA and CELF pretreatments under optimized conditions compared to yields from an Avicel cellulose control at loadings of 5 and 15 mg_{enzyme} g_{glucan}⁻¹ are shown in Figure 7A and B. These results show that although a concern

of solvent-based pretreatment, such as with ionic liquids, can be the toxicity of residual solvent in pretreated solids towards enzymes or microbes that would reduce ethanol yields, simply washing CELF-pretreated solids with water through a vacuum filter was sufficient to achieve high yields by SSF. Furthermore, consistent with our results for just enzymatic hydrolysis of the

same solids, higher SSF ethanol yields were obtained for solids from CELF pretreatment at both enzyme loadings. For the higher loading of 15 mg_{enzyme} g_{glucan}⁻¹, the ethanol yield plateaued at approximately 90 and 83% of the theoretical yield for solids from CELF and DA pretreatments, respectively, and the yield increased much more rapidly for the CELF-pretreated solids (Figure 7A). However, Avicel microcrystalline cellulose was more recalcitrant than corn stover solids produced by either pretreatment, and the ethanol yields were still increasing at ~300 h fermentation time. These results suggest that, unlike CELF-pretreated material, the high lignin content of DA-pretreated corn stover and the lower enzyme accessibility of crystalline Avicel limit the effectiveness of hydrolytic enzymes and reduce overall ethanol yields. The differences are even more dramatic at a more commercially affordable enzyme loading of 5 mg_{enzyme} g_{glucan}⁻¹, for which yields from CELF-pretreated solids peaked at over 90% in only four days, whereas the yield for DA-pretreated solids was only approximately 50% after six days. Thus, CELF pretreatment gave a superior conversion of corn stover to ethanol compared to either of the other two substrates to achieve ~90% or higher ethanol yields consistently at both 15 and 5 mg g_{glucan}⁻¹ enzyme loadings. Future studies will optimize SSF ethanol yields from CELF-pretreated solids at higher solid concentrations to realize more economically attractive ethanol titers.

The yields of metabolites after seven days of culture to close material balances on sugars are shown in Figure 7C. More glycerol was produced by the conversion of CELF- than DA-pretreated corn stover solids, likely because of the greater osmotic stress generated by the higher initial glucose concentra-

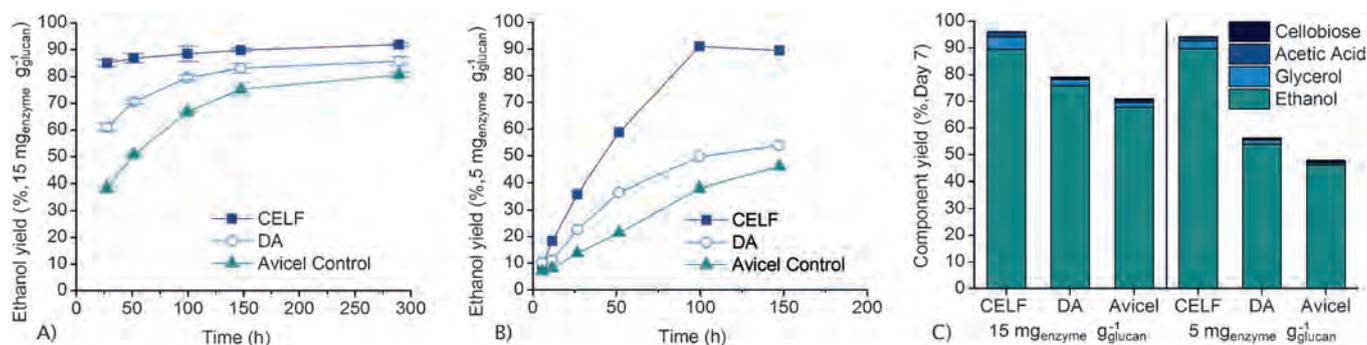


Figure 7. Ethanol yields from SSF of solids from DA and CELF pretreatment of corn stover and Avicel PH-101 cellulose versus culture time at A) 15 mg_{enzyme} g_{glucan}⁻¹ and B) 5 mg_{enzyme} g_{glucan}⁻¹ loading of Accellerase 1500 enzyme. C) Cumulative yields [%] of SSF metabolites from a seven day culture.

tions^[22] or higher ethanol concentrations at the completion of the fermentation. Additional optimization to reduce residual glucose concentrations during culture may further improve ethanol yields. Acetic acid and cellobiose concentrations remained minimal throughout the fermentation for each of the samples. Thus, ethanol yields from SSF were mostly governed by the extent of sugar release by enzymes and not by yeast performance.

The integration of CELF pretreatment with SSF to produce ethanol from lignocellulosic biomass is outlined in Figure 8 in a simplified block flow diagram. THF and sulfuric acid can be introduced directly to wet biomass and fed to a high-solids reactor (i.e., a Pandia reactor) that is heated to reaction temperature by direct steam injection. After the CELF reaction, depressurization of the contents at the reactor exit decreases the temperature rapidly to 100 °C to quench further reaction. The solids from the reactor can then be separated from the liquids and washed with water in a countercurrent belt filter to remove soluble inhibitors released in pretreatment and limit sugar dilution. The liquids are then delivered to a distillation island in which THF can be recovered. Although THF forms a 95% azeotrope with water, further energy-intensive separation is not required because the recycled THF stream does not require a high purity.^[13] Furthermore, the CELF process is inherently aqueous based and does not require distillation of THF to dryness, which avoids the concentration of THF peroxides to dangerous levels. If needed, peroxide destruction methods practiced industrially, such as caustic soda treatment, can be implemented without detriment to the process. Upon the removal and recovery of THF, the dissolved lignin product precipitates as a solid that could potentially be upgraded catalytically to valuable chemicals and fuel products or burned to provide process heat and power. Neutralization and conditioning of the liquid stream by a suitable base (e.g., overliming with calcium hydroxide) is applied to make the dissolved xylose and arabinose released during pretreatment suitable for biological fermentation. Although Figure 8 shows that both the liquid and washed-solid streams feed the same SSF operation, the

liquid stream rich in xylose and arabinose from CELF pretreatment could be fed to one fermentation train and the cellulose-rich solids fed to a separate SSF operation. In the latter case, micro-organisms engineered for the fermentation of dissolved xylose and arabinose sugars could be employed in the first fermenter, and conventional yeast such as *S. cerevisiae* could be employed for the combined enzymatic hydrolysis and fermentation of the cellulose-rich solids in the second, with high ethanol yields expected based on the results in this study. Our future work will comprise fermentation of the soluble sugars from Stage 1 to ethanol.

Conclusions

THF is a biomass-sourced green solvent with catalytic qualities that promotes biomass deconstruction and delignification. We have shown a significant augmentation of traditional DA pretreatment by application of THF as a miscible co-solvent, which represents a significant advancement in pretreatment technology. Optimization of this pretreatment strategy, co-solvent-enhanced lignocellulosic fractionation (CELF), at 150 °C with 0.5 wt% sulfuric acid resulted in total (arabinan, xylan, and glucan) combined (Stage 1+Stage 2) sugar yields of ~95% of the theoretical maximum at a low enzyme loading of 2 mg_{g_{glucan}}⁻¹. Unlike DA pretreatment, yields from the enzymatic hydrolysis of solids from the CELF pretreatment of corn stover remained high over a wide range of pretreatment times, such that optimization of the process could focus on the maximization of the xylose recovery in Stage 1. The exceptional sugar yields from the enzymatic hydrolysis of CELF-pretreated solids also translated into an excellent simultaneous saccharification and fermentation (SSF) performance with *S. cerevisiae* D₅A of over 90% ethanol yields at an enzyme loading of only 5 mg_{protein} g_{glucan}⁻¹. A process strategy was outlined such that CELF pretreatment could be integrated with SSF to produce ethanol directly from lignocellulosic biomass and THF could be recycled. Future characterization of the pretreated solids on multiple feedstocks is planned to better understand how CELF alters the physiochemical features of biomass and enhances sugar yields.

CELF also provided new insights into promising pretreatment strategies that could enhance enzymatic hydrolysis. By tracing the fate of the primary components in biomass after DA and CELF pretreatments, we showed that virtually complete lignin removal was the key difference that could account for the enhanced enzymatic hydrolysis of the solids from CELF pretreatment. Our fractal kinetic analysis of the experimental enzymatic hydrolysis data also pointed to lignin removal by

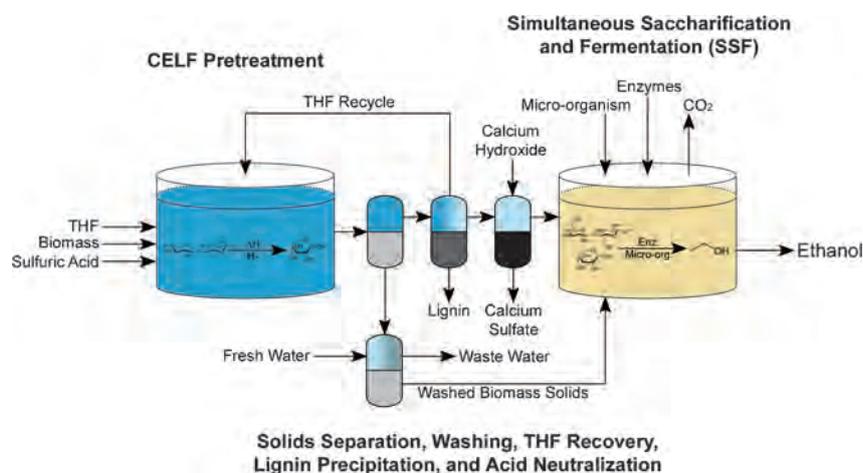


Figure 8. Simplified block flow diagram of a proposed biomass conversion process that integrates CELF pretreatment with SSF to produce ethanol.

CELLF that resulted in higher accessibility and less inhibition towards cellulolytic enzymes than that possible for DA pretreatment. SEM images showed that extensive lignin removal by CELLF pretreatment apparently altered the vascular and support structures of corn stover dramatically such that the cell walls were completely collapsed and the material appeared “wrinkled” after air-drying.

Experimental Section

Compositional analysis of corn stover was performed according to the established NREL procedure (version 8-03-2012) in triplicate. The resulting mass composition was $(37.1 \pm 0.7)\%$ glucan, $(25.1 \pm 0.3)\%$ xylan, $(4.2 \pm 0.1)\%$ arabinan, $(14.5 \pm 0.4)\%$ K-lignin, and 28.0% other materials.^[23] Other materials are usually composed of ash (4–6 wt%), proteins (2–3 wt%), acetic acid (2–4 wt%), sugar acids (1–2 wt%), and extractives (2–8 wt%) that were not quantified in this study.^[24] Frozen stock of *Saccharomyces cerevisiae* (D₅A) was prepared from plate monocultures that were transferred and cultured in a shaker incubator at 150 rpm and 38 °C in yeast peptone dextrose (YPD) media that contained yeast extract (10 g L^{-1}), peptone (20 g L^{-1}), and glucose (50 g L^{-1}). After 24 h incubation, a 40 wt% glycerol in water solution was added, and 1 mL aliquots of the resulting mixture were transferred to sterile cryovials and placed in a -70°C freezer. Before each SSF run, the inoculum was prepared by thawing, transferring, and growing the frozen stock on a shaker incubator at 150 rpm and 37 °C for 12 h in 250 mL baffled flasks with YPD medium. The inoculum was then centrifuged and resuspended in sterile deionized (DI) water twice for washing and prepared for inoculation at a 0.5 optical density (O.D.).

Analytical procedures

All chemical analyses were based on Laboratory Analytical Procedures (LAPs) documented by NREL (Golden, CO; http://www.nrel.gov/biomass/analytical_procedures.html). Liquid samples along with appropriate calibration standards were analyzed by HPLC (Waters Alliance 2695 system equipped with a Bio-Rad Aminex HPX-87H column and Waters 2414 refractive index (RI) detector) with an eluent (5 mM sulfuric acid) flow rate of 0.6 mL min^{-1} . The chromatograms were integrated by using Empower 2 software package (Waters Co., Milford, MA). As developed and practiced consistently in our laboratory,^[16,25] combined total sugar yields (Stage 1+Stage 2) from each pretreatment were determined as the sum of the total mass of soluble glucose, xylose, and arabinose released by pretreatment (Stage 1) plus the total mass of these three sugars released by saccharification of the washed pretreated solids with enzymes (Stage 2, enzymatic hydrolysis). Details of the calculation of sugar yields are outlined in the Supporting Information. As a result of their greater abundance in corn stover composition, total sugars were considered to include glucose, xylose, and arabinose. The total lignin recovered was calculated from the mass of total unwashed K-lignin precipitated upon recovery of THF, whereas delignification was calculated from the percentage of K-lignin that remained in the pretreated material compared to the initial K-lignin content of the raw material.

Pretreatment of corn stover

Pretreatment reactions were performed in a 1 L Hastelloy Parr autoclave reactor (236HC Series, Parr Instruments Co., Moline, IL) equipped with a double stacked pitch blade impeller rotated at

200 rpm. The THF co-solvent mixture for CELLF pretreatment contained a 1:1 volume ratio of THF (>99% purity, Fisher Scientific, Pittsburgh, PA) and water. A 0.5 wt% sulfuric acid (Ricca Chemical Company, Arlington, TX) concentration was found to achieve the highest total glucose+xylose+arabinose yield from DA alone or CELLF pretreatment coupled with subsequent enzymatic hydrolysis.^[16] Before each reaction, corn stover was added to the acid solution and soaked overnight at 4 °C. Corn stover solid loadings were 5 wt% for both the CELLF and DA pretreatments based on the total working mass of liquids and solids in the reaction. All reactions were maintained at temperature ($\pm 2^\circ\text{C}$) by convective heating by using a 4 kW fluidized sand bath (Model SBL-2D, Techne, Princeton, NJ), and the reactor temperature was measured directly by using an in-line thermocouple (Omega, K-type). The sand bath temperature was set to 340 °C to reduce the heat-up time to under 3 min.^[26] At the conclusion of each reaction, the reactor was cooled by submerging quickly it in a large water bath at RT. The solids were then separated from the reaction liquor by vacuum filtration at RT through glass fiber filter paper (Fisher Scientific, Pittsburgh, PA). The mass and density of the liquid fractions were measured to complete accurate yield calculations. As a result of differences in density between the co-solvent mixtures and pure water, final densities were determined by weighing 25 mL of the reacted liquid in a volumetric flask after each reaction. Liquid samples were analyzed by HPLC as described previously.

Enzymatic hydrolysis of pretreated corn stover and Avicel cellulose

As noted in the NREL standard protocol,^[27] enzymatic hydrolysis of pretreated materials was performed in triplicate using 125 mL Erlenmeyer flasks with a 50 g total working mass that contained 50 mM citrate buffer (pH 4.8) to maintain pH, 0.02% sodium azide to prevent microbial growth, and approximately 1 wt% glucan from pretreated solids or Avicel PH-101 cellulose (Sigma Aldrich, St. Louis, MO). The cellulase enzyme (Accellerase1500, DuPont Industrial Biosciences, Palo Alto, CA) loadings were varied from 2–30 $\text{mg}_{\text{enzyme}} \text{g}_{\text{glucan}}^{-1}$ based on the mass of glucan in the raw corn stover, as described elsewhere,^[28] and not according to the glucan content of the pretreated material to provide a useful comparison among different pretreatments.^[5a,6b,29] Enzyme loadings based on the raw material are important because a pretreatment should not be penalized for releasing sugars before enzymatic hydrolysis, as total sugar yields from both Stage 1 (pretreatment)+Stage 2 (enzymatic hydrolysis) should be maximized.^[30] The flasks that contained biomass slurry were placed in a Multitron orbital shaker (Infors HT, Laurel, MD) set at 150 rpm and 50 °C and allowed to equilibrate for 1 h before the addition of enzyme. Samples of approximately 0.75 mL were taken periodically into 2 mL centrifuge vials (Fisher Scientific, Pittsburgh, PA) from each flask and centrifuged at 14000 rpm for 5 min to determine the progress of the enzymatic hydrolysis. The supernatant was then transferred into 500 μL HPLC vials (Grace Davison, Deerfield, IL) for HPLC analysis.

Fractal modeling of hydrolysis kinetics

A fractal model based on first-order chain breakdown to form glucose was found to best describe cellulose hydrolysis with the rate coefficient k_t related to the hydrolysis time raised to the fractal exponent h [Eq. (1)].^[31]

$$\frac{dC}{dt} = k_t C, \text{ in which } k_t = kt^{-h} \quad (1)$$

The model described by Equation (2) was used to fit the experimental data from the enzymatic hydrolysis by nonlinear regression using MATLAB 7.0 (damped least squares) in which X [%] is the conversion and t [h] is time:

$$X = 100 * \left\{ 1 - \exp \left[-k \left(1 + \frac{t^{1-h} - 1}{1-h} \right) \right] \right\} \quad (2)$$

Cell cultivation and SSF

Consistent with NREL standard protocols,^[32] SSF was performed in triplicate in 125 mL flasks with a 50 g working mass that contained citrate buffer (50 mM, pH 4.8), yeast extract (10 g L⁻¹, Becton, Dickinson and Company, Redlands, CA), peptone (20 g L⁻¹, Becton, Dickinson and Company, Redlands CA), tetracycline (40 mg L⁻¹, Sigma Aldrich, St. Louis, MO) as an antimicrobial agent, Accellerase 1500 cellulase (loaded at 5 or 15 mg_{protein} g_{glucan}⁻¹), and D₅A frozen stock culture. DI water and solids were loaded into flasks (with attached bubble traps) to achieve a 4 wt% glucan loading of either the pretreated solid residues or Avicel PH 101 cellulose, and masses of the whole flask assembly were recorded before autoclaving at 121 °C for 30 min. Flasks were then cooled, reweighed, and moved into a laminar flow hood (Baker and Baker Ruskinn, Sanford, ME) for aseptic readdition of presterilized DI water to replenish water loss, yeast extract, citrate buffer, tetracycline, Accellerase 1500 cellulase, and cell inoculum.

SEM imaging

A field-emission scanning electron microscope (Philips XL-30) was used to provide images of the raw, pretreated, and post-enzymatic hydrolysis corn stover. Air-dried samples of each were placed on pin-stub mounts with carbon tape and sputter coated with Pt by using a Cressington 108 Auto system (Ted Pella Inc, Redding CA). The surface macro- and microstructures of the samples were characterized at an acceleration voltage of 2 kV.

Acknowledgements

We are grateful for funding by the Office of Biological and Environmental Research in the Department of Energy (DOE) Office of Science through the BioEnergy Science Center (BESC) at Oak Ridge National Laboratory (Contract DE-PS02-06ER64304), the National Science Foundation (Grant #2013142496), and the University of California Transportation Center (UCTC) for making this research possible. We also acknowledge the Center for Environmental Research and Technology (CE-CERT) of the Bourns College of Engineering for providing the facilities and the Ford Motor Company for funding the Chair in Environmental Engineering that facilitates projects such as this one. We would like to acknowledge Dr. Taiying Zhang for her contribution to the inception of this paper.

Keywords: biomass • enzymes • hydrolysis • renewable resources • solvent effects

[1] a) C. E. Wyman, *Trends Biotechnol.* **2007**, *25*, 153–157; b) C. E. Wyman, G. Huber, *Biofuels Bioprod. Biorefin.* **2009**, *3*, 105–107.

- [2] R. D. Perlack, B. J. Stokes, U.S. Department of Energy, Oak Ridge National Laboratory, Oak Ridge, TN, **2011**, p. 227p.
- [3] a) L. Lynd, N. Greene, B. Dale, M. Laser, D. Lashof, M. Wang, C. Wyman, *Science* **2006**, *312*, 1746–1748; b) L. R. Lynd, M. S. Laser, D. Bransby, B. E. Dale, B. Davison, R. Hamilton, M. Himmel, M. Keller, J. D. McMillan, J. Sheehan, C. E. Wyman, *Nat. Biotechnol.* **2008**, *26*, 169–172.
- [4] a) B. Yang, C. E. Wyman, *Biofuels Bioprod. Biorefin.* **2008**, *2*, 26–40; b) L. R. Lynd, C. E. Wyman, T. U. Gerngross, *Biotechnol. Prog.* **1999**, *15*, 777–793.
- [5] a) N. Mosier, C. Wyman, B. Dale, R. Elander, Y. Y. Lee, M. Holtzapple, M. Ladisch, *Bioresour. Technol.* **2005**, *96*, 673–686; b) R. Kumar, G. Mago, V. Balan, C. E. Wyman, *Bioresour. Technol.* **2009**, *100*, 3948–3962.
- [6] a) L. da Costa Sousa, S. P. S. Chundawat, V. Balan, B. E. Dale, *Curr. Opin. Biotechnol.* **2009**, *20*, 339–347; b) C. E. Wyman, B. E. Dale, V. Balan, R. T. Elander, M. T. Holtzapple, R. S. Ramirez, M. R. Ladisch, N. S. Mosier, Y. Y. Lee, R. Gupta, S. R. Thomas, B. R. Hames, R. Warner, R. Kumar, in *Aqueous Pretreatment of Plant Biomass for Biological and Chemical Conversion to Fuels and Chemicals*, Wiley, Weinheim, **2013**, pp. 239–259.
- [7] a) R. Kumar, C. E. Wyman in *Aqueous Pretreatment of Plant Biomass for Biological and Chemical Conversion to Fuels and Chemicals*, Wiley, Weinheim, **2013**, pp. 281–310; b) V. Arantes, J. Saddler, *Biotechnol. Biofuels* **2011**, *4*, 3.
- [8] Y.-H. P. Zhang, S.-Y. Ding, J. R. Mielenz, J.-B. Cui, R. T. Elander, M. Laser, M. E. Himmel, J. R. McMillan, L. R. Lynd, *Biotechnol. Bioeng.* **2007**, *97*, 214–223.
- [9] K. Karimi, M. Shafiei, R. Kumar in *Biofuel Technologies* (Eds.: V. K. Gupta, M. G. Tuohy), Springer, Heidelberg, **2013**, pp. 53–96.
- [10] A. Schedemann, E. C. Ihmels, J. Gmehling, *Fluid Phase Equilib.* **2010**, *295*, 201–207.
- [11] D. Humbird, R. Davis, L. Tao, C. Kinchin, D. Hsu, A. Aden, P. Schoen, J. Lukas, B. Olthof, M. Worley in *National Renewable Energy Laboratory Technical Report NREL, TP-5100-47764*, **2011**.
- [12] N. Weiss, N. Nagle, M. Tucker, R. Elander, *Appl. Biochem. Biotechnol.* **2009**, *155*, 115–125.
- [13] C. M. Cai, T. Zhang, R. Kumar, C. E. Wyman, *Green Chem.* **2013**, *15*, 3140–3145.
- [14] C. M. Cai, N. Nagane, R. Kumar, C. E. Wyman, *Green Chem.* **2014**, *16*, 3819–3829.
- [15] J. Matous, J. Novak, J. Sobr, J. Pick, *Czech. Chem. Commun.* **1972**, *37*, 2653–2663.
- [16] T. A. Lloyd, C. E. Wyman, *Bioresour. Technol.* **2005**, *96*, 1967–1977.
- [17] a) R. Kumar, F. Hu, P. Sannigrahi, S. Jung, A. J. Ragauskas, C. E. Wyman, *Biotechnol. Bioeng.* **2013**, *110*, 737–753; b) P. Sannigrahi, D. H. Kim, S. Jung, A. Ragauskas, *Energy Environ. Sci.* **2011**, *4*, 1306–1310.
- [18] D. Klein-Marcuschamer, P. Oleskowicz-Popiel, B. Simmons, H. Blanch, *Biotechnol. Bioeng.* **2012**, *109*, 1083–1087.
- [19] a) S.-Y. Ding, Y.-S. Liu, Y. Zeng, M. E. Himmel, J. O. Baker, E. A. Bayer, *Science* **2012**, *338*, 1055–1060; b) B. Yang, C. E. Wyman, *Biotechnol. Bioeng.* **2006**, *94*, 611–617; c) L. Kumar, V. Arantes, R. Chandra, J. Saddler, *Bioresour. Technol.* **2012**, *103*, 201–208.
- [20] Z. Wang, J.-H. Xu, H. Feng, H. Qi, *Bioresour. Technol.* **2011**, *102*, 2890–2896.
- [21] P. Andrić, A. S. Meyer, P. A. Jensen, K. Dam-Johansen, *Biotechnol. Adv.* **2010**, *28*, 308–324.
- [22] E. Nevoigt, U. Stahl, *FEMS Microbiol. Rev.* **1997**, *21*, 231–241.
- [23] J. B. Sluiter, R. O. Ruiz, C. J. Scarlata, A. D. Sluiter, D. W. Templeton, *J. Agric. Food Chem.* **2010**, *58*, 9043–9053.
- [24] D. W. Templeton, C. J. Scarlata, J. B. Sluiter, E. J. Wolfrum, *J. Agric. Food Chem.* **2010**, *58*, 9054–9062.
- [25] T. Zhang, R. Kumar, C. E. Wyman, *Carbohydr. Polym.* **2013**, *92*, 334–344.
- [26] B. Yang, C. E. Wyman, *Methods Mol. Biol.* **2009**, *581*, 103–114.
- [27] M. Selig, N. Weiss, Y. Ji, Laboratory Analytical Procedures (LAPs), National Renewable Energy Laboratory, Golden, CO, **2008**.
- [28] X. Gao, R. Kumar, S. Singh, B. Simmons, V. Balan, B. Dale, C. Wyman, *Biotechnol. Biofuels* **2014**, *7*, 71.
- [29] a) C. E. Wyman, V. Balan, B. E. Dale, R. T. Elander, M. Falls, B. Hames, M. T. Holtzapple, M. R. Ladisch, Y. Y. Lee, N. Mosier, V. R. Pallapolu, J. Shi, S. R. Thomas, R. E. Warner, *Bioresour. Technol.* **2011**, *102*, 11052–11062; b) R. J. Garlock, V. Balan, B. E. Dale, V. Ramesh Pallapolu, Y. Y. Lee, Y. Kim, N. S. Mosier, M. R. Ladisch, M. T. Holtzapple, M. Falls, R. Sierra-Ramirez, J. Shi, M. A. Ebrik, T. Redmond, B. Yang, C. E. Wyman, B. S. Donohoe, T. B.

- Vinzant, R. T. Elander, B. Hames, S. Thomas, R. E. Warner, *Bioresour. Technol.* **2011**, *102*, 11063–11071.
- [30] P. Pollet, E. A. Davey, E. E. Urena-Benavides, C. A. Eckert, C. L. Liotta, *Green Chem.* **2014**, *16*, 1034–1055.
- [31] Z. Wang, H. Feng, *Bioresour. Technol.* **2010**, *101*, 7995–8000.
- [32] N. Dowe, J. McMillan, in *National Renewable Energy Laboratory Technical Report NREL*, TP-510-42630, **2008**.

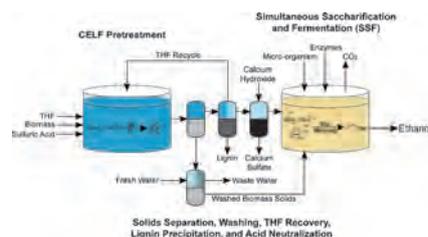
Received: September 24, 2014

Revised: December 7, 2014

Published online on ■ ■ ■■, 0000

FULL PAPERS

Break it down! We describe a new pretreatment approach, co-solvent-enhanced lignocellulosic fractionation (CELf), that reduces plant recalcitrance by solvating biomass lignin using acidified aqueous THF solutions to improve sugar yields and reduce enzyme requirements for saccharification. Our results provide important insights into promising biomass pretreatment strategies.



*T. Y. Nguyen, C. M. Cai, R. Kumar, C. E. Wyman**



Co-solvent Pretreatment Reduces Costly Enzyme Requirements for High Sugar and Ethanol Yields from Lignocellulosic Biomass

