Chapter 20

Advances in Cellulose Solvent- and Organic Solvent-Based Lignocellulose Fractionation (COSLIF)

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Cost-effective release of soluble fermentable sugars from lignocellulose, the most abundant form of renewable biomass, is among the most costly steps for emerging biorefineries. Lignocelluosic biomass is a complicated natural composite, primarily consisting of three biopolymers: cellulose, hemicellulose, and lignin. Distinct from high temperature/pressure required for most lignocellulose pretreatments (e.g., dilute acid, ammonia fiber expansion, ammonia recycle percolation, and so on), cellulose solvent- and organic solvent-based lignocellulose fractionation (COSLIF) has been developed to fractionate lignocellulose components (cellulose, hemicellulose, acetic acid, and lignin) at modest reaction conditions (Zhang Y.-H.P., et al. *Biotechnol. Bioeng.* **2007**, *97*, 214−223). Separation of the three polymers can be implemented based on their different solubility in a cellulose solvent (concentrated phosphoric acid), an organic solvent (e.g., acetone or ethanol) and water; recycling of phosphoric acid and the organic solvent can be conducted based on the solvents' different volatilities. Very high glucan digestibilities (e.g., ~96-97% in hour 24) were obtained for several types

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of biomass, such as corn stover, switchgrass, hemp hurds and hybrid poplar, at a cellulase loading of 15 filter paper units per gram of glucan. At a low enzyme loading (5 filter paper units per gram of glucan), the digestibility remained as high as 93% at hour 24 for the COSLIF-pretreated corn stover but only reached ~60% for the dilute acid (DA)-pretreated biomass. As compared to the DA-pretreated biomass, higher glucan digestibility and faster enzymatic hydrolysis rates for the COSLIF-pretreated corn stover were in good agreement with (i) more efficient biomass structure destruction and (ii) larger cellulose accessibility to cellulase.

Introduction

Cellulose, the most abundant renewable bioresource (ca. 1 x 10^{11} tons/year), is mainly produced by terrestrial plants (*1–4*). Technologies for effectively converting low-cost agricultural and forestry residues (lignocellulosic biomass) to biofuels and biobased products offer many benefits to society, including improved energy security, decreased trade deficits, healthier rural economies, improved environmental quality, nearly zero net greenhouse gas emissions, technology exports, and sustainable utilization of renewable resources (*4–9*). Effectively overcoming lignocellulose recalcitrance to release soluble sugars is still the largest technical and economic challenge for the emerging biofuels and biobased chemical industries (*4*, *10*, *11*).

The conversion of biomass to simple sugars usually involves two sequential steps – lignocellulose pretreatment and enzymatic cellulose hydrolysis (Fig. 1). Two different strategies have been proposed and investigated. The substrate strategy is focused on lignocellulose pretreatment (identification of the best pretreatment methods and optimization of reaction conditions), resulting in increased reactivity of pretreated lignocellulosic feedstock so that commercial available low-cost *Trichoderma* cellulase can work efficiently. The enzyme strategy is focused on improving cellulase performance so that biomass pretreatment could be minimized or avoided. Cellulase development could include (i) construction of artificial cellulosomes that are believed to have much higher specific hydrolysis activity than non-complexed *Trichoderma* cellulase (*12–15*) or recombinant cellulolytic microorganisms, and (ii) the introduction of recombinant cellulase-expressing bioenergy plants, providing a more reactive structure for enzymatic hydrolysis (*16*). Biomass recalcitrance can also be reduced by using genetic engineering tools to modify the composition of energy crop plants (*17*).

Fractionation and co-utilization of all the major components of the lignocellulose feedstock is more and more accepted to be vital for biorefineries because of the tight margins associated with fuel production from cellulose and hemicellulose and feedstock prices (*4*, *18*). Mature industries, such as crude oil refineries and corn-ethanol biorefineries, produce a variety of products from

their multi-component feedstocks. Although corn wet milling-based biorefineries require higher initial capital investment and higher processing costs than dry milling systems, the former is the dominant process for large plants (*19*, *20*) because of higher revenues generated by co-products such as gluten feed, gluten meal, and corn oil (*21*). These value-added products account for approximately a third to a half of the wet milling total revenue (*22*), whereas less effective fractionation in the dry milling process results in co-product revenues accounting for only 20% of the total revenue (*20*).

Given that typical biomass contains 40% glucan, 20% hemicellulose, 20% lignin and 4% acetate (from hemicellulose), biomass-based biorefineries could produce up to 100 gallons of cellulosic ethanol per ton of dry biomass after some process improvements (high sugar liberation yields and high sugar-to-ethanol yields). The delivered biomass costs, including growth, harvesting, collection and delivery, could range from $60 to $120 per dry ton. If the selling price of cellulosic ethanol is around $2.5 per gallon, and no other co-products are produced, the margin between the main product revenue and feedstock costs would be between $130-190 per ton of biomass. It would be challenging for this narrow margin to cover all the required expenditures, such as cellulase costs ($0.2-1.0 per gallon of ethanol = $20-100/ton biomass), distillation ($0.2-0.4 per gallon of ethanol = $20-40 per gallon of biomass), pretreatment, waste treatment, labor, tax, and capital depreciation ($0.4-1.0 per gallon of ethanol = $40-100 ton of biomass). With the co-utilization of lignocellulose components such as hemicellulose, acetic acid and lignin, a more robust and economically feasible biorefinery is possible. Effective isolation of high-value hemicellulose could provide an especially profitable opportunity. Already, plants producing xylitol as a major product from corn cob hemicellulose have good profits in China. Similarly, isolation of acetic acid prior to ethanol fermentation could further increase revenues (~$40 per ton of biomass or $0.40 credit per gallon of ethanol) and would decrease inhibition of ethanol fermentation. Isolation of a large amount of high-quality lignin would generate numerous opportunities for high-end applications, such as carbon fiber polymers (*4*).

This chapter provides a description and research update for a technology called cellulose solvent- and organic solvent-based lignocellulose fractionation (COSLIF), which can separate lignocellulose components from lignocellulosic biomass.

Cellulose Solvent- and Organic Solvent-Based Lignocellulose Fractionation (COSLIF)

COSLIF Mechanism

A new technology called cellulose solvent- and organic solvent-based lignocellulose fractionation (COSLIF) has been shown to separate lignocellulose components under modest reaction conditions (e.g., 50°C and atmospheric pressure) by using a cellulose solvent, an organic solvent, and water (*18*). The key

ideas of COSLIF are (1) removal of partial lignin and hemicellulose (eliminating the major obstacles to cellulose hydrolysis and allowing cellulase to access the substrate more efficiently) (*4*, *23*, *24*), (2) de-crystallization of cellulose fibers by a cellulose solvent (providing better cellulose accessibility to cellulase) (*25*, *26*), and (3) modest reaction conditions (causing a decrease in sugar degradation, less inhibitor formation, lower utility consumption, and less capital investment) (*24*, *27*, *28*).

After searching for a number of cellulose solvents suitable for biomass dissolution and considering their recycling, we find out that concentrated phosphoric acid is a good cellulose solvent for biorefineries. Concentrated phosphoric acid is a modest acid. When its concentration is more than a critical value, it can completely dissolve cellulose under mild conditions (*26*). Different from a strong acid sulfuric acid, formation of esters between phosphoric acid and cellulose is very weak. When acid concentration is decreased by water dilution, derivation effect becomes negligible.

Figure 2 shows the overall processes of the COSLIF technology, using concentrated phosphoric acid as the cellulose solvent and acetone as the organic solvent, including a solvent recycling scheme. The mechanisms for each unit operation are:

(1) in the digestion tank, concentrated H_3PO_4 (> 83%) is mixed with grounded lignocellulose at 50°C for ~30-60 minutes depending on biomass type. The cellulose solvent can
   i) break up all linkages among lignin, hemicellulose, and cellulose;
   ii) dissolve cellulose fibrils and hemicellulose, which breaks up orderly hydrogen bonds among sugar chains;
   iii) weakly hydrolyze cellulose and hemicellulose to modestly reduce their degree of polymerization (DP); and
   iv) provide acidic conditions, which cause removal of acetyl groups from hemicellulose.
(2) in the precipitation tank, an organic solvent (e.g., acetone or ethanol) is added to precipitate the dissolved cellulose and hemicellulose and to dissolve partial lignin in the organic solvent;
(3) in washer-1 (solid/liquid separator), organic solvent washes out ~99.5% of phosphoric acid from the precipitated solids and removes more lignin (by leaching);
(4) in washer-2 (solid/liquid separator), water is used to wash the organic solvent from the solids and to remove water-soluble short-DP hemicellulose fragments from the solid cellulose;
(5) in the hydrolysis reactors, nearly pure amorphous cellulose is hydrolyzed quickly at 50°C with the *Trichoderma* cellulase;
(6) in the distiller, the black liquor containing phosphoric acid, acetone, acetone-soluble lignin, and acetic acid can be separated. Highly volatile acetone and modestly volatile acetic acid are separated by fractionation distillation; after removal of the organic solvent, the precipitated lignin can be separated from the concentrated phosphoric acid at the bottom of the column by a solid/liquid separator; and

(7) in the flash tank, the light liquor containing acetone, a small amount of phosphoric acid and water-soluble hemicellulose can be separated. Acetone can be recovered by flashing. Addition of $CaCO_3$ can neutralize trace phosphoric acid and form a precipitate of $Ca_3(PO4)_2$; the precipitated $Ca_3(PO4)_2$ can be regenerated to concentrated phosphoric acid by adding concentrated sulfuric acid. Water-soluble hemicellulose remains in the liquid phase.

In all, this technology can fractionate lignocellulose into amorphous cellulose (mainly glucose after hydrolysis), lignin, hemicellulose, and acetic acid at modest reaction conditions (50°C, atmospheric pressure) with simple recycling of the organic solvent and phosphoric acid. This new technology isolates lignocellulose components based on their solubility in different solvents, while using low-cost separation operations, e.g., solid/liquid separation. Cellulose is insoluble in water but soluble in concentrated phosphoric acid. Short-DP hemicellulose fragments are isolated from cellulose because of their high solubility in acetone/water mixtures. A fraction of lignin is soluble in the organic solvent but insoluble in the aqueous phase, so it can be separated from the other lignocellulose components when the organic solvent is removed.

Cellulose Solvent Criteria

A number of cellulose solvents have been used to address biomass recalcitrance, but most of them cannot be applied to the production of low-cost commodities, due to cost issues. Ideal cellulose solvents for biocommodity biorefineries must meet the following criteria:

(1) able to dissolve cellulose at low temperatures (reduces utility consumption);
(2) able to dissolve wet cellulose (avoids biomass drying);
(3) low cost (high recycle ratio or low solvent costs);
(4) nonvolatile (prevents solvent loss through evaporation);
(5) thermostable (allowing nearly infinite recycling);
(6) chemostable (compatible with other reagents);
(7) nontoxic to enzymatic hydrolysis and microbial fermentation;
(8) high capacity to dissolve cellulose (> 10 wt. % cellulose/volume);
(9) fast diffusion rate in solid lignocellulosic biomass (resulting in a shorter reaction time), and
(10) relatively low viscosity.

The first attempt to overcome lignocellulose recalcitrance by using cellulose solvents was conducted by Professors Mike Ladisch and George Tsao in 1978 (*29*). After searching for a number of cellulose solvents, they found that Cadoxen, an alkali solution of CdO in aqueous ethylenediamine, could dissolve biomass. The resulting regenerated amorphous cellulose could be hydrolyzed quickly by cellulase (*29*), but the glucan digestibility was modest. Because Cadoxen is corrosive and toxic, any traces of the solvent in the treated biomass may inhibit subsequent hydrolysis and fermentation steps. With the invention of ionic liquids (IL) that dissolve cellulose (*30*), several attempts have been made to pretreat biomass by using different IL cellulose solvents (*31–33*). Enzymatic glucan
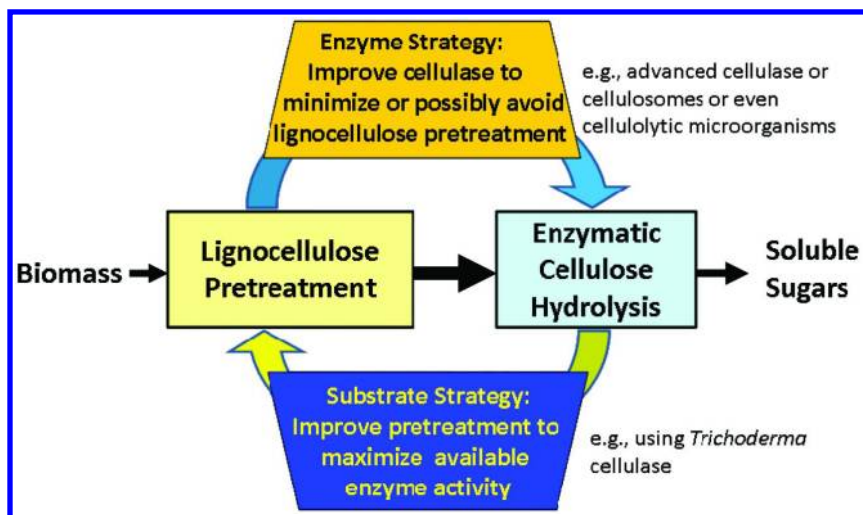
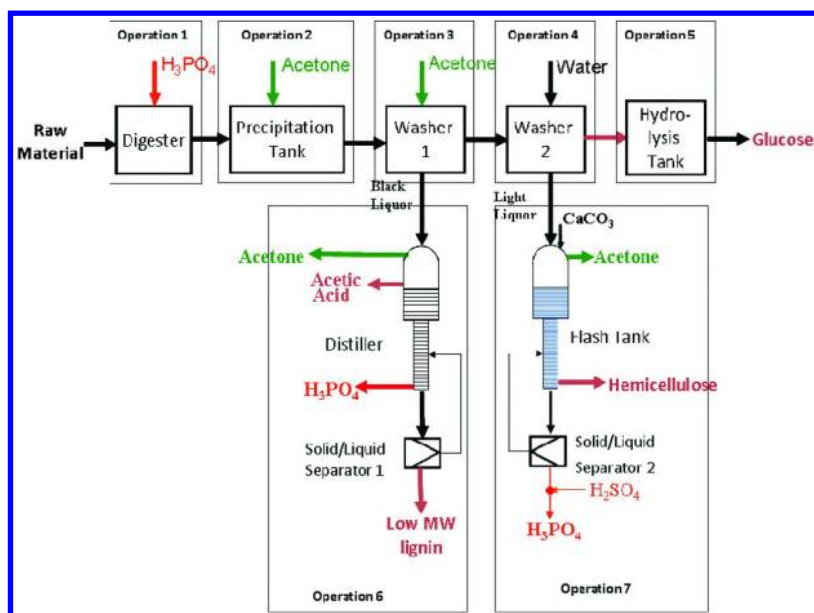*Figure 1. Biomass saccharification paradigms.*

Figure 2. Flowchart of the COSLIF technology with recycling of the cellulose solvent and organic solvent.

digestibility of ionic-liquid pretreated biomass ranges widely (*31*, *32*), suggesting that more research is needed to understand the solvent's mechanisms and develop a cost-effective method to recycle the cellulose solvent. In addition, removing hemicellulose and lignin fractions after biomass dissolution remains a significant challenge.
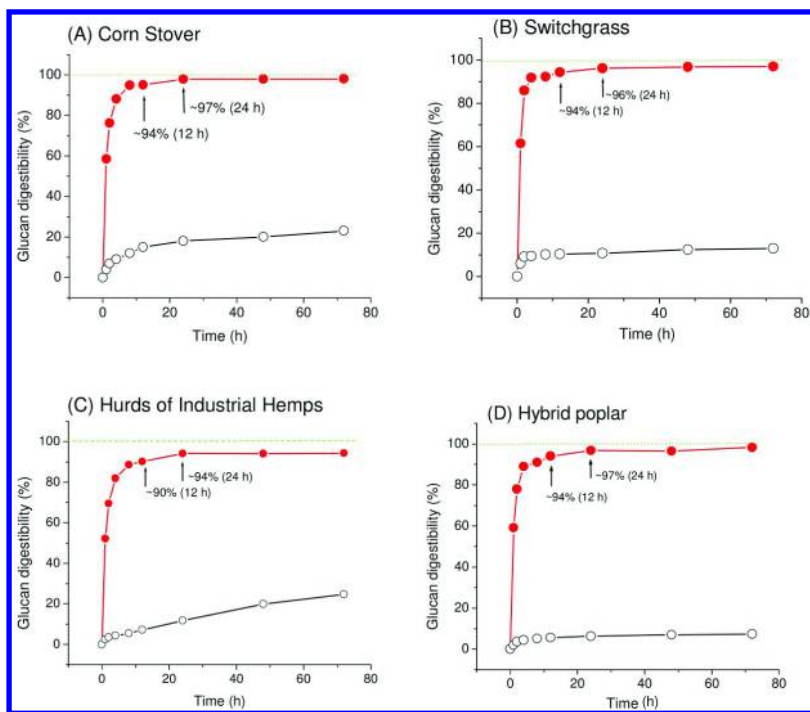
Figure 3. The enzymatic hydrolysis profiles for four examples of COSLIF-pretreated biomass: (A) corn stover, (B) switchgrass, (C) industrial hemp hurds, and (D) hybrid poplar. The hydrolysis conditions were 1% glucan, 15 FPUs of cellulase, and 30 units of β-glucosidase per gram of glucan at 50°C.

Important Roles of the Organic Solvent

Addition of the organic solvent has four goals: 1) to precipitate dissolved cellulose and hemicellulose in amorphous forms, resulting in an easy separation of solid saccharides from liquid cellulose solvent; 2) to dissolve partial lignin in the organic solvent and, after separation of this organic solvent mixture from the biomass, recover solid lignin (due to the insoluability of organic phase-dissolved lignin in acidic aqueous solutions); 3) to recycle concentrated phosphoric acid by avoiding dilution and conducting easy acid re-concentration; and 4) to fractionate oligo-hemicellulose sugars from cellulose due to the solubility of the former, and insolubility of the latter, in the organic solvent/water mixture (*34*).

Enzymatic Hydrolysis, Supramolecular Structures, and Substrate Accessibility

Enzymatic Hydrolysis

Following COSLIF fractionation, nearly pure amorphous cellulose has been obtained for both herbaceous and hardwood lignocellulose, including corn stover, switchgrass, industrial hemp hurds, and hybrid poplar. However, only phosphoric acid beyond the critical concentration (~83%) can efficiently destroy biomass structure; the reaction time ranges from 45 to 60 min, depending on biomass type. Four different well-pretreated biomass types have similar hydrolysis performance at an enzyme loading of 15 filter paper units of cellulase and 30 units of β-glucosidase per gram of glucan. The glucan digestibilities were ~90% at hour 12 and ~94-97% at hour 24 (Fig. 3A-D). These very high sugar digestibilities after enzymatic hydrolysis are attributed to negligible sugar degradation during fractionation and very high enzymatic cellulose digestibility (~97% in 24 hours) during the hydrolysis step. To put this effectiveness in perspective, COSLIF pretreatment can produce more than a 20% increase in sugar yields compared to steam explosion.

Dilute acid (DA) pretreatment has been widely studied (*35*, *36*, *38*). This process is usually conducted at high temperatures and high pressures catalyzed by a dilute acid (often sulfuric acid). Dilute acid at high temperatures removes acid-labile hemicellulose. This results in a disruption of the linkages among cellulose, hemicellulose, and lignin (*38–42*). COSLIF can remove more lignin but retain more hemicellulose than DA (*43*). The higher sugar retention by COSLIF is attractive because this allows a higher release of fermentable sugars during the enzymatic hydrolysis step. Figure 4 presents the different hydrolysis profiles for the same corn stover pretreated by COSLIF and dilute acid. The glucan digestibility of the COSLIF-pretreated corn stover reached more than 90% at hour 12 and 97% at hour 24. In contrast, the DA-pretreated corn stover had much slower hydrolysis rates, and its final digestibility was 84% at hour 72 (Fig. 4A). At a low enzyme loading (5 filter paper units per gram of glucan), the digestibility remained as high as 93% at hour 24 for the COSLIF-pretreated corn stover but only reached ~60% for the dilute acid (DA)-pretreated biomass (Fig. 4B).

Figure 5 presents the mass balance of switchgrass pretreated by the COSLIF technology and enzymatic cellulose hydrolysis at a low enzyme loading (5 FPUs of cellulase and 10 units of β-glucosidase per gram of glucan). Studies of mass balances of pretreatment and enzymatic hydrolysis are highly recommended for evaluating lignocellulose pretreatments (*11*). The overall glucose and xylose yields of the COSLIF-pretreated switchgrass were 85% and 63%, respectively. With technological improvements (e.g., a supplementary hemicellulase in the enzymatic hydrolysis step, optimization of reaction conditions, pre-extraction of water soluble sugars before pretreatment, and adjustment of washing conditions such as solvent temperatures and flow rates), higher xylose recovery yields are anticipated without sacrificing glucose yields.
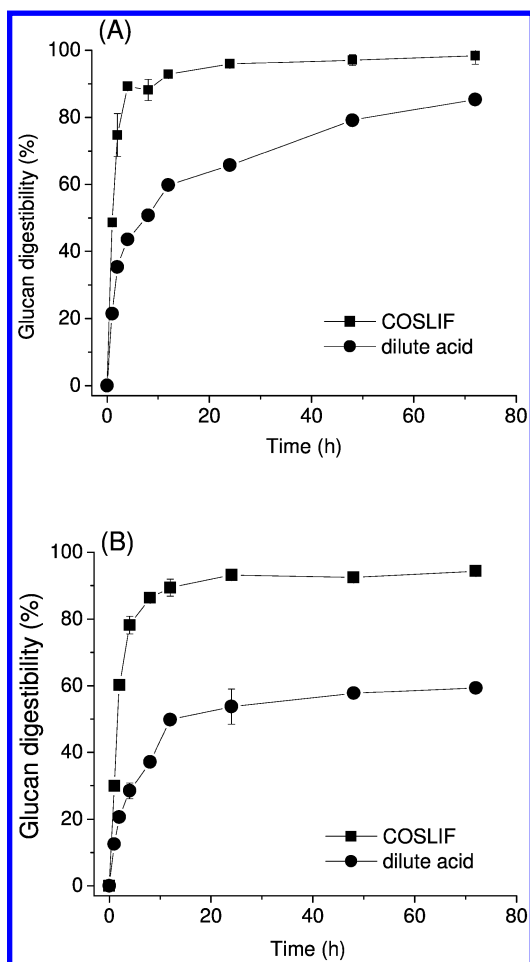
Figure 4. Comparative hydrolysis of corn stover pretreated by COSLIF and dilute acid pretreatments at different enzyme loadings. (A) 15 FPUs of cellulase and 30 units of β-glucosidase per gram of glucan, and (B) 5 FPUs of cellulase and 10 units of β-glucosidase per gram of glucan.

Supramolecular Structures

The supramolecular structural changes for industrial hemp hurds before and after various pretreatments can be observed by using a scanning electron microscope (SEM) (Fig. 6). The plant cell vascular bundles and fibril structure of intact biomass are easily identified under SEM (Fig. 4A, B). Modest pretreatment conditions (e.g., 84.0% H_3PO_4, 50°C and 30 minutes) open larger holes on the surface of plant cell walls by removing the most easily-digested fraction (possibly, hemicellulose and some lignin), but the supramolecular fibril structure is only partly destroyed (C, D). A well-treated lignocellulose sample (84.0% H_3PO_4,
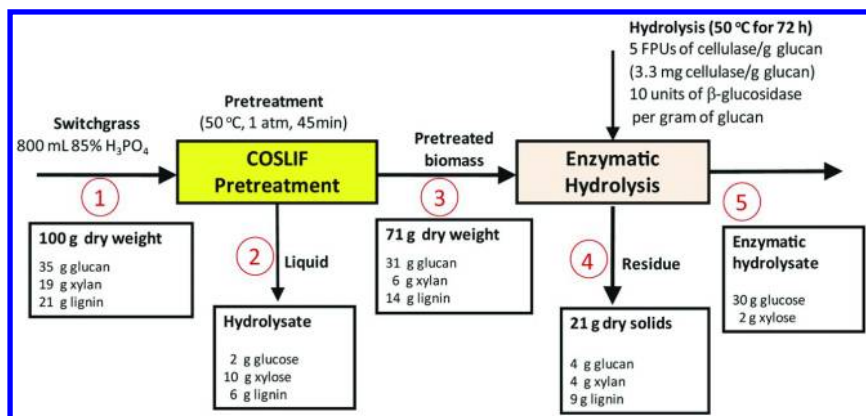
Figure 5. Mass balance of switchgrass pretreated by COSLIF and hydrolyzed enzymatically at an enzyme loading of 5 FPUs of cellulase and 10 units of β-glucosidase per gram of glucan.
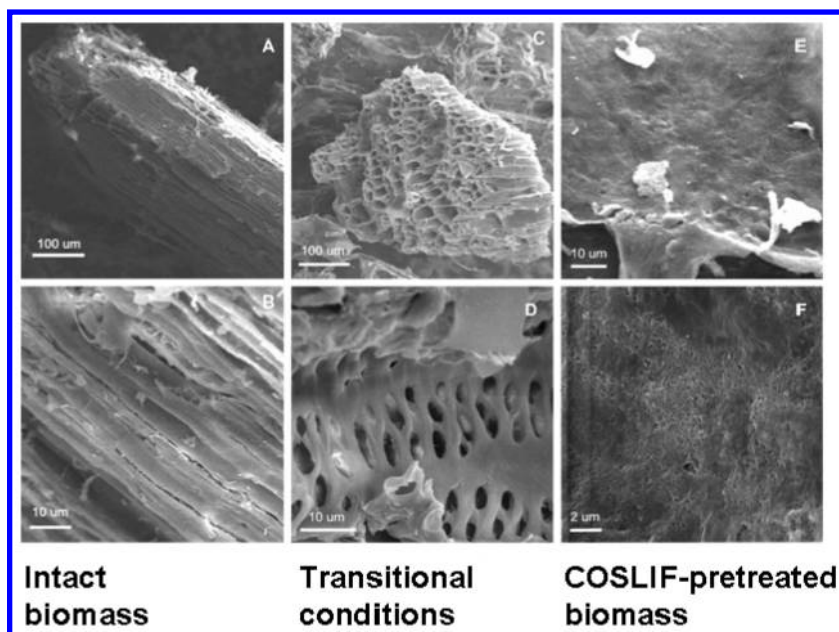

Figure 6. SEM images for COSLIF-pretreated biomass (A, intact, B, modestly-pretreated, and C, well-pretreated)

50°C and 60 minutes) shows all fibrous structures of the lignocellulose completely disrupted (E, F). These images show much more complete structure degradation than similar images taken after treatments such as hot water or ammonia recycle percolation (*44*, *45*).

Substrate Accessibility

Cellulose accessibility to cellulase (CAC, m^2/g cellulose) is calculated based on the maximum cellulase adsorption capacity, as described previously (*3*, *25*, *46*).

$$CAC = \alpha * A_{max} * N_A * A_{G2} \qquad (1)$$

where $\alpha = 21.2$, for the number of cellobiose lattices occupied by a non-hydrolytic protein called TGC (this acronym describes the protein's three components: thioredoxin, a green fluorescent protein and a cellulose-binding model) (*46*), A_{max} = the maximum cellulase adsorption capacity (mole cellulase/g cellulose), N_A = Avogadro's constant (6.023×10^{23} molecules/mol), and A_{G2} = the area of the 110 face of the cellobiose lattice (0.53×1.04 nm = 5.512×10^{-19} m^2) (*3*).

The total (biomass) substrate accessibility to cellulase (TSAC), including CAC and non-cellulose accessibility to cellulase (NCAC), represents the cellulase adsorption capacity for the entire pretreated biomass sample. For pure cellulosic samples, TSAC equals CAC, since NCAC equals zero. Here a scheme is described for quantitatively determining CAC and NCAC for pretreated lignocellulosic substrates (Fig. 7), based on the facts that (i) BSA can irreversibly bind with the lignin fraction of lignocellulosic biomass (*47*, *48*) and (ii) BSA cannot bind with cellulose. First, TSAC (m^2/g biomass) can be estimated from direct adsorption of the TGC protein,

$$TSAC = \alpha * A_{max,TGC} * N_A * A_{G2} \qquad (2)$$

where $A_{max,TGC}$ = the maximum TGC adsorption capacity of the biomass (μmole TGC/g biomass).

Secondly, CAC (m^2/g biomass) can be measured based on the maximum TGC adsorption capacity after competing adsorption sites are blocked by introducing a large amount of BSA (e.g., 5 g/L) that can non-specifically bind on the surface of the lignin (*47*). This maximum TGC adsorption capacity of the BSA-blocked biomass is a close approximation to the cellulose accessibility to cellulase (CAC).

$$CAC = \alpha * A_{max,BSA/TGC} * N_A * A_{G2} \qquad (3)$$

where $A_{max,BSA/TGC}$ = a maximum TGC adsorption capacity of biomass after BSA blocking (μmole TGC/g biomass).

Therefore, NCAC (m^2/g biomass) can be calculated as

$$NCAC = TSAC - CAC \qquad (4)$$

The adsorption results suggest that the values of $A_{max,TGC}$ and $A_{max,BSA/TGC}$ are 2.05 ± 0.15 and 1.64 ± 0.13 μmol/g for COSLIF-pretreated biomass and 1.09 ± 0.08 and 0.84 ± 0.05 μmol /g for DA-pretreated biomass, respectively (*43*). For the COSLIF-pretreated sample, the TSAC was found to be 14.44 ± 1.09 m^2/g
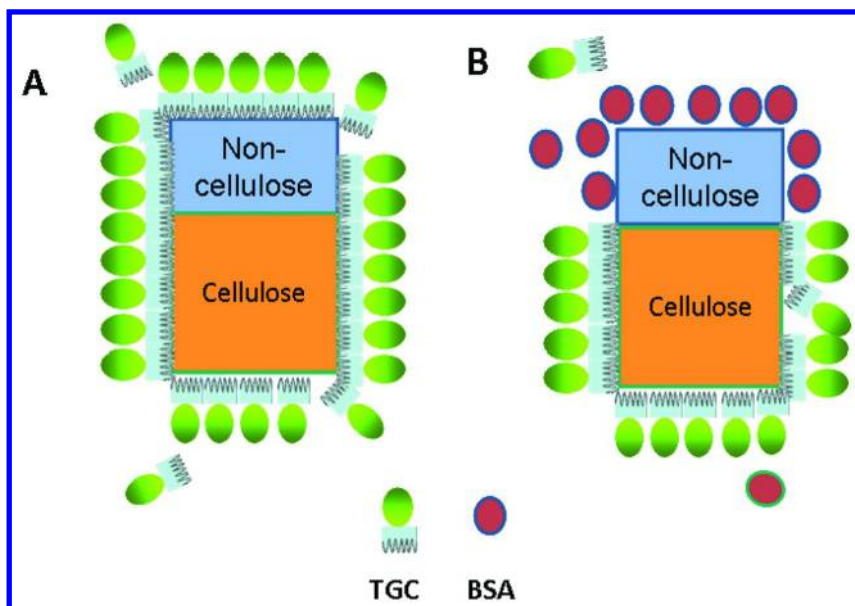
*Figure 7. Scheme for quantitative determination of TSAC and CAC for
the pretreated biomass. (A) Direct TGC adsorption for determining TSAC,
including the cellulose and non-cellulose (lignin) fractions, and (B) Second TGC
adsorption for determining CAC after BSA blocking of the lignin fraction.*

biomass, where CAC and NCAC are 11.57 ± 0.90 and 2.88 ± 0.20 m²/g biomass,
respectively. The much faster hydrolysis rates and higher glucan digestibility
observed with the COSLIF-pretreated corn stover were attributed to a much higher
CAC (11.57 m²/g biomass) than that of the DA-pretreated corn stover (5.89 m²/g
biomass).

Perspectives

Lignocellulose fractionation based on the different solubilities of
lignocellulose components in different solvents is a relatively new concept,
and the COSLIF technology is in its early stage (*18*, *24*). COSLIF has several
advantages, including high glucan digestibility, fast hydrolysis rate, low cellulase
use, effectiveness that is nearly feedstock-independent, higher revenues from
co-products (acetic acid, lignin, and hemicellulose), and minimal formation of
inhibitors. However, this technology also has several challenges, such as the
high ratios of cellulose solvent and organic solvent to biomass, which may result
in high processing costs for efficient recycling of both solvents or high capital
investment. Therefore, further studies of the COSLIF technology will be focused
on:
(1) decreasing cellulose solvent use per unit biomass by finding better cellulose
    solvents,

(2) decreasing organic solvent use per unit biomass by using better organic solvents and more efficient washing methods,

(3) efficiently recycling both solvents through flashing, distillation or fractionation distillation,

(4) identifying suitable solid/liquid unit operations,

(5) efficiently regenerating the cellulose solvent,

(6) characterizing the properties of isolated lignin,

(7) developing new applications for relatively pure lignin,

(8) studying the feasibility of cellulase recycling,

(9) conducting economic analysis based on an ASPEN-Plus model, and

(10) validating technology feasibility with a pilot plant.

Substantial progress will be made in these areas, and the principles of lignocellulose fractionation would have important applications in lignocellulose-based biorefineries. In the short term, cellulosic ethanol production based on cellulose-rich wastes from existing industries, such as corn fiber from corn ethanol biorefineries, wheat hull from flour processing facilities, and sawdust from lumber manufacturers, is attractive, since integrated biorefineries such as these could not only solve solid waste disposal problems but also produce value-added products such as biofuels. Smaller biorefineries that utilize cellulosic waste from on-site manufacturers could be profitable due to the large saving in feedstock costs (~$30-90/ton of biomass, i.e. $0.35-1.00 per gallon of cellulosic ethanol). The application of this nearly feedstock-independent technology on biomass residues from local manufacturers could provide great opportunities to build profitable small-scale biorefineries (i.e. 100 tons of biomass per day) that can produce ~2.8 million gallons of cellulosic ethanol per year, plus acetic acid as a value-added co-product. In the long term, full utilization of all the components of lignocellulosic biomass will be extremely important for the bioeconomy.

Acknowledgements

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