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Solvent fractionation of lignocellulosic biomass

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Abstract: Effectively breaking lignocellulose recalcitrance, releasing the locked polymeric sugars and coutilization of lignocellulose components is the largest technical and economical challenge for the emerging bioeconomy. Cellulose solvent- and organic solvent-based lignocellulose fractionation (COSLIF), has shown to effectively fractionate lignocellulose under modest reaction conditions. The resulting solids, amorphous cellulose, from corn stover, switchgrass, common reed, poplar, and hemp herds, showed high glucan digestibilities and fast enzymatic hydrolysis rates. Utilization of co-products fractionated from lignocellulose (e.g., lignin, acetic acid, and hemicellulose) would greatly increase overall potential revenues of the future biorefineries.

Key words: biomass, cellulosic ethanol, cellulose solvent, COSLIF, enzymatic cellulose hydrolysis, hemicellulose, lignin, lignocellulose fractionation.

4.1 Introduction

Concerns about the accumulation of greenhouse gases and the depletion of cheap fossil fuels (e.g., oil and natural gas) are motivating the use of sustainable primary energy sources, such as biomass, solar energy, nuclear energy, wind energy, tidal energy, and so on. Energy used for transportation usually costs more than stationary energy. Transportation fuels must have high energy density (MJ/kg or MJ/L) and the ability to generate high power density (W/kg) in a compact space (Zhang 2008a). Currently, affordable transportation is available through the combination of liquid fuels (gasoline and diesel) and internal combustion engines. In the future, the majority of automobile powertrain systems will rely on the hydrogen/electricity system because of its high energy utilization efficiency, minimal pollutant emissions, and diverse sustainable primary energy sources (Zhang 2008a; 2008b). However, this transition will take a relatively long time.

Liquid ethanol is a near-term alternative liquid transportation fuel. Currently, most ethanol is produced from soluble sugars made from sugarcane and corn kernels, but competition between food demand and biofuels production has been a cause of soaring food prices. In addition, even complete conversion of the limited supply of corn kernels to first generation biofuels would replace only a small fraction of current transportation fuel demand.

The production of second generation biofuels from more abundant, non-food lignocellulosic biomass is a more sustainable option. Advanced biofuels produced this way would provide many benefits, such as promoting rural economies, enhancing energy security, increasing process energy efficiency (output/input), and decreasing greenhouse gas emissions. Typical lignocellulosic biomass includes:

- agricultural residues (e.g., wheat and rice straws, corn stover, bagasse, etc.)
- forest residues (e.g., wood chips and sawdust)
- dedicated bioenergy crops (e.g., switchgrass, poplar, etc.)
- industrial and municipal solid wastes.

Overcoming lignocellulosic biomass recalcitrance followed by enzymatic hydrolysis of reactive polymeric carbohydrates (i.e., cost-efficient liberation of fermentable sugars from biomass) is perhaps the most challenging technical and economic barrier to biorefinery success (Fortman *et al.* 2008; Lynd *et al.* 2008; Zhang 2008c). Pretreatment is among the most costly steps in biochemical conversion of biomass (Eggeman and Elander 2005; Wyman *et al.* 2005b), accounting for up to 40% of the total processing cost (Lynd 1996; Lynd *et al.* 2005). Also, it affects the costs of other operations including size reduction prior to pretreatment and enzymatic hydrolysis and fermentation after pretreatment. Pretreatment can also strongly influence downstream costs involving detoxification (if inhibitors are generated), enzymatic hydrolysis rate and enzyme loading, mixing power, product concentration, product purification, power generation, waste treatment demands, and other process variables (Wyman *et al.* 2005b; Zhang 2008c).

The main purpose of this chapter is to describe a new technology that uses a combination of a cellulose solvent (concentrated phosphoric acid) and an organic solvent for fractionating lignocellulose components (cellulose, hemicellulose, acetic acid and lignin) under modest reaction conditions. Potential applications of the isolated lignocellulose components are briefly discussed.

4.2 Lignocellulosic biomass

Lignocellulosic biomass is cheaper than crude oil, natural gas, or corn kernels on the basis of energy content (Lynd *et al.* 2008; Zhang 2008c; Zhang and Lynd 2008). The key challenge is cost-effective release of the locked polymeric carbohydrates to fermentable soluble sugars (e.g., glucose and xylose). As

opposed to the relatively mature thermochemical conversion of biomass to syngas, biological conversion of biomass to fermentable sugars has great potential for reductions in processing costs and capital investment. It is believed that with intensive research and development efforts, biological conversion will become the predominant pathway for biomass utilization.

Lignocellulosic biomass is a natural composite containing three main biopolymers (cellulose, hemicellulose, and lignin) that are intertwined chemically and physically. The complex structure of biomass makes chemical and biological degradation difficult. Many factors affect biomass recalcitrance, including substrate accessibility to cellulase, degree of polymerization, cellulose crystallinity, and lignin and hemicellulose contents (Himmel *et al.* 2007; Mansfield *et al.* 1999; Zhang and Lynd 2004). Two major factors are (1) low cellulose accessibility to cellulase (CAC) that hinders the enzymes from working efficiently and (2) lignin and hemicellulose on the surface of cellulose that blocks cellulase from accessing cellulose efficiently (Hong *et al.* 2007; Moxley *et al.* 2008; Zhang *et al.* 2006a; 2007; Zhang and Lynd 2004; 2006).

4.2.1 Cellulose and enzymatic cellulose hydrolysis

Cellulose is the most abundant component of lignocellulosic biomass, comprising around 30–50% of its dry weight. Cellulose is a homopolysaccharide made of anhydroglucopyranose linked by β-1,4-glucosidic linkages, with a degree of polymerization (DP) ranging from several hundred to more than 10 000 (Zhang and Lynd 2004). Highly ordered hydrogen bonds and van der Waal's forces among nearby anhydroglucose units result in the formation of crystalline microfibrils (Zhang 2008c; Zhang and Lynd 2004). The microfibrils form further microfibrils, which constitute the basic framework of the plant cell walls and provide rigidity and strength. These crystalline cellulose chain bundles have very low substrate accessibility to large-size cellulases (Hong *et al.* 2007; 2008; Zhang and Lynd 2004), resulting in low hydrolysis rates occurring on crystalline cellulose (Zhang *et al.* 2006a).

Cellulose hydrolysis requires endoglucanase, cellobiohydrolases, and β-glucosidase to work together (Zhang *et al.* 2006b; Zhang and Lynd 2004). Endoglucanase is responsible for cleaving accessible β-glucosidic bonds of the cellulose chain randomly and creating more ends for the action of cellobiohydrolases. Cellobiohydrolases hydrolyze the ends of cellulose chains and release soluble cellobiose into the aqueous phase. The release of soluble sugars from solid substrate to the aqueous phase is rate-limiting for the whole hydrolysis process (Zhang and Lynd 2004; 2006). β-Glucosidase cleaves cellobiose to form glucose, which reduces cellobiose inhibition for endoglucanase and cellobiohydrolase. In general, cellobiohydrolase is more sensitive to product inhibition than endoglucanase (Zhang and Lynd 2004). Enzymatic cellulose hydrolysis is a very complicated process, involving heterogeneous

substrate properties as well as synergy and competition among several enzymes on limited accessible solid surfaces. A functionally based mathematic model (Zhang and Lynd 2006) has been developed to correlate disparate phenomena into an aggregated system and give some useful predictions for lignocellulose pretreatment and cellulase engineering. For example, the simulation results clearly suggest that the optimal ratio of cellobiohydrolase to endoglucanase for maximal synergy is a dynamic rather than constant value, depending on substrate properties (degree of polymerization and substrate accessibility), enzyme loadings, and reaction time (Zhang and Lynd 2006). One of the model's most important predictions is that increasing substrate accessibility to cellulase is the most important factor for increasing enzymatic hydrolysis rates (Zhang and Lynd 2006). This insight was the driving force for the development of a new technology called cellulose solvent- and organic solvent-based lignocellulose fractionation (COSLIF) (Moxley *et al.* 2008; Zhang *et al.* 2007).

Quantitative determination of cellulose accessibility to cellulase (CAC) is valuable for investigating complicated enzymatic cellulose hydrolysis mechanisms. None of the previous methods for measuring cellulose accessibility – nitrogen adsorption-based Brunauer-Emmett-Teller (BET), size exclusion chromatography, small angle X-ray scattering (SAXS), microscopy – is perfectly applied in the enzymatic cellulose hydrolysis process because: (1) 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) (Zhang and Lynd 2004); (2) cellulases are large-size molecules; and (3) cellulase is preferentially adsorbed on the 110 face of cellulose fibers that cellulase can hydrolyze (Chanzy *et al.* 1984; Hong *et al.* 2007; Lehtio *et al.* 2003). Small-size molecule adsorption methods, such as BET, water vapor sorption, alkali swelling, or the exchange of H to D atoms with D_2O, result in an overestimation of CAC (Hong *et al.* 2007; Zhang and Lynd 2004). Cellulase-size exclusion chromatography can neither differentiate the effective cellulose surface for adsorption and hydrolysis nor account for the external surface (Hong *et al.* 2007).

Maximum cellulase adsorption capacity has been suggested to represent CAC (Zhang and Lynd 2004) and the data in the literature have been summarized and used before (Zhang and Lynd 2004; 2006). However, it is relatively difficult to obtain reliable and accurate data based on the adsorption of active cellulases on cellulose because cellulase can hydrolyze substrate during the adsorption measurement, especially for easily-hydrolyzed cellulose (Steiner *et al.* 1988), resulting in rapid changes in substrate characteristics (Fan *et al.* 1980; Tanaka *et al.* 1986). Therefore, many adsorption studies have been conducted at a decreased temperature (e.g., 4 °C) in order to minimize hydrolysis effects (Kyriacou *et al.* 1989; Medve *et al.* 1997; Ooshima *et al.* 1983; Reinikainen *et al.* 1995; Ryu *et al.* 1984; Steiner *et al.* 1988). The drawback of this strategy is that active cellulase adsorption at low temperatures may be significantly

different from that at hydrolysis temperatures (Kyriacou *et al.* 1988; 1989; Ooshima *et al.* 1983).

Quantitative determination of cellulose accessibility to cellulase (m^2/g cellulose) was established based on the Langmuir adsorption of a fusion protein containing a cellulose-binding module (CBM) and a green fluorescent protein (GFP) (Hong *et al.* 2007). One molecule of the recombinant fusion protein occupied 21.2 cellobiose lattices on the 110 face of bacterial cellulose nanofibers (Hong *et al.* 2007). The CAC values of several cellulosic materials – regenerated amorphous cellulose (RAC), bacterial microcrystalline cellulose (BMCC), Whatman No. 1 filter paper, fibrous cellulose powder (CF1), and microcrystalline cellulose (Avicel FMC PH105) – are 41.9, 33.5, 9.76, 4.53, and 2.38 m^2/g, respectively. The CAC value of RAC made from Avicel is 17.6-fold larger than that of Avicel (Hong *et al.* 2007). The fastest hydrolysis of amorphous cellulose was observed for 10 g RAC/L at an enzyme loading of 15 filter paper units (FPUs) per gram of cellulose within three hours (Zhang *et al.* 2006a).

4.2.2 Hemicellulose

Hemicellulose, the second most abundant polysaccharide in lignocellulosic biomass, is a heteropolymer containing primarily pentoses (xylose), as well as some hexoses (e..g., glucose and mannose). The main role of hemicellulose is to interact with cellulose and lignin to cross-link the cellulose microfibrils to the lignin matrix. Since hemicellulose is a small-size branched polysaccharide with DP ranging from ~200 to 400, it is more vulnerable than cellulose to catalysts and enzymes. For example, complete hydrolysis of hemicellulose to monomeric sugars can be implemented at 121 °C for 1 hour in the presence of 1% sulfuric acid, but 4% sulfuric acid is needed for cellulosic fragments (Moxley and Zhang 2007). In general, dilute acid or alkali can efficiently hydrolyze hemicellulose, resulting in a disruption of the linkages among cellulose, hemicellulose, and lignin.

Complete enzymatic hydrolysis of hemicellulose requires more enzymes than cellulose (more than three enzymes) to work together, due to the more complicated structure of hemicellulose. More information about these hemicellulases has been reviewed elsewhere (Shallom and Shoham 2003; Singh *et al.* 2003). Enzymatic hemicellulose hydrolysis is much faster than enzymatic cellulose hydrolysis, due to its amorphous structure and high turn-over number of hemicellulases.

Hemicellulose has broad applications. Hemicellulose has been utilized in the form of plant gum in thickeners, adhesives, protective colloids, emulsifiers, stabilizers as well as biodegradable oxygen barrier films (Hartman *et al.* 2006; Kamm and Kamm 2004; Zhang 2008c). Oligosaccharides may provide a source of even higher value-added products, such as animal feed additives (Davis *et al.* 2002; Fernandez *et al.* 2002). This polymer's primary monomeric sugar, xylose, can be easily fermented to sugar alcohol, xylitol (Mussatto *et al.* 2005; Woodyer

et al. 2005). Fufural, a xylose degradation product, can be used in the production of lubricants, coatings, adhesives, and furan resins. Effective fermentation of xylose to ethanol also could provide an unlimited market for xylose (Gray *et al.* 2006; Zhang *et al.* 1995).

4.2.3 Lignin

Lignin is the most abundant phenolic biopolymer found in nature. Low-quality industrial lignin is isolated by the paper pulp industry, but this material is usually burned for energy generation and chemical recycling. The research and development of lignin utilization is lagging because of limited supplies of high-quality lignin. Likewise, the lack of ready markets for high-quality lignin limits the motivation to conduct R&D pertaining to its isolation.

On the laboratory scale, high-quality lignin has been demonstrated to work as a substitute for polymeric materials, such as phenolic powder resins, polyurethane, and polyisocyanuratre foams as well as epoxy resins. Because it is a good adsorbent and has excellent adhesive, rheological, and collidial properties, lignin can also be used as a partial replacement for phenolic binders for oriented-strand board production (Lora and Glasser 2002; Zhang 2008c). Lignin is a raw chemical precursor for DMSO, vanilla, phenol, and ethylene (Eckert *et al.* 2007; Lora and Glasser 2002; Reddy and Yang 2005) and can also be converted to value-added carbon fiber with a selling price ranging from ~$5–20/kg (Zhang 2008c). Broad potential lignin applications also exist for agricultural chemicals. For example, lignin, a biodegradable UV-light antioxidant absorbent, is appropriate for release-controlled pesticides and slow-release fertilizers containing ionically or organically bound nitrogen or other fertilizers. This use offers an important ecological benefit, as slow-release fertilizers are crucial for decreasing non-point groundwater pollution. One of the largest-scale uses of lignin could be as a soil conditioner to aid in formation of humus. In the future, lignin could also be used for coating easily degradable biomass for carbon sequestration purposes.

Pre-isolation of lignin prior to ethanol fermentation can provide a good feedstock for further conversion to fuel additives or for thermochemical conversion to synthetic diesel (Zhang 2008c). Nearly pure lignin can be processed much more easily and efficiently by thermochemical catalysis (e.g., pyrolysis or gasification) than crude lignocellulosic biomass.

Lignin applications for high-end markets, such as polymer substitutions and carbon fiber, are expected to be pursued before low-end lignin markets. Although lignin-based products do not currently compete with products derived from petrochemicals, the technical and economic situations are changing rapidly.

4.3 Cellulose solvent-based lignocellulose pretreatment

A number of pretreatment technologies have been studied and developed over the years. Nearly all lignocellulose treatments can be divided into three categories: (1) physical methods, including dry milling, wet milling, irradiation, and microwave treatment; (2) chemical methods, using dilute acids (dilute H_2SO_4, H_3PO_4, HCl, acetic acid, formic acid/HCl), alkalis (NaOH, lime, ammonia, amine, etc.), organosolv, oxidizing agents (O_3, NO, H_2O_2, $NaClO_2$), steam explosion with or without catalysts, CO_2 explosion, ammonia fiber explosion (AFEX), hot water, hot water with flow-through, supercritical fluid extraction (CO_2, CO_2/H_2O, CO_2/SO_2, NH_3, H_2O) and so on; and (3) biological methods, such as the use of white rod fungi.

Many current mainstream lignocellulose pretreatments (e.g., steam explosion, dilute acid, ammonia based pretreatment) cannot efficiently disrupt orderly hydrogen bonds among glucan chains in crystalline cellulose. Instead, they aim at removing hemicellulose or lignin. The pretreated biomass has relatively slow hydrolysis rates and modest cellulose digestibility (Wyman *et al.* 2005a; 2005b). Moreover, many pretreatment methods are feedstock-specific. For example, ammonia fiber explosion (AFEX) has been found to be relatively ineffective for pretreating woody biomass (Alizadeh *et al.* 2005; Chundawat *et al.* 2007; Dale *et al.* 1996; Murnen *et al.* 2007). Until now nearly all intensively studied pretreatments share one or more common shortcomings:

(1) severe pretreatment conditions (except AFEX), resulting in sugar degradation and inhibitor formation (Klinke *et al.* 2004; Mussatto and Roberto 2004);
(2) low or modest cellulose digestibility because of the presence of residual lignin and hemicellulose (Wyman *et al.* 2005a);
(3) high cellulase loading required;
(4) slow hydrolysis rate because a significant fraction of pretreated lignocellulose remains crystalline;
(5) large utility consumption (Eggeman and Elander 2005);
(6) huge capital investment because of poor economy of scale (Zhang 2008c);
(7) low co-utilization of all the major components of lignocellulose (except organosolv) (Zhang 2008c; Zhang *et al.* 2007).

Because hemicellulose is the most vulnerable component in lignocellulosic biomass, many of lignocellulose pretreatments are focused on hemicellulose removal. Such pretreatments include steam explosion and dilute acid. Although most hemicellulose can be removed efficiently in these processes, the condensed lignin on the crystalline cellulose surface still slows down hydrolysis rates and decreases cellulose digestibility. Subsequent lignin-targeting processes have been tested to remove this remaining lignin by using oxidative reagents, flow-through, and organic solvents.

Hydrolysis of pure crystalline cellulose has a relatively slow hydrolysis rate and low glucan digestibility (Zhang *et al.* 2006a; 2007), suggesting that the efficient removal of hemicellulose and lignin is not enough for high yield enzymatic cellulose hydrolysis. Therefore, there is an urgent need to find new pretreatment technologies that can overcome the recalcitrance of lignocellulose by targeting the most recalcitrant component – cellulose.

4.3.1 Cellulose solvent-only lignocellulose pretreatment

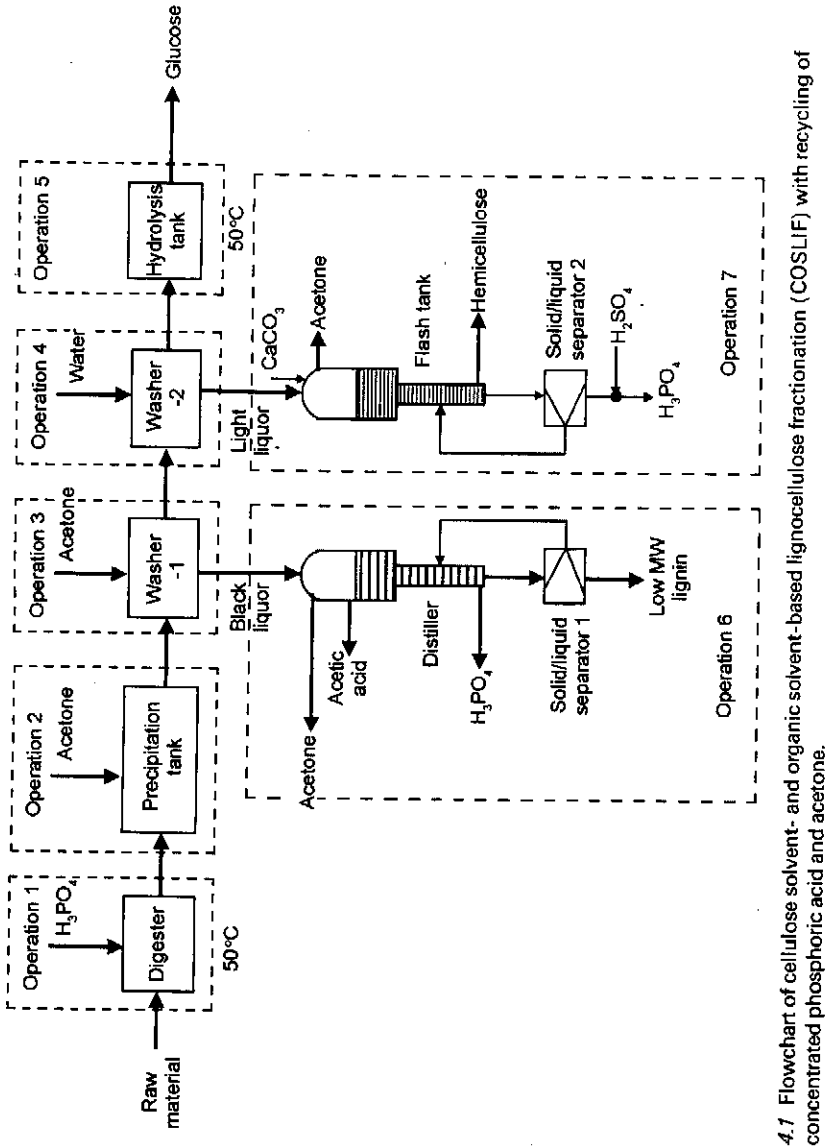
The first attempt to overcome biomass recalcitrance by using cellulose solvents was taken by Professors Mike Ladisch and George Tsao in 1978 (Ladisch *et al.* 1978). After searching for a number of cellulose solvents, they found that Cadoxen, an alkali solution of CdO in aqueous ethylenediamine, can dissolve biomass. The resultant regenerated amorphous cellulose (RAC) from pure cellulose is hydrolyzed quickly by cellulase with a very high digestability, while the glucan digestibility of pretreated biomass is modest (Ladisch *et al.* 1978). A drawback of this technology is that Cadoxen is corrosive and toxic, and any remaining toxic components in the solid biomass may inhibit subsequent enzyme hydrolysis and fermentation steps.

With the invention of ionic liquids that dissolve cellulose (Swatloski *et al.* 2002), several attempts have been made to pretreat biomass by using different cellulose solvents (Dadi *et al.* 2006; Kilpeläinen *et al.* 2007; Zhu 2008). Enzymatic glucan digestibility of ionic-liquid pretreated biomass ranges widely (Dadi *et al.* 2006; Kilpeläinen *et al.* 2007; Zhu 2008), suggesting that more research is needed to understand its mechanisms. Remaining hemicellulose and lignin fractions on the surface of cellulose could be an obstacle to efficient cellulose hydrolysis. In addition, efficient recycling of costly ionic solvents remains challenging.

4.3.2 Cellulose solvent- and organic solvent-based lignocellulose fractionation (COSLIF)

In order to deal effectively with two root causes of the recalcitrance of lignocellulose – breaking up orderly hydrogen bonds in crystalline cellulose and removing lignin and hemicellulose from the surface of cellulose – a novel pretreatment process has been developed, cellulose solvent- and organic solvent-based lignocellulose fractionation (COSLIF) (Zhang *et al.* 2007). COSLIF not only fractionates lignocellulose components based on the significantly different solubility of cellulose, hemicellulose, and lignin in the cellulose solvent, organic solvent, and water, respectively, but also recycles the solvents due to a large difference in solvent volatility (Zhang *et al.* 2007).

Figure 4.1 shows the overall flowchart of the COSLIF technology where concentrated phosphoric acid is used as the cellulose solvent and acetone as the



4.1 Flowchart of cellulose solvent- and organic solvent-based lignocellulose fractionation (COSLIF) with recycling of concentrated phosphoric acid and acetone.

organic solvent (Zhang *et al.* 2007). The key design principles of COSLIF are (1) de-crystallization of the cellulose fibers (i.e., more cellulose accessibility so that cellulase can work on the substrate more efficiently), (2) removal of partial lignin and hemicellulose from cellulose (i.e., fewer substrate obstacles to the enzymes, so that cellulase can access the substrate more efficiently), and (3) modest reaction conditions (i.e., a decrease in sugar degradation, less inhibitor formation, lower utility consumption, and less capital investment).

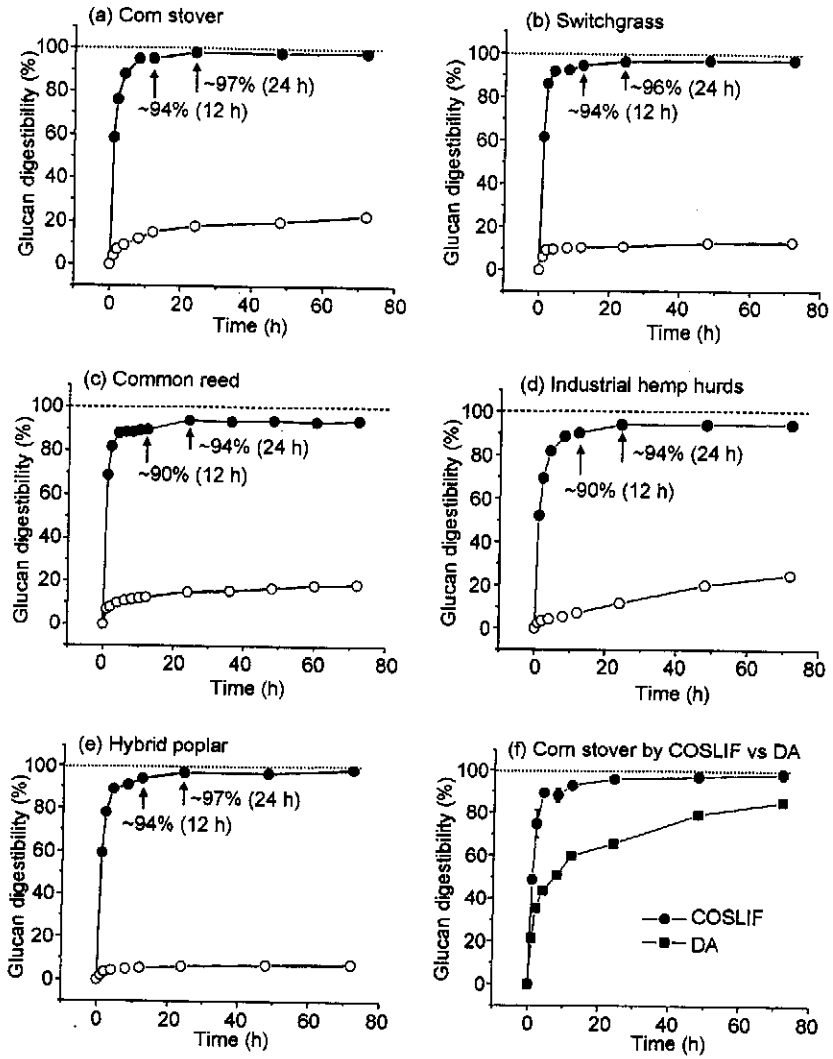
After testing five lignocellulosic feedstocks: corn stover, switchgrass, hurds of industrial hemps, common reed, and hybrid poplar, phosphoric acid was discovered to destroy biomass structure efficiently only beyond the critical concentration (i.e., ~83%); the reaction time ranges from 45 to 60 min, depending on the type of biomass. Five different well-pretreated biomass types have similar hydrolysis performance at an enzyme loading of 15 FPUs of cellulase and 30 units of β-glucosidase per gram of glucan. The glucan digestibilities were around 90% at hour 12 and ~94–97% at hour 24 (Fig. 4.2(a)–(e)).

One of the most widely studied pretreatments has been dilute acid (DA) (Bernardez *et al.* 1993; Grethlein 1985; Ooshima *et al.* 1990; Schell *et al.* 2003). 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. By doing so, the linkages among cellulose, hemicellulose, and lignin are disrupted (Converse 1993; Kumar and Wyman 2008; Lloyd and Wyman 2005; Moxley and Zhang 2007; Schell *et al.* 2003). In a comparison study between the COSLIF and DA technologies, glucan, hemicellulose, and lignin contents of COSLIF-pretreated and DA-pretreated corn stover were examined (Zhu *et al.* 2009). The results are shown in Table 4.1.

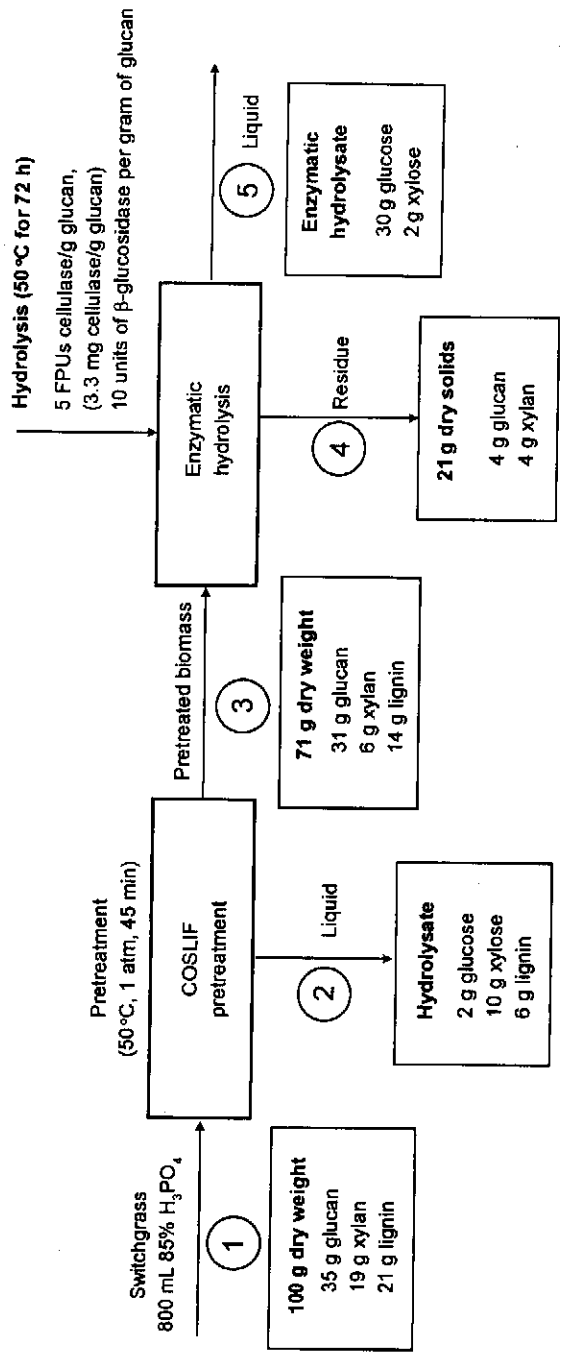
As is evident from Table 4.1, COSLIF can remove more lignin than DA, while retaining more glucan and hemicellulose. The higher solid sugar retention by COSLIF is attractive because this allows a more rapid release of fermentable sugars during the enzymatic hydrolysis step. Figure 4.2(f) presents the 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 stove had much slower hydrolysis rates, and its final digestibility was 84% at hour 72.

Table 4.1 Comparison of the effects of COSLIF and DA on biomass composition

	COSLIF	Dilute acid (DA)
Glucan content	58.2 ± 2.5%	53.7 ± 1.5%
Hemicellulose content	6.2 ± 0.3%	3.4 ± 0.2%
Lignin content	19.7 ± 0.3%	30.3 ± 0.7%



4.2 Hydrolysis curves for different feedstocks at 15 FPUs of cellulase/g glucan and 50 °C. Pretreatment conditions: (a) corn stover (85% H_3PO_4, 50 °C and 40 min), (b) switchgrass (85% H_3PO_4, 50 °C and 40 min), (c) common reed (85% H_3PO_4, 50 °C and 60 min), (d) industrial hemp hurds (85% H_3PO_4, 50 °C and 60 min), (e) poplar (85% H_3PO_4, 50 °C and 60 min) and (f) corn stover (COSLIF, 85% H_3PO_4 at 50 °C for 60 min) vs. dilute acid (DA) pretreatment of the same feedstocks (1.4% H_2SO_4, 165°C, and 8 min).



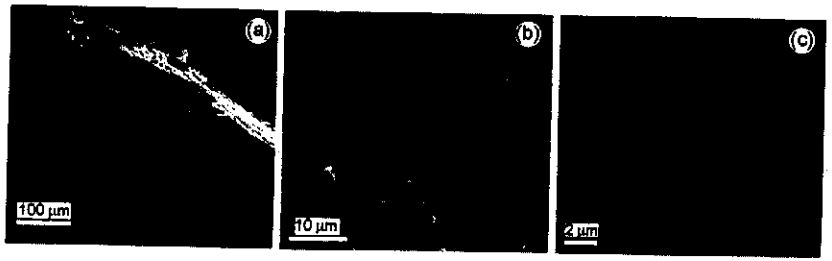
4.3 Mass balance for switchgrass via the COSLIF technology and enzymatic hydrolysis at 50 °C with 10 g/L glucan, 15 FPUs of cellulase and 30 units of β-glucosidase per gram of glucan.

Figure 4.3 presents the material balance of swithgrass pretreated by the COSLIF technology and enzymatic cellulose hydrolysis at an enzyme loading of 15 FPUs of cellulase and 10 units of β-glucosidase per gram of glucan. The overall glucose and xylose yields were calculated to be 85% and 63%, respectively. With technological improvements (e.g., a supplementary hemicellulase for enzymatic hemicellulose hydrolysis, optimization of reaction conditions, pre-extraction of water soluble sugars before the pretreatment, and washing methods and conditions such as solvent temperatures and flow rates), higher xylose recovery yields are expected without the sacrifice of glucose yields.

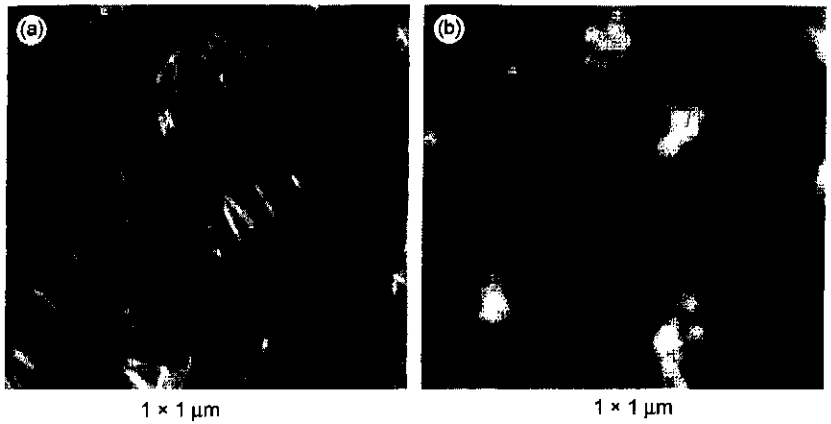
4.3.3 Supramolecular structures

The supramolecular structural changes in industrial hemp hurds before and after the different pretreatments are shown by using a scanning electron microscope (SEM) (Fig. 4.4). The intact biomass presents its plant cell vascular bundles and its fibril structure under SEM (Fig. 4.4(a)). Modest pretreatment conditions (84.0% H_3PO_4, 50 °C and 30 min) open larger holes on the surface of plant cell walls by removing the easily digested lignocellulose fraction (i.e., hemicellulose) but the supramolecular fibril structure receives only partial damage (Fig. 4.4(b)). A well-treated lignocellulose sample (84.0% H_3PO_4, 50 °C and 60 min) shows all fibrous structures of the lignocellulose completely disrupted (Fig. 4.4(c)). These images are completely different from those after treatments such as dilute acid and ammonia recycle percolation, which show residual structures even after very long treatment times (Kim and Lee 2005; Zeng *et al.* 2007).

The dramatic changes in the supramolecular structures of corn stover before and after pretreatment are also shown by the atomic force microscopy (AFM) images in Fig. 4.5. Before pretreatment, the corn stover cell wall structures and elementary cellulose fibers are clearly identified (Fig. 4.5(a)). After pretreatment, no fibril structures are observed (Fig. 4.4(b)), indicating that concentrated phosphoric acid not only disrupts all the linkages among cellulose, hemicellulose, and lignin, but also breaks up the orderly hydrogen bonds among glucan chains. The much faster hydrolysis rates and higher glucan digestibility



4.4 SEM micrographs of hurds of industrial hemps. (a) intact biomass, (b) modestly treated biomass, and (c) well-treated biomass.



4.5 AFM images for corn stover. (a) intact biomass and (b) well-pretreated biomass.

of the COSLIF-pretreated corn stover are attributed to more effective biomass structure destruction (Figs 4.4 and 4.5).

4.4 Future trends

Lignocellulose fractionation based on the different solubilities of lignocellulose components in different solvents is a new concept. The COSLIF technology, which exploits this idea, is in its infancy (Moxley *et al.* 2008; Sathitsuksanoh *et al.* 2009; 2010; Zhang *et al.* 2007). COSLIF pretreatment has several advantages, such as high glucan digestibility, a fast hydrolysis rate, low cellulase use, nearly feedstock-independent pretreatment results, greater potential revenues from co-products (acetic acid and lignin or even hemicellulose), and minimal formation of inhibitors. Several challenges remain, however, 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 and possibly high capital investment. Therefore, further studies of the COSLIF technology will be focused on:

- decreasing cellulose solvent use per unit biomass by finding better cellulose solvents,
- decreasing organic solvent use per unit biomass by using better organic solvents and efficient washing methods,
- efficiently recycling both solvents through flashing, distillation or fractionation distillation,
- identifying suitable solid/liquid unit operations,
- efficiently regenerating the cellulose solvent,
- characterizing the properties of isolated lignin,

- developing new applications for relatively pure lignin,
- studying the feasibility of cellulase recycling,
- conducting economic analysis based on an ASPEN-Plus model, and
- validating technology feasibility with a pilot plant.

Substantial progress can be made in these areas, and the principles of lignocellulose fractionation have potential 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 more attractive, since integrated biorefineries could not only solve solid waste disposal problems but also produce value-added products such as biofuels. Much smaller biorefineries that utilize cellulosic waste from on-site manufacturers could be profitable due to the large savings in feedstock costs (~$30–90/ton of biomass, i.e., $0.35–1.00 per gallon of cellulosic ethanol). The applicability of this nearly feedstock-independent COSLIF technology for biomass residues from local manufacturers could provide great opportunities to build profitable small-size biorefineries (i.e., 100 tons of biomass per day) that could produce ~2.8 million gallons of cellulosic ethanol per year, as well as acetic acid as a value-added co-product. In the long term, full utilization of lignocellulose components other than carbohydrates, such as lignin, will be extremely important for the bioeconomy.

4.5 Sources of further information and advice

- Classic reviews of cellulose solvents (Fengel and Wegener 1984; Jayme and Lang 1963; Pereira *et al.* 1988; Zhang and Lynd 2003).
- Ionic liquids as a cellulose solvent (ElSeoud *et al.* 2007; Swatloski *et al.* 2002).
- The cellulose solvent-only lignocellulose pretreatment (Ladisch *et al.* 1978).
- The COSLIF technology (Moxley *et al.* 2008; Zhang *et al.* 2007).

4.6 References

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Part II

Hydrolysis (saccharification) processes for lignocellulose-to-bioalcohol production