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Fundamentals of Biomass Pretreatment by Fractionation

Poulomi Sannigrahi[1,2] and Arthur J. Ragauskas[1,2,3]

[1] *BioEnergy Science Center, Oak Ridge, USA*

[2] *Institute of Paper Science and Technology, Georgia Institute of Technology, Atlanta, USA*

[3] *School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, USA*

10.1 Introduction

With the rise in global energy demand and environmental concerns about the use of fossil fuels, the need for rapid development of alternative fuels from sustainable, non-food sources is now well acknowledged. The effective utilization of low-cost high-volume agricultural and forest biomass for the production of transportation fuels and bio-based materials will play a vital role in addressing this concern [1]. The processing of lignocellulosic biomass, especially from mixed agricultural and forest sources with varying composition, is currently significantly more challenging than the bioconversion of corn starch or cane sugar to ethanol [1,2]. This is due to the inherent recalcitrance of lignocellulosic biomass to enzymatic and microbial deconstruction, imparted by the partly crystalline nature of cellulose and its close association with hemicellulose and lignin in the plant cell wall [2,3]. Pretreatments that convert raw lignocellulosic biomass to a form amenable to enzymatic degradation are therefore an integral step in the production of bioethanol from this material [4]. Chemical or thermochemical pretreatments act to reduce biomass recalcitrance in various ways. These include hemicellulose removal or degradation, lignin modification and/or delignification, reduction in crystallinity and degree of polymerization of cellulose, and increasing pore volume. Biomass pretreatments are an active focus of industrial and academic research efforts, and various strategies have been developed.

Among commonly studied pretreatments, organosolv pretreatment, in which an aqueous organic solvent mixture is used as the pretreatment medium, results in the fractionation of the major biomass components, cellulose, lignin, and hemicellulose into three process streams [5,6]. Cellulose and lignin are recovered as separate solid streams, while hemicelluloses and sugar degradation products such as furfural and hydroxymethylfurfural (HMF) are released as a water-soluble fraction. The combination of ethanol as the solvent and

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sulfuric acid as the delignification catalyst has been applied to several common biomass feedstocks [7–11]. This approach allows for an efficient utilization of all of the major biomass components, making it potentially attractive from an economic perspective if good value can be obtained from each in a true biorefinery concept. Many other pretreatments such as steam explosion, dilute acid, and AFEX produce cellulose-rich solids that can be hydrolyzed with enzymes to produce glucose but do not result in a clean fractionation into separate lignin, cellulose, and hemicellulose streams [4,5,12]. While hemicellulose can be potentially utilized by fermentation with pentose fermenting microbes, lignin is usually too degraded to be useful as a co-product. In these pretreatments, the lignin is usually expected to be burned as an energy source.

In this chapter, we focus on organosolv pretreatment as a method for biomass fractionation to recover high-quality streams of each of the major biomass components. We provide a short historical perspective on organosolv pulping and pretreatment, followed by a more detailed overview of the latter. Different solvents and catalysts used in organosolv pretreatment and results from biomass fractionation are discussed with an emphasis on ethanol, as it is an excellent solvent when the production of bio-ethanol is desired. The chemistry of organosolv delignification, a key component of biomass fractionation by this method, and the nature of organosolv lignin are described. Other aspects, including modifications in the structure and crystallinity of cellulose after organosolv pretreatment and the co-products of biomass fractionation by ethanol organosolv pretreatment, are also discussed.

10.2 Organosolv Pretreatment

10.2.1 Organosolv Pulping

Kleinert and Tayenthal proposed the use of aqueous ethanol for the delignification of wood in 1931, as described by Johansson *et al.* [13]. Along with addressing some of the environmental concerns associated with traditional Kraft and sulfite pulping, the use of organic solvents enabled the recovery of lignin and other dissolved components such as extractives in an un-degraded form, thus enabling a more efficient use of the lignocellulosic feedstock [13]. The most common organosolv pulping systems apply ethanol or methanol with mineral acids as catalysts. Alkaline organosolv pulping with methanol-water-NaOH has been investigated, but the requirement of an additional chemical recovery system for the alkali is a major drawback. Comparison of results from organosolv pulping to conventional Kraft and sulfite pulping shows that yields of organosolv softwood pulps are higher than that of conventional pulps at equivalent values of the Kappa number [13]. The Kappa number is a parameter representing the residual lignin content of a wood pulp. The strength properties of these pulps are comparable to Kraft and sulfite pulps and do not show much variation based on solvents and cooking methods. Organosolv pulp mills can be operated on a smaller scale (300 tons of pulp/day) than Kraft pulp mills (1000 tons of pulp/day) and still remain economically attractive [14]. The pulp produced can be bleached easily without chlorine, adding to the environmental benefits of this process. Further details on organosolv pulping have been reviewed by Aziz and Sarkanen [15], Johansson *et al.* [13] and Muurinen [16].

10.2.2 Overview of Organosolv Pretreatment

Organosolv pretreatment is similar to organosolv pulping, but does not require the equivalent degree of delignification as the latter. Further, while slight degradation of the cellulose structure can aid in enzymatic hydrolysis during the production of biofuels, preservation of fiber quality is important during pulping. Different cooking conditions may therefore be optimal for organosolv pulping versus pretreatment of the same lignocellulosic feedstock. In order to lower costs, both processes require that most of the solvent be recycled. This is usually achieved by flashing to atmospheric pressure, precipitation of lignin by dilution with water, and distillation of the precipitation liquor. Up to 98% of methanol and 99% ethanol can be recovered during organosolv pulping [17].

The ethanol organosolv process was originally designed to produce a clean biofuel for turbine generators by researchers at General Electric and University of Pennsylvania in the 1970s [18]. It was subsequently developed by the Canadian pulp and paper industry in to the Alcell pulping process for hardwoods. The Lignol Corporation adapted this technology as part of a commercial lignocellulosic biorefinery platform [19]. While the pretreatment conditions in the Lignol process vary with the feedstock being processed, the general ranges are: cooking temperature of 180–195 °C; cooking time of 30–90 min; ethanol concentration of 35–70% (w/w) and a liquor to solids ratio from 4:1 to 10:1 (w/w). Lignin is recovered as a precipitate by flashing the pulping liquor to atmospheric pressure, followed by dilution with water. Hemicellulose sugars and furfural are recovered as co-products from the water-soluble stream.

10.2.3 Solvents and Catalysts for Organosolv Pretreatment

A wide range of solvents and catalysts has been studied for their suitability to organosolv pulping and pretreatment. The following section describes the application of different solvent-catalyst systems and their application to lignocellulosic biomass. Ethanol and methanol are commonly used as solvents for organosolv pretreatment primarily due to their low cost, low boiling point, and ease of recovery. The Hildebrand solubility parameter or δ-value of a solvent can be used to estimate the solubility of lignin or other polymers. In general, the Hildebrand solubility parameter provides a numerical estimate of the interaction between different materials, with similar values indicating good solubility. Solvents which display good lignin solubility have δ values close to 11 [20], with acetic acid ($\delta = 10.1$), formic acid ($\delta = 12.1$), ethanol ($\delta = 12.9$), and acetone ($\delta = 9.7$) being good examples. 75% of a mixture of dioxane, ethanol, and acetone with 25% water were also found to have δ-values close to lignin and exhibited the ability to dissolve both high- and low-molecular-weight lignin fractions [21].

Ethanol and Methanol

From the viewpoint of bioethanol production, ethanol is a good solvent as losses can be made up by feeding back some of the ethanol produced by fermentation and the ethanol lost in pretreatment could end up in the product. This choice also reduces process complexity by eliminating an additional solvent stream. A general schematic of the ethanol organosolv pretreatment is given in Figure 10.1. Ethanol organosolv pretreatment can be performed with or without a catalyst with auto-catalyzed pretreatments being performed at higher temperatures (185–210 °C). The severity of the pretreatment conditions can be represented by the combined severity factor CS, which is a function of pH, cooking time, and temperature [22] and is calculated:

$$\mathrm{CS} = \log\left[t \exp\left(\frac{T - T_{\mathrm{ref}}}{14.7}\right)\right] - \mathrm{pH}$$

where t is pretreatment time (min); T is pretreatment temperature (°C); and T_{ref} is 100 °C.

A wide range of catalysts have been explored for ethanol and methanol organosolv pretreatment including mineral acids, magnesium sulfate, magnesium, calcium or barium chloride or nitrate, sodium bisulfate, and sodium hydroxide [23]. While organosolv delignification benefits by addition of mineral acids as catalysts, the lignin recovered is also more degraded, potentially making it less useful as a co-product. The factors affecting organosolv delignification, mechanisms of delignification, and results from characterization of organosolv lignin and the residual lignin on the biomass are discussed in Section 10.3.

Four main reactions or processes occur during ethanol or methanol organosolv pretreatments: (1) hydrolysis of lignin hemicellulose linkages and internal lignin bonds results in hemicellulose and lignin

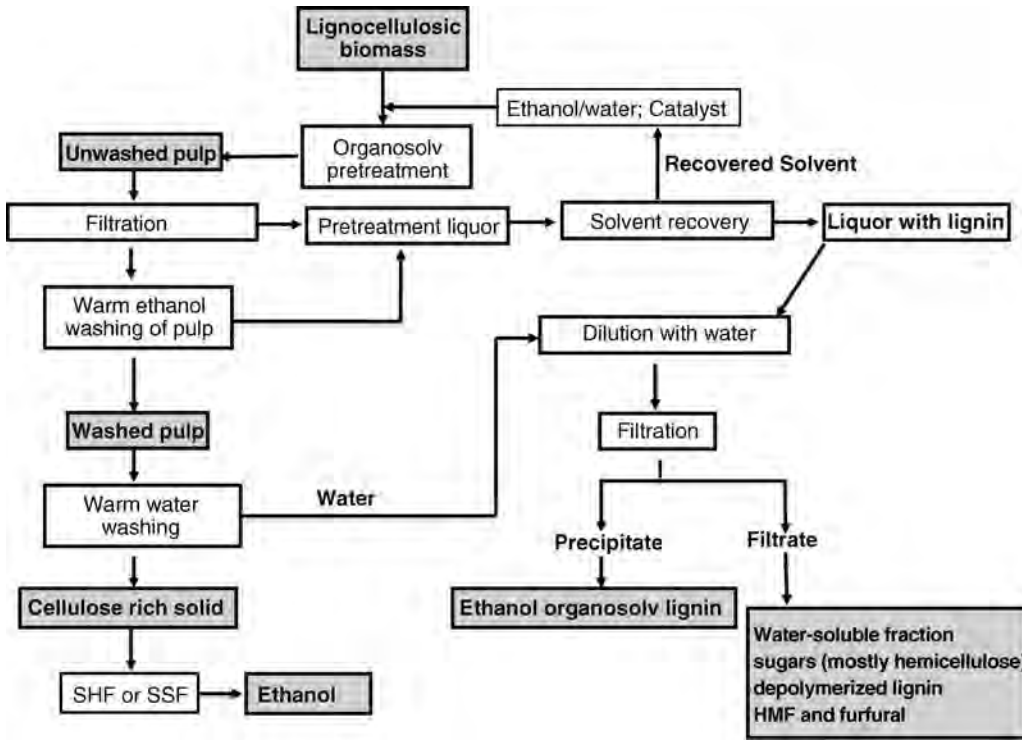

Figure 10.1 *Schematic representation of an ethanol organosolv pretreatment approach.*

solubilization by cleavage of 4-*O*-methylglucuronic acid ester bonds to the α-carbons of lignin and cleavage of α and β-*O*-aryl ether linkages respectively; (2) glycosidic bonds are cleaved in hemicelluloses and less frequently in cellulose, with the extent of cellulose degradation a function of the pretreatment severity as discussed further in Section 10.4; (3) acid-catalyzed degradation of monosaccharides to furfural, HMF, and further degradation products such as levulinic acid and formic acid; and (4) the lignin condensation reactions also discussed in Section 10.3 can occur (especially in acid-catalyzed organosolv pretreatment).

Ethanol organosolv pretreatment has been applied to a wide range of lignocellulosic feedstocks including softwoods, hardwoods, agro-energy crops, and agricultural residues [7,8,10,11,24]. Pretreatment conditions used for different types of biomass are compiled in Table 10.1. Sulfuric acid is the most frequently used catalyst for ethanol organosolv pretreatment and has been applied to several commonly used feedstocks including pine, hybrid poplar, Miscanthus, and switchgrass. Sulfuric acid concentrations are based on percentage dry weight of the biomass and are usually between 0.5 and 1.75%. Higher acid concentrations lead to greater delignification, but greater hemicellulose degradation may be an undesired side effect. In case of Miscanthus, an additional dilute sulfuric acid (0.15 M) presoaking step in which the biomass is extracted overnight with acid under reflux, was beneficial in recovering hemicellulose sugars prior to organosolv pretreatment [7]. Sulfur dioxide (SO_2) behaved similarly to sulfuric acid in terms of lignin removal and cellulose recovery when used as a catalyst for the organosolv pretreatment of Lodgepole pine [28]. When subjected to enzyme hydrolysis, the acid-catalyzed organosolv substrate showed faster and greater (100% vs. 70%) cellulose to glucose conversion than the SO_2 catalyzed substrate. The lower weight-average degree of polymerization (DP_w) of cellulose after sulfuric-acid-catalyzed organosolv pretreatment may be an important factor in determining its enzymatic digestibility and is discussed further in Section 10.4.

Table 10.1 *Solvent concentrations, pretreatment conditions, and catalysts used for ethanol organosolv pretreatment of different biomass feedstocks. The conditions given are optimal for high cellulose recovery and cellulose to glucose conversion after enzymatic hydrolysis.*

Biomass	Ethanol concentration (% by volume)	Catalyst (% dry wt. biomass)	Temperature, time (°C, min)	Reference
Eucalyptus	75	1% acetic acid	200, 60	[25]
Baggase	75	1% acetic acid	200, 60	[25]
Hybrid poplar	60	1.3% sulfuric acid	180, 60	[8]
Lodgepole pine	65	1.1% sulfuric acid	170, 60	[9]
Loblolly pine	65	1.1% sulfuric acid	170, 60	[11]
Miscanthus	80	0.5% sulfuric acid	170, 60	[7]
Kanlow switchgrass	75	0.9% sulfuric acid	180, 60	[26]
Buddleja davidii	50	1.8% sulfuric acid	180, 40	[24]
Lodgepole pine	78	0.03M $MgCl_2$	200, 60	[28]
Lodgepole pine	65	20% NaOH	170, 69	[28]
Lodgepole pine	65	1% sulfur dioxide	170, 60	[28]
Barley straw	50	0.1M $FeCl_3$	170, 60	[27]

For most feedstocks, the cellulose-rich substrate produced during ethanol organosolv pretreatment exhibits high glucose yields after enzymatic hydrolysis. One of the exceptions is base- (NaOH) catalyzed pretreatment of Lodgepole pine, which resulted in high lignin removal but low glucose recovery and xylan removal and showed low cellulose to glucose conversion of the resulting substrate [28]. Results from enzymatic digestibility of cellulose obtained after organosolv pretreatment are included in Table 10.2, which compiles results from biomass fractionation. In most cases, 98% cellulose to glucose conversion is achieved in 48 hours of enzyme hydrolysis with an enzyme loading on 20 FPU cellulase and 40 IU beta-glucosidase per gram of cellulose. These studies were mostly carried out at a solids loading of 2 g cellulose in 100 mL solution. Several characteristics of organosolv-pretreated biomass such as low hemicellulose and lignin content, decreased cellulose chain length and molecular weight [24,28], and increased pore volume contribute to this improved digestibility.

Fermentation of organosolv-pretreated substrates has been performed with simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF). At an industrial scale, SSF is likely to be the preferred approach as it leads to overall lower processing times, less enzymatic inhibition by hydrolysis products (e.g., cellulase inhibition by cellobiose), and hence lower capital and operating costs [29]. Pan *et al.* [18] compared the ethanol yields upon SSF and SHF of ethanol-organosolv-pretreated mixed softwoods. SHF on a substrate with 6.4% residual lignin resulted in 90% of the theoretical ethanol conversion (assuming 1 g glucose yields 0.51 g ethanol) in 8 hours. A substrate with 8.5% residual lignin produced 84% of its theoretical yield in 24 h with SSF [18]. At a low concentration of fermentation inhibitors, SSF has been seen to yield 99.5% theoretical ethanol yield from acetone-organosolv-pretreated *Pinus radiata* [30]. Thus SSF and SHF both appear suitable for the production of ethanol after biomass pretreatment and fractionation using the organosolv method. It should be mentioned that all the above studies employed the hexose-fermenting yeast *Saccharomyces cerevisiae*. Fermentation of the pentose sugars with specialized microorganisms or other high-value uses of these sugars is vital to good process economics.

Butanol

Butanol is an excellent delignification agent due to its hydrophobicity [28]. It can also be produced from lignocellulosic biomass by a fermentation of sugars released after pretreatment and enzymatic hydrolysis,

Table 10.2 *Results from biomass fractionation by ethanol organosolv pretreatment according to conditions presented in Table 10.1. All results are given as % dry weight of untreated biomass.*

Biomass feedstock	Cellulose			Hemicellulose			Lignin			
	Untreated	Liquid fraction[a]	Solid fraction[b]	Untreated	Liquid fraction	Solid fraction	Untreated	EOL[c]	Liquid fraction	Solid fraction
Loblolly pine [11]	42.0	3.0	33.3 (55)	21.6	0.5	15.3	29.9	5.4	12.4	11.6
Lodgepole pine [10]	50.5	4.2	37.6 (100)	23.9	1.2	11.1	25.1	19.6	4.8	4.2
Hybrid poplar [8]	48.9	0.6	43.2 (98)	22.4	4.9	11.2	23.3	15.5	5.2	6.2
Miscanthus [7]	37.7	1.7	35.5 (98)	37.3	3.3	25.1	26.3	18.1	0.2	7.8
B. davidii [24]	38.9	1.4	32.3 (98)	26.1	5.7	11.0	30.2	8.9	3.4	19.0

[a] Effluent and wash solution after pretreatment

[b] Solid substrate recovered after pretreatment. Numbers in parentheses represent the % cellulose to glucose conversion after 48 h enzymatic hydrolysis with 20 FPU cellulase and 40 IU beta-glucosidase per gram cellulose. 8 FPU cellulase and 16 IU beta-glucosidase per gram of cellulose were used in this study.

[c] Ethanol organosolv lignin

using the microorganism *Clostridium acetobutylicium* [31]. Butanol has value as a fuel additive and as a platform chemical for producing materials and value-added chemicals. When an aqueous butanol mixture is used as a solvent for organosolv pretreatment, given the limited miscibility of butanol in water it is possible to concentrate hemicelluloses in the aqueous layer, lignin in the butanol layer, and cellulose in the solid fraction. Thus, pretreatment with butanol may also be an efficient means of biomass fractionation. Del Rio *et al.* [28] studied the effects of different catalysts on ethanol and butanol organosolv treatment of Lodgepole pine. Pretreatment with butanol/water consistently yielded substrates that were more readily hydrolysable with enzymes. The limited miscibility of butanol and water was suggested to lead to higher pretreatment severity, which in turn led to the formation of substrates with lower hemicellulose content, lower cellulose DP_w, and increased pore size. Among the solvent-catalyst systems studied, butanol-SO_2 resulted in the fastest cellulose to glucose conversion with 82% conversion in 12 hours [28].

Polyhydroxy Alcohols

Ethylene glycol and glycerol are the most commonly employed higher-boiling-point alcohols for organosolv pretreatments. One of the main advantages of using such solvents is that the pretreatment can be performed at atmospheric pressure, which reduces energy costs and the need for pressure vessels. Aqueous glycerol was found to be effective for delignification of wood chips [32]. Auto-catalyzed aqueous organosolv pretreatment at 240 °C for 4 h resulted in 95% cellulose recovery and 70% lignin removal from wheat straw [33]. Results from recovery of lignin and hemicellulose in glycerol pretreatment are not available. Similar to steam explosion, aqueous glycerol pretreatment dissociates the guaiacyl lignin subunits and is seen to have a smaller effect on syringyl lignin [23]. In lieu of using high-grade glycerol, crude glycerol produced as a by-product of biodiesel generation can be used in these pretreatments, leading to further cost reduction. The high-energy costs of solvent recovery, which is considered a disadvantage of glycerol pretreatment, can also be partially offset by using crude glycerol.

Organic Acids and Peracids

As mentioned in Section 10.2.3, acetic acid and formic acid have solubility parameter values similar to lignin and are good lignin solvents. However, their use in biomass pretreatment has been somewhat limited due to their corrosive nature. Vazquez *et al.* [34] pretreated Eucalyptus wood chips with an HCl-catalyzed 70% acetic acid solution. Xylan removal and delignification were observed as the main effects of this pretreatment. However, high rates or extent of xylan and lignin removal did not translate into faster or greater enzymatic cellulose hydrolysis. Acetylation of cellulose, in which hydroxyl groups of cellulose are substituted by acetyl groups, inhibited productive binding of cellulase to cellulose via hydrogen bonds. Pretreatment of beech hardwood with 80% formic acid under different conditions showed that it was capable of extensive delignification (up to 90% at 130 °C and 150 min) and high cellulose recovery (average of 98%; [35]). Further, xylose was reported as the main hemicellulose hydrolysis product and did not undergo significant conversion to furfural. The enzymatic digestibility of fractionated cellulose was not examined but, in a manner analogous to acetic acid pretreatment, formylation of cellulose during formic acid pretreatment can hinder enzymatic digestibility of the pretreated biomass.

Peracetic and performic acid have also been investigated as organosolv pretreatment reagents. Performic acid is used in the Milox pulping process; however, there are major concerns about its stability and the associated safety issues [36]. Peracetic acid is produced by reacting acetic acid with hydrogen peroxide in the presence of sulfuric acid. While peracetic acid pretreatments can be performed at ambient temperatures for longer time periods to save on energy costs, increasing the temperature to 80–90 °C can significantly decrease the pretreatment time. Treatment with sodium hydroxide to remove part of the lignin and swell the biomass prior

to peracetic acid pretreatment was found to be very effective in producing material from hybrid poplar and sugarcane baggase that was very amenable to enzymatic deconstruction [37]. This two-step method had high carbohydrate yields together with negligible formation of furfural and HMF due to low carbohydrate dehydration at low pretreatment temperatures. The corrosive nature of peracetic acid, reagent costs in producing it, and operational concerns are some of the drawbacks to its large-scale application.

Phosphoric Acid

Zhang *et al.* [38] exploited the differential solubilities of cellulose, hemicelluloses, and lignin in different solvents to develop a novel pretreatment strategy by which lignocellulosic biomass could be fractionated into amorphous cellulose, hemicellulose, lignin, and acetic acid. In this method, the biomass is brought in contact with concentrated phosphoric acid (>82%) at 50 °C for 30–60 min, which acts to disrupt the lignin-carbohydrate complex bonds, break up hydrogen bonding in carbohydrate chains, weakly hydrolyze cellulose and hemicellulose to low degree of polymerization (DP) fragments, and remove acetyl groups from hemicellulose to produce acetic acid [38]. The cellulose and hemicellulose dissolved in acetic acid are precipitated by the addition of acetone, which also results in the partial dissolution of lignin. Phosphoric acid is washed from the precipitated solids with the addition of more acetone and the liquor comprising phosphoric acid, acetone, acetone-soluble lignin, and acetic acid derived from the hemicellulose fraction is distilled to separate the acetone and acetic acid based on their different volatility. This pretreatment was shown to effectively produce enzyme hydrolysable cellulose from diverse feedstocks including corn stover, switchgrass, hybrid poplar, and Douglas fir [38]. With the exception of Douglas fir cellulose, which showed a 75% conversion to glucose in 24 h, cellulose from the other biomass materials showed 97% conversion to glucose in the same time period. While this process has promise as a method for biomass fractionation, the corrosive nature of phosphoric acid stream may impede its implementation on an industrial scale.

Acetone and Methyl Isobutyl Ketone

Acetone is an excellent lignin solvent, and both auto-catalyzed and catalyzed (usually with mineral acids) acetone organosolv pretreatments have been successfully applied to the fractionation of a variety of biomass feedstocks. Araque *et al.* [30] optimized the conditions for organosolv pretreatment of *Pinus radiata* with aqueous acetone (50%) and 0.9% sulfuric acid. After pretreatment at an H factor (a parameter representing pretreatment severity which is a function of heat-up time, cooking temperature, and cooking time) of 939, up to 70.9% of the glucan could be recovered in the solid fraction. Almost all the hemicelluloses were solubilized or degraded, and about 47% of the lignin was recovered as organosolv lignin, leading to a relatively efficient fractionation of the biomass. While modest (72%) enzymatic glucose yields were obtained, the extent of delignification was not found to have a clearly discernible effect on the extent of cellulose hydrolysis. Huijgen *et al.* [39] fractionated a key agricultural residue, wheat straw, using auto-catalyzed-acetone-organosolv pretreatment. A 50% acetone-water mixture gave the highest lignin recovery (61%), but it decreased at higher acetone concentrations due to higher solution pH. Slowing lignin bond cleavage at higher pH lowered the extent of delignification. Huijgen *et al.* [39] conducted a series of pretreatments with 50% acetone-water at different reaction times and temperature. Good biomass fractionation was obtained after acetone pretreatment at 205 °C for 1 h, which resulted in 82% hemicellulose hydrolysis, 79% delignification, and 93% cellulose recovery. A glucose yield of 87% was obtained after enzymatic hydrolysis. Delignification increased with process severity up to a temperature of 205 °C. At 220 °C, lignin condensation reactions were suggested to increase the residual lignin content in the pulp. However, the amount of lignin which could be recovered from the organosolv liquor increased with process severity, and 100% could be recovered after pretreatment at 220 °C for 120 minutes.

Hemicellulose hydrolysis is auto-catalyzed by the pH drop resulting from the formation of acetic acid by dehydration of hemicellulose acetyl side groups. Increased pH resulting from higher acetone concentrations in the cooling liquor also results in reduced hemicellulose hydrolysis. At high temperatures, xylose was found to be increasingly dehydrated to furfural [39]. Separate reaction conditions were found to be optimal for the recovery of cellulose, hemicellulose, and lignin. Pretreatment conditions should therefore be chosen after taking into account the net revenues of the different process streams.

Acetone-based fractionation of biomass has also been carried out in a two-step process in which biomass is first treated with water at 180 °C to recover hemicellulose. This is followed by extraction with a flowing water-acetone mixture at 230 °C and 10 MPa pressure to separate the lignin and the cellulose [40]. These results suggest that acetone is a promising solvent for biomass fractionation.

In a recent study, Bozell *et al.* [41] developed a novel organosolv biomass fractionation process which they termed "Clean Fractionation." In this method, lignocellulosic material is separated with a ternary mixture of methyl isobutyl ketone, ethanol and water in the presence of sulfuric acid, which selectively dissolves lignin and hemicellulose, leaving a cellulose residue. Treatment of the single-phase liquor with water results in the separation of a lignin-rich organic phase and a hemicelluloses-rich aqueous phase. For woody feedstocks, the yield of the cellulose fraction across all separations averaged 47.7 wt% ($\pm$1.1). The authors reported that while the cellulose and lignin fractions obtained using this method were quite pure, the impurities in the hemicellulose stream could be removed by ion exchange chromatography.

10.2.4 Fractionation of Biomass during Organosolv Pretreatment

Organosolv pretreatment, especially with ethanol as a solvent, has been shown to be very effective in fractionating lignocellulosic biomass into a cellulose-rich solid, a water-soluble hemicellulose stream, and a solid organosolv lignin fraction. Information on the mass balance of the major biomass components available for several promising feedstocks is compiled in Table 10.2. As the results in Table 10.2 indicate, the cellulose recovered in the solid fraction can range from 74% [9] to 94% [7]; when combined with that dissolved in the pretreatment liquor, up to 99% of the cellulose in the untreated biomass can be accounted for. Results from glucose released after 48 hours of enzymatic hydrolysis of this cellulose-rich solid substrate are also included in Table 10.2, providing further evidence of the effectiveness of organosolv pretreatment. The hemicellulose content of the pretreated solids was usually low, and a greater proportion of these sugars are measured in the liquid stream. A much greater fraction of hemicelluloses are degraded during organosolv pretreatment compared to cellulose, with hemicellulose recoveries ranging from 51% [9] to 76% in Table 10.2 [7]. Hemicelluloses are easily degraded in pretreatments carried out at low pH to form furfural and HMF and acetic acid. Organosolv pretreatment conditions can be optimized further to improve hemicellulose yields, but the net revenue of the hemicellulose stream or from its conversion to ethanol by pentose-fermenting organisms compared to that from greater cellulose recovery and corresponding ethanol yield should be considered.

The generation of a relatively pure, undegraded, and sulfur-free lignin stream is one of the main advantages of organosolv pretreatment; at appropriate processing conditions, most of the lignin in the biomass can be recovered. However, conditions that result in the highest degree of delignification usually involve higher acid concentrations and cooking temperatures [8], which may not be optimal for producing substrates amenable to enzymatic hydrolysis. Under conditions which generated pretreated biomass with good enzymatic sugar release ($\geq$70%), 20–78% of the total lignin (acid insoluble + acid soluble) in the untreated biomass was recovered as ethanol organosolv lignin (Table 10.2). Lignin depolymerization during pretreatment leads to its partial solubilization in the cooking and wash solutions, and this lignin is not recovered in the organosolv lignin stream. However, it still leads to delignification of biomass and aids in enzymatic hydrolysis. As seen in the results compiled in Table 10.2, up to 42% of the lignin may be dissolved in the

combined ethanol and water wash solutions. Overall, the mass closure for lignin was much higher than for hemicellulose and cellulose and, in some cases, exceeded 100% due to the presence of extractives, lignin fragments, tannins, and related phenol compounds in the water-soluble fraction, resulting in inflated measurements of the acid-soluble lignin content [8].

10.3 Nature of Organosolv Lignin and Chemistry of Organosolv Delignification

Lignin is an amorphous, cross-linked phenolic polymer that is biosynthesized from three mono-lignols: coniferyl alcohol, sinapyl alcohol, and *p*-coumaryl alcohol [42]. The proportions of different mono-lignols involved depend on the plant species and undergo radical polymerization to form lignin inter-unit linkages. Some common lignin inter-unit linkages are β-*O*-aryl ether (β-*O*-4), resinol (β-β′), phenylcoumaran (β-5′), biphenyl (5-5′) and 1,2-diarylpropane (β-1′) [42].

10.3.1 Composition and Structure of Organosolv Lignin

Organosolv pretreatment results in lignin solubilization into the pretreatment solvent and washing liquor, from which it is precipitated by lowering the solution pH for recovery as a separate fraction. The lignin recovered in this process is generally termed organosolv lignin. To understand pathways for organosolv delignification, native (milled wood) lignin from biomass and organosolv lignin can be characterized using elemental analysis, quantitative ^{1}H, ^{13}C and ^{31}P nuclear magnetic resonance (NMR), and molecular weight analysis. Wet chemistry techniques, such as thioacidolysis and nitrobenzene oxidation, coupled with gas chromatography can also be applied for precise determination of specific structural moieties in lignin. However, unlike spectroscopic techniques, these methods do not have the ability to analyze the whole lignin structure. Recently, results from detailed characterization of these two lignin fractions from Loblolly pine, Miscanthus, and *Buddleja davidii* have been obtained [43–45]. Table 10.3 summarizes quantitative ^{13}C NMR results, which shed information on the proportions of different lignin functional groups and compositional parameters such as degree of condensation that can be calculated from these results. For all three biomass feedstocks, the largest change after organosolv pretreatment was a decrease (∼50%) in Cγ in β-*O*-4. This is consistent with acid-catalyzed scission of these linkages, which has been proposed as the major mechanism for lignin dissolution in organosolv systems. Such distinct changes are not seen for the β-β′ and β-5′ linkages. In the aromatic region, ^{13}C NMR can provide information on the protonated (aromatic C—H), oxygenated (aromatic C—O), and condensed (aromatic C—C) functional groups. In lignin isolated after organosolv pretreatment, the oxygenated aromatic region does not change compared to milled wood

Table 10.3 *Lignin functional group content and degree of condensation for milled wood lignin (MWL) and ethanol organosolv lignin (EOL).*

Equivalences per aromatic ring	Pine MWL [43]	Pine EOL[a] [43]	Miscanthus MWL [44]	Miscanthus EOL[b] [44]	*B. davidii* MWL [45]	*B. davidii* EOL[a] [45]
Methoxyl	1.0	0.9	1.0	1.1	1.2	1.1
β-*O*-4	0.6	0.3	0.5	0.3	0.6	0.3
C_β in β-β and β-5	0.2	0.1	—	—	0.2	0.1
Aromatic C-O	2.0	2.1	1.9	1.5	2.2	2.2
Aromatic C-C	1.5	2.1	1.7	2.0	1.4	1.8
Aromatic C-H	2.6	2.0	2.5	2.3	2.4	2.1
Condensation	0.4	1.1	—	—	0.6	1.0

[a] The organosolv pretreatment conditions are given in Table 10.1.

[b] Organosolv pretreatment of Miscanthus was performed at 65% ethanol, 1.2% sulfuric acid, 190 °C, and 60 min.

Table 10.4 *Hydroxyl group contents of MWL and EOL determined by ^{31}P NMR spectroscopy.*

Lignin	Hydroxyl (OH) content (mmol/g lignin)			Carboxylic OH (δ 136.6–133.6)
	Aliphatic OH (δ 150.0–145.5)	Condensed phenolic OH (δ 144.7–141.0)	Guaiacyl OH (δ 141.0–138.0)	
Pine MWL [43]	7.3	0.1	0.5	0.0
Pine EOL[a] [43]	4.2	0.6	1.4	0.3
B. davidii MWL [45]	4.5	0.3	0.4	0.0
B. davidii EOL[a] [45]	2.5	1.0	1.5	0.2
Miscanthus MWL [44]	4.0	0.2	0.7	0.1
Miscanthus EOL[b] [44]	1.2	0.1	1.3	0.2

[a] The organosolv pretreatment conditions are given in Table 10.1.

[b] Organosolv pretreatment of Miscanthus was performed at 65% ethanol, 1.2% sulfuric acid, 190 °C, and 60 min.

lignin. However, for all three biomasses, an increase in the condensed aromatic C content and a decrease in protonated aromatic C is seen in the organosolv lignin (Table 10.3). This implies an increase in the degree of condensation due to the formation of C—C linkages. The methoxyl (OCH_3) content of lignin does not change appreciably, except after severe pretreatments [44,45], indicating no demethylation.

Following phosphitylation, the different free hydroxyl (OH) functionalities in lignin can be quantitatively estimated with ^{31}P NMR spectroscopy [46–48]. Quantification is performed based on the known OH content of an internal standard such as cyclohexanol. ^{31}P NMR experiments require less instrument time than quantitative ^{13}C NMR, and the spectra display nicely resolved peaks which can be ascribed to aliphatic, condensed and uncondensed phenolic, and carboxylic OH groups. ^{31}P NMR data from organosolv lignin and their comparison to milled wood lignin showed a decrease in aliphatic OH content after organosolv pretreatment (Table 10.4). This was accompanied by an increase in the phenolic OH and an increase in degree of condensation as shown by ^{13}C NMR results and also evident from the ^{31}P results. Carboxylic OH content of organosolv lignin fractions was higher than the corresponding milled wood lignin (MWL), indicating the hydrolysis of ester bonds during pretreatment.

The molecular weight distribution of lignin can be determined with gel permeation chromatography (GPC), with acetylated lignin dissolved in tetrahydrofuran and results quantified against polystyrene standards. Number average $\left(\overline{M_n}\right)$and weight average $\left(\overline{M_w}\right)$ molecular weights and the polydispersity index $\left(D = \overline{M_w}/\overline{M_n}\right)$ of lignin can be estimated using this method. The polydispersity index provides an estimate of the distribution of molecular weights in a polymeric material. Results listed in Table 10.5 show that the molecular weight of organosolv lignin is lower than milled wood lignin, even at low pretreatment severity. This leads to better solubility of organosolv lignin in various solvents and allows for a variety of practical applications that are discussed later. The polydispersity does not vary significantly but is often lower in

Table 10.5 *Molecular weight distributions and polydispersities of MWL and EOL.*

Lignin	$\overline{M_n}$ (g/mol)	$\overline{M_w}$ (g/mol)	D $\left(\overline{M_n}/\overline{M_w}\right)$
Pine MWL [43]	7590	13 500	1.77
Pine EOL[a] [43]	3070	5410	1.77
B. davidii MWL [45]	7260	16 800	2.31
B. davidii EOL[a] [45]	661	2350	3.56
Miscanthus MWL [44]	8300	13 700	1.65
Miscanthus EOL[b] [44]	4690	7060	1.51

[a] The organosolv pretreatment conditions are given in Table 10.1.

[b] Organosolv pretreatment of Miscanthus was performed at 65% ethanol, 1.2% sulfuric acid, 190 °C, and 60 min.

organosolv lignin. Low molecular weights coupled with low polydispersity (indicating a narrow distribution of molecular weights) are ideal for further valorization of organosolv lignin. With an increase in pretreatment severity, the molecular weight of organosolv lignin falls to a certain extent, after which it does not change significantly [24]. Thus, ethanol organosolv pretreatment appears to attack certain lignin linkages and solubilize a fraction of the lignin. As severity increases, more of the same linkages are hydrolyzed leading to greater delignification. A drop in molecular weight with increasing severity has also been seen for formic acid and acetic acid organosolv pretreatment of wheat straw [49].

Effects of Pretreatment Severity on Composition of Organosolv Lignin

With increasing pretreatment severity, a greater proportion of lignin in a biomass feedstock is recovered as organosolv lignin [7–9,24,50,51]. Under conditions of optimum glucose recovery, up to 70% of the lignin could be removed from Miscanthus [7]. When the effects of the different pretreatment parameters, temperature, ethanol concentrations, and acid concentrations are considered separately, temperatures above 195 °C result in decreased lignin yield from hybrid poplar due to excessive depolymerization which reduces the lignin recovery [50]. The maximum lignin recovery was obtained at 65% ethanol concentration. While low ethanol concentrations lead to lower solution pH and promote the cleavage of lignin aryl ether linkages, the degraded lignin has limited solubility due to the low solvent concentration. Lignin solubility in ethanol-water mixtures is highest at *c.* 70% ethanol concentration [52]. Organosolv pretreatment at higher ethanol concentrations therefore leads to lower lignin recovery due to lower solution pH and limited lignin solubility. Longer reaction times and higher acid concentrations both lead to greater delignification [50].

With increasing pretreatment severity, the organosolv lignin recovered has higher phenolic content due to the enhanced cleavage of α- and β-aryl ether linkages between lignin units. This leads to the formation of new phenolic units [53]. A decrease in the number of these linkages observed in ^{13}C and ^{1}H NMR results and in aliphatic OH groups from ^{31}P NMR provides evidence for this mechanism (Table 10.6). An increase in lignin condensation is also seen to occur at high severity, and the reaction responsible is discussed in the following section. Other structural trends with increased severity include increased carboxylic content in organosolv lignin as a result of enhanced ester hydrolysis. The molecular weight distribution and polydispersity of organosolv lignin decreased with increasing temperature and acid concentration (Table 10.6). Increasing ethanol concentration produced organosolv lignin with higher molecular weights and polydispersity, due to the reduced cleavage of aryl ether linkages. Reaction time did not have a significant effect on molecular weight [49].

Table 10.6 *Variations in ethanol organosolv lignin compositions and molecular weights as a function of CS.*

Lignin Parameter	Miscanthus [53]			*B. davidii* [43]			Hybrid Poplar [51]	
	CS 1.75	CS 2.39	CS 2.93	CS 2.40	CS 2.92	CS 1.68	CS 2.47	CS 2.87
% biomass recovered as EOL	3.7	11.0	13.1	4.3	19.0	5.7	17.2	4.4
β-*O*-4 (#/Ar group)	0.9	0.2	0.1	0.4	0.2	—	—	—
Aliphatic OH[a] (mmol/g lignin)	3.1	1.1	1.3	2.7	1.9	5.0	3.2	3.3
Phenolic OH[a] (mmol/g lignin)	2.3	4.0	3.9	2.6	2.7	2.2	4.1	4.6
$\overline{M_n}$(g/mol)	2500	3100	2300	578	645	1515	1123	783
$\overline{M_w}$(g/mol)	6500	4300	3200	2740	2490	3877	1890	1105
D	2.6	1.4	1.4	4.7	3.9	2.6	1.7	1.4

[a] Aliphatic and phenolic OH contents for Miscanthus and *B. davidii* were determined with ^{31}P NMR spectroscopy, while those for Hybrid poplar were determined with ^{1}H NMR spectroscopy.

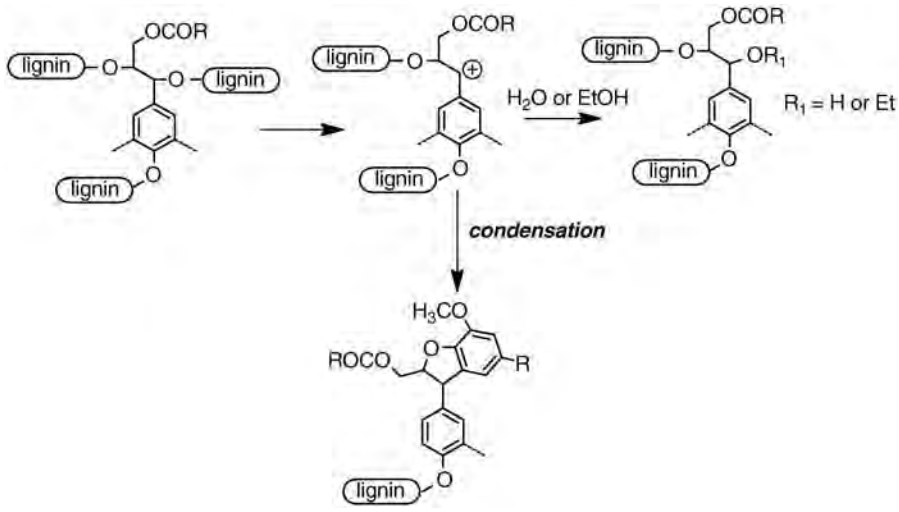

Figure 10.2 *Solvolytic splitting of α-O-aryl ether linkages in lignin [53].*

10.3.2 Mechanisms of Organosolv Delignification

It is widely acknowledged that the cleavage of aryl ether linkages is primarily responsible for lignin breakdown during the organosolv pretreatment. Of these, α-*O*-aryl ether bonds are cleaved more easily, whereas β-*O*-aryl ether bonds are broken under more severe conditions, especially at higher acid concentrations [54]. The cleavage of α-*O*-aryl ether bonds is the rate-controlling step in organosolv delignification [55], and several pathways have been proposed for this reaction [54] including: (1) solvolytic splitting of α-*O*-aryl ether linkages via the quinone methide intermediate; (2) solvolytic cleavage by nucleophilic substitution benzylic position by an S_N2 mechanism; and (3) the reaction via formation of a benzyl carbocation under acidic conditions (Figure 10.2). β-*O*-aryl ether linkages can be broken by homolytic cleavage with the loss of terminal γ-methylol groups as formaldehyde. This mechanism has been shown to give rise to stilbenes [45]. Formation of Hibbert's ketones (evidenced by the presence of carbonyl groups) can also occur via cleavage of β-*O*-aryl ether bonds [53]. Mechanisms of solvolytic splitting of β- and γ-*O*-aryl ether linkages are shown in Figure 10.3. Under more acidic conditions, lignin condensation reactions occur which are counter-productive to organosolv delignification, as observed by several researchers [43,54,56]. Condensation reactions lead to the formation of higher-molecular-weight lignin fractions which are not readily soluble in the organosolv pretreatment solvent and hence cannot be recovered. The benzyl carbocation intermediate of α-*O*-aryl and

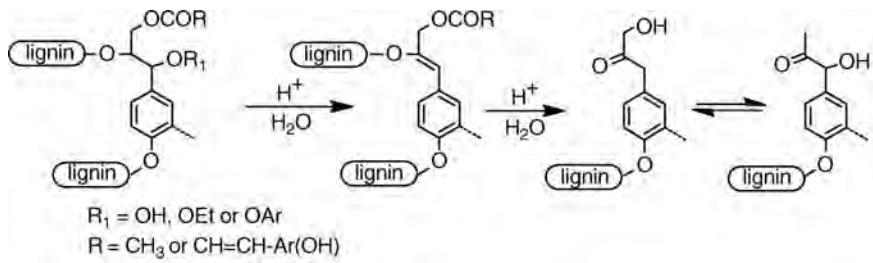

Figure 10.3 *Solvolytic splitting of β- and γ-O-aryl ether linkages in lignin [53].*

β-*O*-aryl ether cleavage can form a bond with another electron-rich carbon atom in a neighboring lignin unit, thus increasing lignin condensation (Figure 10.2). In organosolv pretreatments with phenolic solvents, lignin condensation can be prevented by reaction of the benzyl carbocations by electrophilic aromatic substitution on the aromatic ring of the solvent. This blocks the reactive benzyl position, preventing it from undergoing condensation reactions with other lignin fragments [54].

10.3.3 Commercial Applications of Organosolv Lignin

As seen in Section 10.3.1, organosolv lignin has higher phenolic and carboxylic content than native lignin from biomass. Under most pretreatment conditions, these isolated lignin fractions also have lower molecular weight and hence higher solubility. Moreover, organosolv lignins have very low sulfur content (as opposed to lignin obtained from Kraft pulping) and have lower oxygen content than native biomass lignin. These characteristics could allow for a variety of commercial applications of organosolv lignin, thus enhancing process economics of biomass fractionation by this method. The high phenolic OH content of organosolv lignin can be exploited to produce phenolic, epoxy, and isocyanate resins [57]. Phenolic powder resins have been successfully applied as a binder for the commercial-scale manufacturing of automotive brake pads and molding. Polyurethane and polyisocyanate foams, with 17% and 26% lignin substitution for polyols, have high density [58]. Investigation of the antioxidant activity of different organosolv lignin preparations has shown that free phenolic groups contribute to higher antioxidant activity [51]. Stabilization of phenoxyl radicals by conjugated double bonds or substituents such as methoxyl groups at the *ortho* position can further enhance antioxidant activity. Lower molecular weight and polydispersity of organosolv lignin also correlates with higher antioxidant activity [51]. Polyphenols can be beneficial for human health in various ways. These compounds can inhibit the oxidation of low-density proteins and decrease the risk of heart disease. Polyphenols also have anti-inflammatory and anti-carcinogenic properties.

High-purity lignin such as organosolv lignin, which has low carbohydrate content, can be used as a precursor for the production of chemicals such as vanilla, phenol, and ethylene and can be converted to carbon fibers which are of high value [58]. Other large-scale applications of organosolv lignin include dispersants, soil-conditioning agent, adsorbents, and adhesives. As an energy source, organosolv lignin has a higher heating value of 26 MJ/kg and, upon combustion, can provide energy in excess of that required for the pretreatment and ethanol distillation [59]. For added value, organosolv lignin can be valorized by catalytic hydrogenation to low-molecular-weight molecules, which are more amenable to liquefaction and production of fuels and fuel additives. Depolymerization and deoxygenation of organosolv lignin is essential for its successful conversion to a form usable as a fuel or fuel additive. Organosolv lignin can also be thermally depolymerized by pyrolysis to produce pyrolytic lignin oil [60]. These oils have some limitations such as low pH, high corrosiveness, and instability, which necessitate upgrading of the crude bio-oil to impart favorable fuel properties. Advances in catalyst research have made important contributions to this field [61].

10.4 Structural and Compositional Characteristics of Cellulose

Native cellulose comprises crystalline regions with long-range molecular ordering, amorphous regions with only short-range molecular ordering, and intermediate or *para*-crystalline regions [62]. Crystalline cellulose is a composite of cellulose Iα, which has a triclinic unit cell structure, and cellulose I_β, which has a monoclinic unit cell. Cellulose I_α is the dominant form of crystalline cellulose in bacterial and algal cellulose and is energetically less stable than cellulose I_β which is predominant in higher plants [63]. The different forms of crystalline cellulose, *para*-crystalline cellulose, amorphous cellulose at accessible fibril surfaces (i.e., in contact with water), and amorphous cellulose at inaccessible fibril surfaces (fibril-fibril contact surfaces and

surfaces resulting from distortions in the fibril interior) can be characterized by solid-state ^{13}C NMR spectroscopy. The parameter crystallinity index (CrI) is a measure of the relative proportion of crystalline cellulose and can be estimated by various analytical techniques including solid-state ^{13}C NMR, Fourier transform infrared (FTIR) spectroscopy, and X-ray diffraction [64]. Due to their greater level of molecular ordering, crystalline cellulose is thought to be more resistant to chemical and enzymatic degradation. The effect of cellulose crystallinity on enzymatic action is however a subject of considerable debate and is beyond the scope of this chapter.

The molecular weight distribution of cellulose can be determined by GPC after derivatization by tricarbanilation and can in turn provide information on the DP. The weight-average degree of polymerization (DP_w) of cellulose, which is most commonly reported, can be calculated from its molecular weight data [65]. Cellulose DP values can also be rapidly determined from viscometry measurements and denoted DP_v. If a pretreatment reduces cellulose DP without causing extensive structural degradation, it produces more reducing ends available for exoglucanase enzymes to act on, thus enhancing sugar release. In case of organosolv pretreatment, cellulose DP is seen to vary with solvent, catalyst and, to some extent, pretreatment severity [24,28,66]. Values of cellulose DP_w compiled from the literature are given in Table 10.7. Organosolv pretreatment of Lodgepole pine with butanol as the solvent and $MgCl_2$ and SO_2 as catalysts resulted in much lower cellulose DP_w than the equivalent pretreatment with ethanol [28]. However, sulfuric-acid-catalyzed organosolv pretreatment produced cellulose with similar DP_w for both solvents. In general, for the different solvent-catalyst systems investigated, lower cellulose DP_w translated to greater cellulose hydrolysis after 12 hours. Organosolv pretreatment of wheat straw with ethanol-produced cellulose with the lowest DP, followed by similar values for methanol and acetic acid and the lowest cellulose depolymerization (highest DP) resulted from combined acetic acid/formic acid pretreatment [66]. For the same solvent, an increase in the proportion of solvent helped preserve cellulose DP_w (Table 10.7). Overall, an increase in pretreatment severity results in lower DP of the cellulose to some extent [10,24], which can help in enzymatic cellulose hydrolysis; however, care should be taken to avoid extremely severe conditions that cause cellulose loss.

The effect of organosolv pretreatment on cellulose CrI has not been extensively investigated but, from the limited amount of data available, it appears to be a function of the initial CrI of biomass and pretreatment severity. The cellulose CrI values discussed in this section were determined with solid-state ^{13}C NMR of cellulose isolated from biomass. Ethanol organosolv pretreatment of Switchgrass [26] and Buddleja [24],

Table 10.7 *Degree of polymerization (DP_w) of cellulose before and after organosolv pretreatment of various biomass feedstocks. (EtOH: ethanol; SA: sulfuric acid; AcOH: acetic acid; FA: formic acid; MeOH: methanol; BuOH: butanol.).*

Biomass	Pretreatment conditions	Cellulose DP_w	
		Untreated	Pretreated
Kanlow Switchgrass [26]	75% EtOH, 0.9% SA, 180 °C, 1 h	2900	2412
B. davidii [24]	50% EtOH, 1.8% SA, 180 °C, 40 min	1000	530
Wheat straw [66]	75% AcOH, 0.1% HCl, 85 °C, 4 h	2600	1594
	90% AcOH, 0.1% HCl, 85 °C, 4 h	2600	1952
	20% AcOH, 60% FA, 0.1% HCl, 85 °C, 4 h	2600	2182
	60% MeOH, 0.1% HCl, 85 °C, 4 h	2600	1519
	60% EtOH, 0.1% HCl, 85 °C, 4 h	2600	1356
Lodgepole pine [28]	78% EtOH, 0.025M $MgCl_2$, 200 °C, 1 h	—	1400
	78% BuOH, 0.025M $MgCl_2$, 200 °C, 1 h	—	848
	65% EtOH, 1.1% SA, 170 °C, 1 h	—	1062
	65% BuOH, 1.1% SA, 170 °C, 1 h	—	1060
	65% EtOH, 1.1% SO_2, 170 °C, 1 h	—	1200
	65% BuOH, 1.1% SO_2, 170 °C, 1 h	—	769

both of which have cellulose CrI values of around 0.50, resulted in an insignificant change in CrI after pretreatment. However, in the case of Loblolly pine [11] with cellulose CrI of 0.63, ethanol organosolv pretreatment produced cellulose with CrI of 0.53, suggesting that pretreatment is capable of altering the crystalline cellulose component. The effect of pretreatment severity on cellulose crystallinity is not well defined as contradictory results have been reported in the literature. While ethanol organosolv pretreatment of Lodgepole pine produced cellulose with higher CrI at higher severities, the opposite effect was seen for Buddleja which exhibited an 11% reduction in CrI at higher pretreatment severity. Cellulose isolated from organosolv-pretreated Lobolly pine after 72 hours of enzymatic hydrolysis in which 70% of the cellulose was hydrolyzed had a CrI of 0.81, indicating preferential hydrolysis of amorphous cellulose [11]. No dominant trends were seen in switchgrass cellulose CrI after 2, 4 and 8 hours of enzymatic hydrolysis [26].

Organosolv pretreatment of biomass can therefore result in good cellulose recovery coupled with high cellulose to glucose conversion by enzymes. This is likely a combined effect of reducing cellulose DP and altering crystallinity. However, with the limited information available in the literature, clear trends in variations in these parameters with pretreatment conditions cannot be discerned, and additional studies are necessary to fully understand the effects of organosolv pretreatment on biomass cellulose.

10.5 Co-products of Biomass Fractionation by Organosolv Pretreatment

In order to maximize revenues from a lignocellulosic biorefinery, the three major biomass components should be utilized to the maximum extent possible. In the simplest biorefinery scenario for ethanol production, all sugars are converted to ethanol and the residual lignin is burned to provide a heat source for pretreatment, distillation, and other operations. One idealized scenario would convert glucose to ethanol; while xylose and the other minor sugars and their degradation products are recovered and sold separately for conversion to value-added products, lignin is used as a high-value polymeric material and acetic acid is sold separately [58]. Such an ideal scenario could potentially increase revenues from $150/ton (for the simple case) to $640/ton when most of the biomass is valorized. Along with ethanol, which is produced from the cellulosic fraction, biomass fractionation by organosolv pretreatment produces several valuable co-products such as organosolv lignin, hemicellulose sugars, acetic acid, furfural, HMF, and levulinic acid. Under conditions optimized for cellulose recovery and good enzymatic digestibility from hardwoods, furfural is formed at slightly higher concentrations (0.5 g/100 g biomass) than HMF (0.1 g/100 g biomass), as there is more xylose than in softwoods [8]. During organosolv pretreatment of the softwood Lodgepole pine [10] however, the hexose degradation products HMF (2.1 g/100 g biomass), levulinic acid (13.5 g/100 g biomass), and formic acid (1.5 g/100 g biomass) are formed at much higher concentrations than furfural (2.1 g/100 g biomass). While most of these degradation products are fermentation inhibitors, they have a variety of potential uses which increases the importance of extracting them prior to SSF and utilizing them as co-products. They could also be used as reactants to make alkanes and other products by catalytic processes now being developed [67–69].

10.5.1 Hemicellulose

The composition of the hemicellulose stream obtained after biomass fractionation is determined by the biomass feedstock. Softwoods generally have lower hemicellulose contents (25–35%) and mannose is the main component of the hemicellulose fraction [70,71]. Hardwoods and herbaceous biomass have higher hemicellulose contents (20–40%), and xylose is the primary constituent of the hemicellulose stream [70,71]. Arabinose and galactose are the least-abundant sugars in most biomass hemicelluloses. During biomass pretreatment and fractionation, most of the hemicelluloses are fractionated into the water-soluble stream; about 50% of the sugars within this fraction are oligomers, with the rest being monomers.

Pentose sugars can be fermented to ethanol with specialized microorganisms but also have several other potential practical applications. Hemicelluloses have been used as a plant gum for thickeners, adhesives, protective colloids, emulsifiers, and stabilizers [72]. They have recently been applied to produce biodegradable oxygen-barrier films [73]. Animal feed additives are also a common use for hemicellulose sugars. Xylose, which is one of the major monomeric sugars in the hemicellulose from a variety of feedstocks, can be fermented to produce xylitol which is used as a sucrose replacement for diabetics and in dental hygiene products. Quesada-Medina *et al.* [20] explored the use of different organosolv systems for lignin extraction from almond shells which had been previously utilized for xylose production by acid hydrolysis. In this manner, two value-added streams could be extracted from almond shells, an agricultural waste.

As mentioned earlier, hemicellulose sugars are typically the fraction with lowest recovery yields as they are easily degraded even under moderately severe pretreatment conditions. In order to utilize these sugars, pre-extraction of the hemicelluloses before organosolv fractionation is an option that should be considered. In case of Miscanthus, presoaking with dilute sulfuric acid (0.15 M) followed by extraction overnight with acid under reflux was beneficial in recovering 57% of the hemicellulose sugars prior to organosolv pretreatment [7].

10.5.2 Furfural

Furfural, a key co-product of biomass pretreatment, is formed by the acid-catalyzed dehydration of pentose sugars (Figure 10.4). It has a wide range of industrial applications as a solvent in lubricants, coatings, adhesives, and furan resins [74] and for production of polytetramethylene ether glycol, which is used to manufacture Lycra and Spandex. Earlier, furfural was used in the production of Nylon but was replaced by petrochemicals [58]. With the advent of lignocellulosic biorefineries, furfural can now return as a green replacement for petrochemicals in Nylon manufacture.

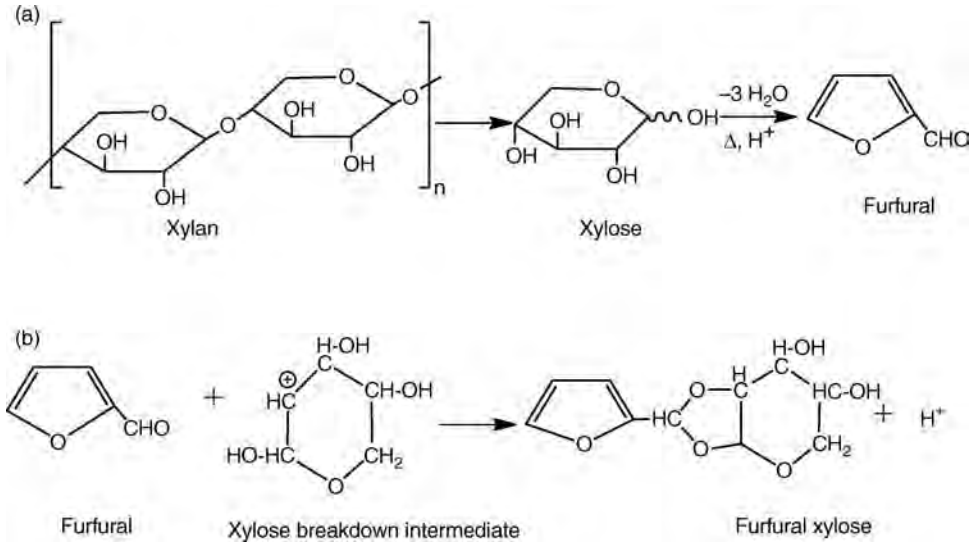

Figure 10.4 *Reactions showing (a) the hydrolysis of xylan to xylose and its dehydration to furfural and (b) condensation reaction of furfural and xylose, which can occur at high temperatures [74].*

Results from organosolv biomass fractionation have shown that xylose dehydration to furfural mostly occurs at temperatures of 190–205 °C [39,50]. Below 190 °C, xylose formation by the hemicellulose hydrolysis is the predominant reaction. At higher temperatures (>205 °C), furfural concentration in the water-soluble fraction decreases due to self-condensation or reaction of xylose with furfural to produce insoluble polymeric material (Figure 10.4), which have been referred to as pseudo-lignin or humins [50,75]. This material may behave similarly to lignin and account for the >100% lignin mass closures for organosolv pretreatments at high severities [39]. The effect of the organosolv cooking parameters of ethanol concentration, acid concentration, and temperature on the generation of furfural has been recently studied [50]. At low temperatures, xylose to furfural conversion is favored only at low ethanol concentrations as it results in lower pH at the same acid loading. Increasing temperature enhanced furfural condensation and lowered its yield. For softwoods, the highest xylose to furfural conversion (70% molar yield) was obtained at 200 °C, 25 min, 60% ethanol and 3% sulfuric acid [50]. Higher ethanol concentrations retarded furfural condensation at high temperatures.

10.5.3 Hydroxymethylfurfural (HMF)

Acid-catalyzed dehydration of hexose sugars results in the formation of HMF, which can further degrade to levulinic acid and formic acid or form condensation products similar to those from furfural (Figure 10.5). HMF is also an inhibitor of fermentations, but can be converted to liquid transportation fuels through the pathways of aldol condensation and hydrogenation [76]. The molar conversion yield of HMF is highest at high temperatures (up to 200 °C), low pH, and longer pretreatment times [50]. Beyond 200 °C, formation of levulinic acid and condensation starts to occur. Ethanol concentration in organosolv has been reported as not having a significant effect on HMF production.

10.5.4 Levulinic Acid

Levulinic acid can be used for several value-added products such as dyes, polymers, flavoring agents, plasticizers, solvents, and fuel additives [50]. Levulinic acid is produced from acid-catalyzed carbohydrate dehydration and is formed by the degradation of HMF, as seen above. Its formation is therefore favored at temperatures above 190 °C. In order to generate levulinic acid during biomass pretreatment, the reaction forming it from HMF should be faster than its consumption by self-condensation, which occurs at high temperature. Under commonly used organosolv pretreatment conditions, levulinic acid is detected only in low concentrations in the water-soluble fraction.

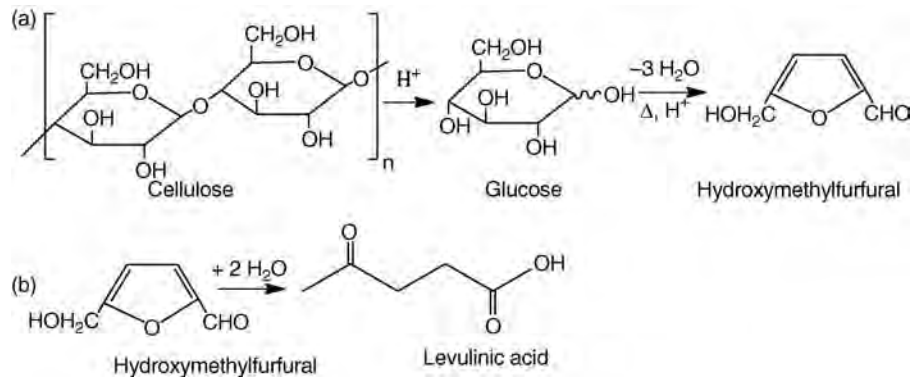

Figure 10.5 *Reactions showing (a) cellulose hydrolysis to glucose and its dehydration to HMF and (b) levulinic acid formation from HMF.*

10.5.5 Acetic Acid

The acetyl groups in hemicelluloses are hydrolyzed during acid-catalyzed pretreatments to form acetic acid. Acetic acid further lowers the pH of the cooking liquor and promotes acid-catalyzed hydrolysis of the biomolecules in biomass. Acetic acid is one of the highest-volume chemical commodities traded throughout the world [19]. It is used to manufacture acetic anhydride for production of cellulose acetate fibers and membranes. Acetic acid is also converted to vinyl acetate, which is used in making paint and paper coatings. In the Lignol process, acetic acid is one of the major co-products of biomass fractionation and ethanol production [19]. In order to avoid problems associated with high acetic acid concentrations in the cooking liquor after solvent reuse (as the acetic acid is also volatilized during flashing to recover the solvent), soda is added to the base of the distillation tower, and acetic acid is recovered as sodium acetate.

10.6 Conclusions and Recommendations

Organosolv pretreatment is capable of fractionating lignocellulosic biomass into separate streams rich in lignin, hemicellulose, and cellulose. The cellulosic fraction is very amenable to enzymatic deconstruction and subsequent fermentation to ethanol. Of the three major biocomponents, the hemicelluloses are the most degraded during fractionation, but once recovered can be fermented to ethanol or utilized as a separate value-added stream. Hemicellulose and glucose degradation products such as furfural, acetic acid, hydroxymethyl furfural, levulinic acid, and formic acid also have a wide range of industrial applications. One of most economically attractive advantages of biomass fractionation by organosolv pretreatment is the ability to recover a large proportion of biomass lignin as a pure sulfur-free low-molecular-weight material. Compositional characteristics of organosolv lignin, such as its high phenolic content and low molecular weight, enable its valorization to a wide range of products including antioxidants, phenolic resins, and chemicals. Other notable advantages of the organosolv pretreatment include increased cellulose enzymatic digestibility and pore volume and reduced DP.

There are tremendous opportunities for future research in this area, including development of efficient solvent and co-product recovery systems and catalysts for lignin conversion to fuels and fuel additives. The effect of pre-extraction of hemicelluloses and extractive compounds and their contribution to improving process economics should be investigated. In terms of fundamental research, the changes in cellulose structure and crystallinity during organosolv pretreatment and their effect on the enzymatic digestibility of the substrate are not fully understood and should be further explored.

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