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MINI-REVIEW

# Pretreatment of woody biomass for biofuel production: energy efficiency, technologies, and recalcitrance

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**Abstract** This mini review discusses several key technical issues associated with cellulosic ethanol production from woody biomass: energy consumption for woody biomass pretreatment, pretreatment energy efficiency, woody biomass pretreatment technologies, and quantification of woody biomass recalcitrance. Both total sugar yield and pretreatment energy efficiency, defined as the total sugar recovery divided by total energy consumption for pretreatment, should be used to evaluate the performance of a pretreatment process. A post-chemical pretreatment wood size-reduction approach was proposed to significantly reduce energy consumption. The review also emphasizes using a low liquid-to-wood ratio (L/W) to reduce thermal energy consumption for any thermochemical/physical pretreatment in addition to reducing pretreatment temperature.

**Keywords** Recalcitrance · Cellulosic ethanol · Woody/Forest biomass · SPORL
Enzymatic hydrolysis/saccharification · Pretreatment

## Introduction

Woody biomass from forestlands and intensively managed plantations can be sustainably produced in large quantities in many regions of the world, including the United States (Perlack et al. 2005). To promote biodiversity, to meet local and regional bioenergy needs, and to achieve healthy and sustainable forest and ecosystem management, woody biomass will be an important part of the feedstock supply mix for the future biobased economy. Woody biomass as a feedstock has many advantages in terms of production, harvesting, storage, and transportation compared with herbaceous biomass for bioconversion (Zhu and Pan 2010). Barriers specifically to woody biomass conversion need to be addressed to translate these advantages into tangible economic benefits for cellulosic ethanol production.

Woody biomass has tough and strong physical structure and high lignin content that make it very recalcitrant to microbial destruction. This suggests that research efforts on woody biomass should focus on upstream processing (e.g., wood size reduction and pretreatment) to reduce recalcitrance and enhance microbial destruction of polysaccharides. Specifically, more research should be devoted to robust thermochemical pretreatment processes to improve wood cellulose enzymatic saccharification and fermentable sugar yield, as well as to significantly reduce energy consumption during pretreatment and wood size reduction from logs to the levels of fibers or fiber bundles for efficient enzymatic saccharification (Zhu and Pan 2010). This is one

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crucial component of sustainability because the goal of cellulosic ethanol production is net energy output. It should be pointed out, however, that it is not difficult to achieve good sugar and ethanol yields, but it is very difficult to achieve good yields at low energy input. However, the issue of pretreatment energy input (i.e., energy consumption for wood size reduction) and the associated net energy output and process energy efficiency have largely been overlooked in the biorefining community. Many expert reviews emphasized maximizing the release/recovery of sugars and ethanol yield from biomass but neglected the importance of minimizing energy consumption for achieving maximal sugar and ethanol yield (Gable and Zacchi 2007; Jorgensen et al. 2007; Kumar et al. 2009; Lynd et al. 2008; Yang and Wyman 2008). To fill this gap, we recently defined the term *pretreatment energy efficiency* and proposed to use both sugar yield and pretreatment energy efficiency to evaluate the performance of any given pretreatment process (Zhu and Pan 2010).

Feedstock pretreatment processes not only affect the enzymatic digestibility of the resultant substrate but also dictate its downstream processing, commercial scalability, and pathway of biorefinery process. For example, dilute acid pretreatment is an extensively investigated technology. Furfurals generated during the pretreatment inhibit the fermentation of hemicellulose sugars. In addition, the nature of condensed lignin limits its use for value-added coproducts other than boiler fuel. Different pretreatments also require different approaches for chemical recovery and wastewater treatment to comply with various environmental regulations. Commercial scalability is another issue rarely discussed in previous expert reviews evaluating the performance of different pretreatment processes (Kumar et al. 2009; Lynd et al. 2008; Yang and Wyman 2008). It is a major technological and ingenuity challenge to scale research technologies to production capacities of up to 1,000 tons of dry mass per day, which requires intensive capital investment as well as research and development.

In this mini review, we address important aspects of ethanol production from woody biomass. First, we outline the difficulties in woody biomass pretreatment for cellulosic ethanol production. We quantitatively define an expression for characterizing biomass "recalcitrance" to differentiate woody biomass from herbaceous biomass. Second, we present several potentially promising pretreatment technologies for woody biomass pretreatment. We emphasize energy consumption (input) as well as potential for maximizing sugar yield, that is, process energy efficiency (Zhu and Pan 2010), when evaluating pretreatment technologies. We discuss woody biomass availability and sustainable production. Last, we discuss the strategies for enhancing enzymatic saccharification of woody substrates.

## Woody biomass availability and sustainable production

It is difficult to quantify the volume of current and potential woody biomass available for energy production given complex social issues such as the debate over shifting land uses and biological questions about present and future productivity levels under traditional and progressive forest management prescriptions (Alig et al. 2003; Berndes et al. 2003; Johnson et al. 2007). This problem is further complicated with the accelerated need to reduce consumption of nonrenewable resources to maintain economic and ecological sustainability (Hill et al. 2006). In the United States, 1,584 million acres are within the lower 48 contiguous states comprising forests (35%), grassland, pasture, and range (37%), and cropland (28%) (Lubowski et al. 2006). Despite having potential for woody biomass production, socioeconomic and ecological factors preclude the use of most of this acreage for energy feedstocks. Nevertheless, using a variety of high-yield scenarios, it was estimated that forestlands in the contiguous United States have the capability to produce 368 million dry tons of biomass annually (Perlack et al. 2005). The estimated biomass of perennial crops on agricultural lands was 377 million dry tons, with a substantial proportion allocated to short-rotation woody crops (Perlack et al. 2005). Overall, both woody biomass from forestlands and intensively managed plantations are necessary components of the renewable energy supply chain. Evaluation of the quantity of woody biomass available from forests and plantations is beyond the scope of this review; such information is available from expert syntheses such as those conducted by Perlack et al. (2005) and Smith et al. (2009). Nevertheless, it is prudent to discuss general trends in availability and sustainability across both feedstock groups.

Woody biomass from forestlands comes from a number of different sources, including but not limited to logging residues from harvest operations, fuel treatments (removing excess biomass), fuelwood, primary and secondary processing mill residues, and urban wood residues (Perlack et al. 2005). There are potential ecological consequences on the overall ecosystem sustainability of removing forest residues and excess biomass during harvesting (Fox 2000). Therefore, numerous studies have been completed or are under way to assess the effects of removing coarse and fine woody debris on soil health and quality, as well as plant, animal, and insect communities (Ares et al. 2007; Powers et al. 2005; Yanai et al. 2003). In addition, economic sustainability issues must be addressed. For example, economically sustainable systems for removal of residual tops, branches, and other material historically left on site must be developed and tested (Rummer 2008). Loggers need to be assured that supplying residual woody biomass for energy production is profitable, which is largely dependent upon available markets. Lastly, the magnitude of the impact of harvesting forest biomass for energy production on biodiversity will vary greatly across temporal and spatial scales, with sustainability being site- and species-dependent.

Woody biomass from intensively managed plantations comes from short-rotation woody crops such as species and hybrids within the genera *Populus* (i.e., poplars) and *Salix* (i.e., willows), as well as other hardwoods (e.g., eucalyptus, *Eucalyptus* spp.; sycamore, *Platanus occidentalis* L.; red alder, *Alnus rubra* Bong.) and softwoods (e.g., loblolly pine, *Pinus taeda* L.; slash pine, *Pinus elliottii* Engelm.; red pine, *Pinus resinosa* Ait.; Douglas-fir, *Pseudotsuga menziesii* [Mirb.] Franco; ponderosa pine, *Pinus ponderosa* Laws.; western hemlock, *Tsuga heterophylla* [Raf.] Sarg.) (Johnson et al. 2007). In the United States, the production of woody biomass from plantations is regionally influenced (Vance et al. 2010), with the majority of feedstocks from the Pacific Northwest with 13.6 million acres of plantations mostly consisting of Douglas-fir but also poplar, alder, ponderosa pine, and western hemlock (Stanturf and Zhang 2003) and the southeast with 32 million acres mostly consisting of loblolly and longleaf pine but also eucalyptus and sycamore (Wear and Greis 2002). In addition, the midwest (poplar, red pine) and northeast (willow) have substantial areas of intensively managed forests that are at least an order of magnitude smaller than the Pacific Northwest and Southeast. Plantation-grown trees have been very productive in the United States, with productivity levels approaching 20 Mg ha$^{-1}$year$^{-1}$ when matching adapted genotypes to optimal local and/or regional site conditions (Stanturf and Zhang 2003; Zalesny et al. 2009). In addition to their potential to produce much higher biomass per unit land area than forestlands, plantations provide opportunities to reduce pressure on native forests (Gladstone and Ledig 1990; Joslin and Schoenholtz 1997). Overall, short-rotation woody crops and other intensively managed trees are one of the most sustainable sources of biomass, provided they are strategically placed on the landscape and managed with cultural practices that conserve soil and water, recycle nutrients, and maintain genetic diversity (Hall 2008). These woody biomass sources also provide secondary benefits such as carbon sequestration, wildlife habitat, and soil stabilization (Hansen 1993; Isebrands and Karnosky 2001; Moser et al. 2002).

A combination of woody biomass from forestlands and intensively managed plantations will be necessary to help achieve U.S. policy that mandates the production of 16 billion gallons of cellulosic biofuels by 2022 (U.S. Energy Independence and Security Act of 2007). Paramount to the success of this achievement will be testing and identification of woody biomass feedstocks that grow fast, accumulate substantial biomass, and break down to sugars easily with energy-efficient technologies. As described above, the availability and sustainability of such woody biomass is possible, with further selection for cellulosic ethanol production dependent upon energy efficiency of conversion, effectiveness of pretreatment technologies, specific levels of recalcitrance, and useful strategies for enhancing enzymatic saccharification.

Two major species of woody biomass have some major differences. In general, hardwood species have much lower recalcitrance to enzyme and microbial processing than do softwood species and therefore easily convert biochemically. Hardwood species can be intensively cultured to improve productivity as discussed. However, hardwoods have higher xylan and low mannan content than softwoods (Zhu and Pan 2010). Xylose (a five-carbon sugar from xylan) is much more difficult to ferment than six-carbon sugars with current microbial technologies. Therefore, a robust pretreatment technology to effectively remove the strong recalcitrance of softwood species is advantageous for biofuel production from woody biomass.

## Woody biomass pretreatment

### Energy consumption for pretreatment (pretreatment energy efficiency)

Woody biomass pretreatment involves both physical and thermochemical processes (Zhu and Pan 2010). Physical pretreatment refers to size reduction (increasing surface area) of wood to the level of fibers and/or fiber bundles from chips or chops to increase enzyme accessibility to wood cellulose. Unlike herbaceous biomass, the size reduction of woody biomass is very energy-intensive. The typical energy consumptions of milling wood chips into fibers are approximately 500 to 800 Wh/kg (Schell and Harwood 1994; Zhu et al. 2009b; Zhu et al. 2010b). Assuming ethanol yield of 300 L/ton wood with current technology and ethanol high heating value of 24 MJ/L, 500 to 800 Wh/kg is equivalent to 25–40% of the thermal energy stored in the ethanol produced. Because mechanical wood size reduction consumes mechanical energy and the conversion efficiency from thermal energy (stored in ethanol) to electric–mechanical energy is only about 30%, the thermal energy in ethanol produced is just sufficient for wood-size reduction. Therefore, any thermochemical/physical pretreatment processes that require prior significant wood size reduction to the level of fiber/fiber bundles, such as ionic liquid (IL) pretreatment (Lee et al. 2009; Sun et al. 2009), will need to overcome this energy barrier for viable commercial cellulosic ethanol production from woody biomass. To reduce mechanical energy consumption for wood size-reduction through milling, we proposed an approach of

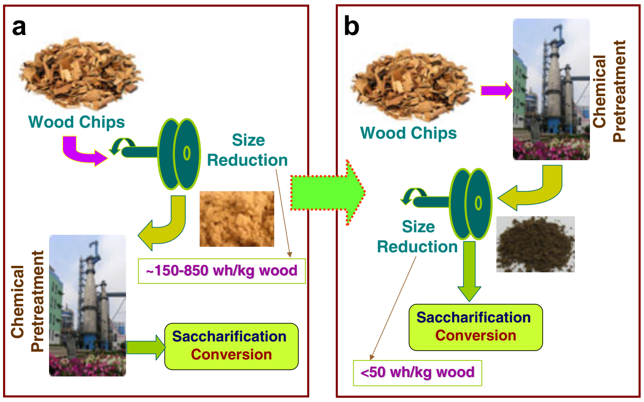

**Fig. 1** Schematic diagram shows the approach for mechanical wood size reduction operation from **a** pre- to **b** post-chemical pretreatment to reduce energy consumption

post-chemical pretreatment size-reduction (Fig. 1). The benefits in energy savings achieved using this post-chemical pretreatment size-reduction approach are significant based on our previous laboratory study (Zhu et al. 2010b). All pretreatment reduced energy consumption for wood size reduction through disk-milling for the four pretreatments examined (Table 1). Energy consumption for disk-milling lodgepole pine wood chips was reduced by as much as about 80% while achieving greater than 90% enzymatic digestion of the resultant substrate when a low pH SPORL pretreatment (Wang et al. 2009; Zhu et al. 2009a) was employed (Table 1). Unfortunately, the method of size reduction before chemical pretreatment (Fig. 1**a**) has been proposed as a standard process flow for cellulosic ethanol production in several key pieces of the literature (Lynd 1996; Yang and Wyman 2008), as well as the U.S. Department of Energy biofuels research roadmap (U.S. DOE 2005). This standard approach has also been adopted for woody biomass pretreatment (Wyman et al. 2009; Yu et al. 2010) despite significant negative impacts on pretreatment energy efficiency (Zhu and Pan 2010; Zhu et al. 2010b). In addition to reducing energy consumption for wood size reduction, the demonstrated post-chemical pretreatment size reduction approach has the following benefits: (1) reducing the resources needed for separating pretreated solids from liquid as the wood remains as chips after pretreatment; (2) eliminating the energy-intensive mixing operation required for pretreating fiberized/pulverized materials; and (3) reducing liquid-to-wood ratio (L/W) in pretreatment to reduce pretreatment thermal energy for heating up water because wood chips have much less water intake than fiberized materials due to the porous and hydrophilic nature of wood fibers.

The total energy consumption for pretreatment also includes thermal energy used for thermochemical/physical pretreatment conducted at elevated temperatures. The thermal energy consumption depends on two factors: (1) L/W and (2) pretreatment temperature. When steam pretreatment is employed, the heat of latent to produce steam should also be included. Because the thermal energy consumption for pretreatment is almost linearly proportional to L/W, reducing L/W is very critical to improve energy efficiency. This poses a significant challenge for IL pretreatment because this method requires a very large L/W of 10 to 20 or higher for efficient pretreatment as demonstrated in the literature (Lee et al. 2009: L/W=20 at 80°C; Sun et al. 2009: L/W=20 at 90°C). Although IL is often carried out at low temperatures below 100°C, the L/W of 10 to 20 significantly increased thermal energy consumption to more than traditional aqueous thermochemical pretreatments that use L/W=3 at temperature around 180°C. The thermal energy required for aqueous pretreatments at three different temperatures with different L/Ws were used to illustrate this point (Fig. 2). The results in Fig. 2 are based on thermodynamic calculations of the enthalpy of saturated pulp suspension without taking thermal energy recovery into account (spreadsheet is available upon request). As can be seen clearly, the energy for aqueous pretreatments at T=75°C with L/W=10 is 18% more than that required for pretreatment at 180°C (much higher temperature) but with L/W=3. To reduce the energy to that for pretreatment at 180°C with L/W=3, L/W needs

**Table 1** Effects of chemical pretreatment on lodgepole pine wood chip size reduction energy consumption and the resultant SED

| Pretreatment at 180°C for 30 minutes[a] | Initial liquor pH | Disk-milling energy (kWh/ton wood) | Size reduction energy savings (%) | SED (%) |
|---|---|---|---|---|
| Untreated | | 699 | | 12.7 |
| Hot water | 5.0 | 680 | 2.7 | 16.0 |
| Acid | 1.1 | 412 | 41.0 | 41.6 |
| SPORL | 4.2 | 594 | 15.0 | 75.1 |
| SPORL | 1.9 | 153 | 78.1 | 91.6 |

Pretreatment L/W=3, disk-milling solids loading =30% (≈ the solid contents of pretreated wood chips), and disk plate gap =0.76 mm

[a] Sodium bisulfite charge was 8% on oven dry wood for the two SPORL runs; sulfuric acid charge was 2.21 (*w*/*w*) on oven dry wood for the dilute acid and low pH SPORL runs, and 0 for the hot water and high pH SPORL runs

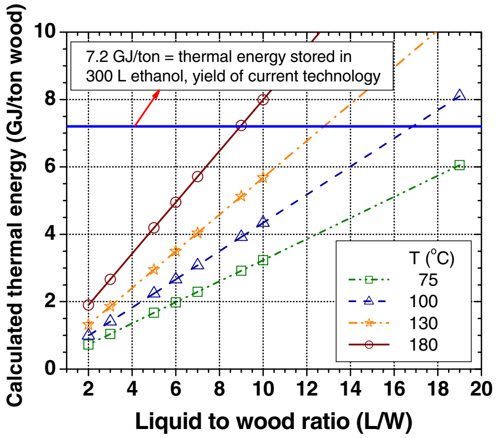

**Fig. 2** Calculated (based on enthalpy of saturated pulp suspension) thermal energy consumptions for aqueous pretreatment at different temperatures and L/W

to be less than 8 and 6 for pretreatment conducted at 75 and 100 °C, respectively.

We define pretreatment energy efficiency using the following expression:

$$\eta_{Pretreatment} = \frac{Total\ sugar\ recovery}{Total\ energy\ consumption\ for\ pretreatment} \qquad (1)$$

where total sugar recovery is the sugar yield as the fraction of total wood sugar (theoretical sugar yield). Eq. (1) is slightly different from our previous definition (Zhu and Pan 2010), where absolute total sugar "yield (kg)" rather than "recovery (fraction)" was used. Therefore, the present definition is independent of the wood carbohydrate content by normalizing the total sugar yield using the sugar content in the feedstock. The pretreatment energy efficiency defined in Eq. (1) has a unit of inverse energy, for example, 1/MJ. Both total sugar recovery and pretreatment energy efficiency should be used in evaluating and comparing the performance of pretreatment processes. Unfortunately, this was not done in most published works, including a recent major study on comparisons of the performance of different pretreatment processes (Wyman et al. 2009) conducted by the Biomass Refining Consortium for Applied and Fundamental Innovations sponsored by the U.S. Departments of Energy and Agriculture.

## Thermochemical pretreatment technologies

Given its strong recalcitrance, only a few pretreatment technologies have proven to be applicable to woody biomass. This is especially true of softwood species. Alkaline-based pretreatments are not generally suitable for cellulosic ethanol production from woody biomass because severe conditions such as high alkali loading and temperatures are required to extensively remove lignin as it is done in chemical pulping. For example, sodium hydroxide pretreatments require an expensive chemical recovery process to reduce the cost of high chemical loading (Zhao et al. 2008). Lime pretreatment (Sierra et al. 2009) is less effective and can cause severe equipment scaling problems. Furthermore, size reduction of wood chips pretreated under high pH (alkaline) through disk-milling consumes more energy than those pretreated under low pH (acid) (Zhu et al. 2010b, also Table 1). Moreover, size reduction of pretreated wood under high pH tends to produce long fibers/bundles (Zhu et al. 2010b), which can increase both difficulties and energy consumption during mixing in high-solids enzymatic saccharification. Finally, ammonia-based pretreatments, such as ammonia-fiber expansion (Balan et al. 2009) and ammonia-recycle percolation (Gupta and Lee 2009), are not effective for woody biomass. IL pretreatment suffers from the difficulties in IL recycling and high-energy input because of very large L/W of 10 20 (Lee et al. 2009; Sun et al. 2009), as discussed previously.

*Dilute acid pretreatment* Dilute acid pretreatment can achieve some level of satisfactory enzymatic saccharification of cellulose when applied to certain hardwood species at high temperatures (Wyman et al. 2009). However, most of the reported work used materials with significant size reduction, which is a major pitfall for practical applications because of the high energy consumption in wood size reduction discussed above. In a recent study (Wyman et al. 2009), size-reduced poplar wood of less than 6 mm was pretreated at 190 °C with sulfuric acid charge of 2% on wood. Total sugar recovery of 82.8% was achieved with an enzyme loading of 15 FPU/g cellulose. The study did not provide adequate information to determine ethanol yield from unit ton of poplar wood. Fermentation efficiency of the enzymatic hydrolysate was 81.4% when a genetically modified *Sacharomyces cerevisiae* 424A(LNH-ST) was used. The study did not provide information about energy consumption for reducing wood size to 6 mm. It should be pointed out that a flashing step (similar to a steam explosion to be discussed in the next subsection) was used to further reduce the substrate size and enhance enzymatic hydrolysis, which was not mentioned in the study (Wyman et al. 2009). In a recent study conducted at our laboratory, we used commercial-sized wood chips (6–38 mm) pretreated at 180 °C with sulfuric acid charge of 1.84% on oven dry wood, followed by disk-milling to produce a solid substrate. The post-chemical pretreatment disk-milling approach significantly reduced size reduction energy consumption to less than 100 Wh/kg (Zhu et al. 2010b). We achieved 80% substrate enzymatic digestibility (SED), which is defined as the percentage of glucan on solid substrate converted to glucose enzymatically.

Dilute acid pretreatment is not able to remove the strong recalcitrance of softwood to achieve satisfactory enzymatic cellulose saccharification. For example, SED was only about 40% when softwood (spruce and lodgepole pine) wood chips were pretreated at 180°C with acid charge of 1.84% on oven dry wood followed by disk-milling (Zhu et al. 2009a; Zhu et al. 2010b). Glucose recovery of 80% was achieved when a two-stage dilute acid pretreatment at 190 and 210°C was applied to size-reduced spruce wood of 2 to 10 mm at the expense of increased thermal energy cost for pretreatment (Monavari et al. 2009b). Continuous circulation of fresh acid solution can increase sugar recovery as practiced in the 1940s using the so-called "Madison Process" at the expense of high L/W or thermal energy cost (Harris and Beglinger 1946).

*Acid-catalyzed steam explosion* Most of the current research on acid-catalyzed steam pretreatment of woody biomass was conducted by two research groups, Professor Zacchi at Lund University of Sweden (Gable and Zacchi 2002; Monavari et al. 2009a; Sassner et al. 2008; Soderstrom et al. 2004) and Professor Saddler at University of British Columbia of Canada (Cullis et al. 2004; Ewanick et al. 2007). In a previous publication, we provided a comprehensive review on catalyzed steam explosion for woody biomass pretreatment (Zhu and Pan 2010). The steam explosion combines chemical pretreatment and size reduction in one step through acid catalyzed steaming followed by a thermal flashing process (quick releasing). This method uses a low L/W with the potential to recover a high-concentration hemicellulose stream. Satisfactory enzymatic saccharification of acid-catalyzed steam pretreated hardwood substrates was achieved. One of the major drawbacks of steam explosion is relatively low sugar recovery of about 65% when applied to softwood, although it can be improved by two-step explosion (Monavari et al. 2009a; Soderstrom et al. 2004). Another drawback is that it is also energy-intensive, especially when applied to softwood species at elevated temperature of above 200°C. Finally, commercial scalability of steam explosion devices needs to be demonstrated.

*Ethanol organosolv pretreatment* The ethanol organosolv process was developed in the 1970s and adopted as the Alcell® process in the late 1980s to produce pulp from hardwoods (Williamson 1988; Pye and Lora 1991; Stockburger 1993). The process uses a blend of ethanol and water with a small amount of mineral acid to extract lignin from wood chips. The Alcell® pulp has comparable strength properties with kraft pulp. In addition to the pulp, the ethanol organosolv pulping process generates a fraction of high-quality lignin (Lora et al. 1993; Hepditch and Thring 1997; Liu et al. 2000). The lignin has coproduct potential for various industrial applications such as adhesives and biodegradable polymers (Boocock and Balatineez 1992; Thring et al, 1997; Kubo and Kadla 2004).

Recently, the ethanol organosolv process was modified into a biorefining platform for pretreating woody biomass for cellulosic ethanol production (Pan et al. 2005a). Different from the pulping application, the pretreatment does not require preventing destruction of fibers and depolymerization of cellulose but rather the enzymatic susceptibility of the substrate and overall sugar recovery. In fact, destructed fibers with smaller size (larger surface area) and lower cellulose degree of polymerization are desirable for enzymatic hydrolysis (Pan et al. 2006a). In our previous studies (Pan et al. 2005a; Pan et al. 2006a; Pan et al. 2007; Pan et al. 2008), the ethanol organosolv pretreatments of mixed softwood, hardwood hybrid poplar, and lodgepole pine killed by mountain pine beetle indicated that the ethanol organosolv process is a unique and promising pretreatment technology for bioethanol production from lignocellulosic biomass, in particular wood. The substrates produced by the process had superior enzymatic digestibility over those pretreated by alternative processes. Over 90% cellulose-to-glucose conversion yield could be achieved within 24 hours at a low enzyme loading of 15 to 20 FPU/g cellulose. The ethanol organosolv lignin generated had high purity, low molecular weight, narrow distribution, and more functional groups (Pan et al. 2005a, b, 2006b), providing potential applications in antioxidants, adhesives, polyurethane foams, and carbon fibers. High-value chemicals, such as furfural, hydroxymethylfurfural (HMF), and formic, acetic, and levulinic acids, were also derived from the hemicellulose fraction (Pan et al. 2007). Hemicellulose recovery is very difficult with ethanol organosolv process. Furthermore, the process requires an energy-intensive step—distillation—to recover solvent, ethanol. The high ethanol loading of 50% (*v*/*v*) in pretreatment requires an ethanol recovery yield of over 99% to be economical because the ethanol concentration in the final fermentation broth is less than 8%, a significant challenge that has not been addressed. Therefore, development of high-value coproducts from lignin and hemicellulose is one of the keys to successful commercialization of the ethanol organosolv process.

*Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL)* The SPORL process, recently developed by the present authors at the U.S. Forest Service, Forest Products Laboratory, and the University of Wisconsin-Madison (Wang et al. 2009; Zhu et al. 2009a), showed robust performance for woody biomass (both hardwoods and softwoods) saccharification. The SPORL process is very similar to traditional dilute acid pretreatment except that an additional catalyst is used: sulfite or bisulfite. Typical acid and bisulfite charges on oven dry wood are about 0.5–1% and 1–3% for hardwood and 1–2% and 4–8% for softwood, respectively. SPORL is a mild pretreatment conducted at a temperature of 160 to 190°C for a period of 10 to 30 minutes in a batch mode. The sulfite addition increases the pretreatment pH under similar acid dosages compared with dilute acid pretreatment. As a result, the SPORL process generates significantly lower amounts of fermentation inhibitors, such as furfural and HMF (Shuai et al. 2010; Wang et al. 2009; Zhu et al. 2009a), than dilute acid pretreatment. More importantly, the partial sulfonation of lignin by sulfite enhances lignin hydrophilicity and consequently softens wood chips. The wood softening can reduce energy consumption for size reduction as discussed previously (Table 1). The improved hydrophilicity of lignin weakens the hydrophobic interaction between lignin and enzymes and therefore facilitates the saccharification of cellulose. In addition, the dissolved lignosulfonate in pretreatment liquor is a potential high-value coproduct with existing markets. SPORL pretreatment can be directly applied to wood chips, which affords to use at a low L/W of about 3 (Zhu et al. 2010a, b). Post SPORL size reduction significantly reduced energy consumption by an order of magnitude (Zhu et al. 2010b, Table 1). SPORL produces a readily digestible substrate. About 90 to 95% enzymatic saccharification of SPORL substrates from softwoods were achieved within 48 hours with enzyme loading of 15 FPU/substrate (Zhu et al. 2009a; Zhu et al. 2010b). Overall sugar recovery of about 85% achieved from lodgepole pine without detailed optimization (Zhu et al. 2010a). When compared with acid catalyzed steam explosion and organosolv pretreatment, the pretreatment energy efficiency of SPORL is about 30 and 15% greater, respectively (Zhu and Pan 2010). Recently, we reported an ethanol yield of 276 L/ton lodgepole pine with the SPORL process. The net energy output was 4.55 GJ/ton wood (before distillation). The net ethanol (lignin energy excluded) energy production efficiency, defined as net energy output divided by total energy input, was 237% (before distillation) from lodgepole pine (Zhu et al. 2010a). This work represents the state of the art in cellulosic ethanol research and development. Furthermore, SPORL produces sulfonated lignin with the potential as a directly marketable coproduct within existing and new markets. Finally, SPORL is developed based on sulfite pulping process with proven commercial scalability with low technological and environmental risks. Therefore, SPORL can be easily implemented using capital equipment practiced for decades in the pulp and paper industry, a significant advantage over many competing technologies.

## Woody biomass recalcitrance

Mother nature produces plant biomass as a structural material with strong recalcitrance against microbial destruction to sustain its intended purpose. This recalcitrance is sustained by the hierarchical structure of plant biomass made of cellulose, hemicelluloses, lignin, etc. This hierarchical structure for wood can be simply described as the basic cellulose unit of elementary fibrils on the order of 3 to 5 nm in diameter that forms microfibrils up to 20 nm in diameter (Wegner and Jones 2009). The microfibrils are bundled together to form a matrix structure, which is the basic unit of fiber (cell) wall. Hemicelluloses and lignin serve as glue or reinforcing materials to bind/build this hierarchical cellulose-based structure from bottom (elementary fibrils) up (fiber) in each level as a biocomposite. Each growth ring (year) usually consists of several or tens of fiber layers. The recalcitrance of plant biomass results from physical and chemical barriers posed by plant fiber (cell) walls to prevent microbes or cellulases from direct access to structural cellulose (Himmel et al. 2007). The concept of plant biomass recalcitrance (PBR) is complex, which is related not only to the physical structure and strength of the biomass matrix but also to the chemical composition of the biomass. For example, woody biomass has greater recalcitrance than herbaceous biomass because of its tough and strong structure and high lignin content. In addition, PBR is also dependent on the physical and chemical features and distribution of the major components within the cell walls of the biomass: hemicellulose, cellulose, and lignin. For example, crystalline cellulose is more recalcitrant to enzymatic destruction than amorphous cellulose; condensed lignin may have a greater resistance to cellulases. Furthermore, molecular-level structure and functional groups of lignin can play an important role, especially in enzyme–lignin interactions that were recently observed (Pan 2008; Liu et al. 2010). Despite that much research has been carried out to remove PBR through pretreatment (Gable and Zacchi 2007; Jorgensen et al. 2007; Yang and Wyman 2008; Zhu and Pan 2010), PBR has never been quantitatively defined in the biomass and microbiology research community. As a result, quantitative comparisons of PBR of different plant biomass have not been made.

There are several ways to define "recalcitrance." Because PBR is a property of plant biomass, it is ideal to define it using physical and chemical characteristics of the plant biomass itself. Such a definition is a daunting task at present given that cell-wall components of plant biomass at the molecular level have not been well understood. Furthermore, there is still a lack of complete knowledge about what and how cell wall structure and components retard efficient saccharification of the structural carbohydrates. Therefore, another way to define PBR is to use enzymatic digestibility of a plant biomass after a given pretreatment coupled with energy input and process parameters during the pretreatment. This definition is certainly affected by the pretreatment process and conditions applied. Nevertheless, it can provide some quantitative information about the nature of PBR when proper pretreatment conditions are taken into account. For example, under a given set of pretreatment conditions, the enzymatic saccharification efficiency of cellulose retained in solid substrate after pretreatment, or SED, can be a surrogate measure of the PBR. The higher the SED, the lower the PBR. The problem with this definition of recalcitrance is that process parameters such as energy consumption for pretreatment that affects the results of SED are not taken into account. One can achieve high SED with high-energy input. In addition, hemicellulose sugar recovery is not considered. Another measure of recalcitrance can be based on the pretreatment energy efficiency, defined as the total sugar yield divided by total energy consumption for pretreatment, or sugar yield on unit energy (Zhu and Pan 2010). The higher the pretreatment energy efficiency, the lower the recalcitrance. This definition does not reflect the achievable maximal sugar yield from biomass as one may be able to achieve high energy efficiency by using less energy but at low sugar yield.

In view of the above discussion, we use the following expression to quantitatively characterize PBR:

$$PBR = \frac{100}{SED} \bullet \frac{\text{Total energy consumption for pretreatment}}{\text{Total sugar recovery}} \tag{2}$$

where total sugar recovery is the fraction of theoretical total sugar yield after the given pretreatment at a given enzyme loading, for example, 15 FPU/g cellulose. We suggest using hot water pretreatment at 170 °C to eliminate complication by chemistry. We also suggest that all feedstock should go through the same size reduction process with known size reduction energy consumption. Furthermore, all pretreatment should be conducted under same liquid to oven-dry biomass solid ratio, for example, L/W=3 for wood. Based on this definition, PBR has a unit of energy. The higher the energy input and the lower the SED and total sugar recovery, the higher the PBR. This definition is proportional to the inverse of the product of SED and pretreatment energy efficiency.

Let us carry out a simple calculation to illustrate the definition of PBR proposed above. Six lodgepole pine trees (Table 2) from forest thinnings at two different sites (B and F) are compared. There are one live tree (L) and two dead trees (D) killed by mountain pine beetle infestations at each site. The digit in the sample label represents the age of the trees after beetle infestation. The sample FDD was a windfall and severely deteriorated. Two additional hardwood samples were from aspen and eucalyptus. For this calculation, all woods were pretreated by SPORL, not hot water as suggested. The enzyme loadings were not constant, that is, 15 and 7.5 FPU/g substrate for softwood and hardwood, respectively, because data were obtained not for the present purpose. The pretreatment energy included thermal energy for pretreatment conducted at 170 °C with L/W of 3, wood chipping energy (assumed at 50 Wh/kg), and measured wood chip milling energy. The results show that the beetle-killed lodgepole pine has low PBR, which agrees with previous obervations that beetle infestation increased the wood susceptibility to chemical pretreatment (Luo et al. 2010; Pan et al. 2008). Furthermore, the greater the beetle infestation age, the lower the PBR. The hardwood has much lower recalcitrance than all lodgepole samples (softwood). Eucalyptus has a greater PBR than aspen. The results validate the definition of PBR described above.

## Strategies for enhancing enzymatic saccharification of woody substrates

High lignin content (20–25% in hardwoods and 25–30% in softwoods) differentiates woody from herbaceous biomass. Furthermore, the removal of hemicelluloses through pretreatment enriches lignin in the substrates. For example, the lignin content in steam exploded Douglas-fir could be as high as 42% (Pan et al. 2004). Lignin is recognized as the primary source of PBR of lignocellulosic biomass to enzymes. It was proposed that lignin inhibits enzymes by

**Table 2** Recalcitrance of different woody biomass feedstocks

| Wood sample[a] | Substrate enzyme digestibility (%) | Total sugar recovery (%) | Total pretreatment energy (GJ) | Pretreatment energy efficiency (1/GJ) | Recalcitrance (GJ) |
|---|---|---|---|---|---|
| Softwood (lodgepole pine from forest thinning) | | | | | |
| BL | 50.8 | 48.6 | 2.57 | 0.189 | 10.4 |
| BD1 | 55.4 | 51.3 | 2.49 | 0.206 | 8.8 |
| BD4 | 58.6 | 54.4 | 2.50 | 0.218 | 7.8 |
| FL | 51.5 | 50.0 | 2.82 | 0.177 | 11.0 |
| FD5 | 50.0 | 48.4 | 2.44 | 0.198 | 10.1 |
| FDD | 59.3 | 54.8 | 2.61 | 0.210 | 8.0 |
| Hardwood | | | | | |
| Aspen | 85.1 | 79.4 | 1.49 | 0.533 | 2.2 |
| Eucalyptus | 79.1 | 75.5 | 1.79 | 0.422 | 3.0 |

All samples were pretreated by SPORL in a laboratory pulping digester at 170 °C for 20 minutes with an L/W of 3 and sodium bisulfite and acid charge of 4 and 1.1%, respectively. See text for detailed about sample description physical block of cellulose and nonproductive adsorption of enzymes (Mansfield et al. 1999). Although delignification can remove PBR (Pan et al. 2004; Yang et al. 2002), all processes for delignification are expensive. Therefore, extensive delignification is not a suitable practice in woody biomass pretreatment for ethanol production.

Nonproductive adsorption of enzymes by lignin reduces the efficiency of cellulose hydrolysis (Eriksson et al. 2002; Mansfield et al. 1999; Sewalt et al. 1997; Yang and Wyman 2006; Zheng et al. 2008). Physically blocking or covering lignin prior to enzyme loading is a strategy to reduce lignin–enzyme interactions and to enhance cellulose saccharification. For example, exogenous protein (for example, bovine serum albumin, BSA) has been used to saturate lignin for reducing adsorption of cellulase and enhancing enzymatic cellulose saccharification (Pan et al. 2005b; Yang and Wyman 2006; Zheng et al. 2008). Surfactants have also been used as lignin-blocking agents to improve enzymatic hydrolysis (Borjesson et al. 2007; Eriksson et al. 2002; Ooshima et al. 1990; Tu et al. 2009). However, both techniques have potential problems. For example, proteins are too expensive to be economically feasible in commercial applications, and surfactants can cause foaming that may affect yeast activity. These techniques need to be verified, especially with respect to the potential effect on the efficiency of simultaneous saccharification and fermentation. Recently, we discovered that certain metal compounds, such as $CaCl_2$, $CaSO_4$, $MgSO_4$, can be used to significantly reduce nonproductive adsorption of enzymes by lignin (both bound on solid substrate and unbound dissolved in pretreatment hydrolysate) through lignin–metal complexation (Liu et al. 2010). With the application of these metal compounds, enzymatic hydrolysis of lignocelluloses was greatly enhanced (Liu et al. 2010).

Selectively removing active fractions of lignin or chemically modifying lignin is another strategy to reduce lignin inhibition. For example, extracting steam-exploded Douglas-fir with 1% NaOH at room temperature only reduced lignin content from 43% to 36%, but enzymatic hydrolysis of the substrate was enhanced by 30% (Pan et al. 2005b). This suggested that the alkali-extractable lignin was more inhibitive to enzymes. It was reported that free phenolic hydroxyl groups on lignin benzene rings played an important role in inhibiting enzymes and that selectively blocking (etherifying) the hydroxyl groups could remove the inhibition of the lignin (Pan 2008).

The inhibitive effect of lignin on enzymes is dependent on the structure of the lignin. For example, SPORL pretreated spruce wood contained as high as 33% lignin (Shuai et al. 2010), but the substrate had ready digestibility. Over 90% cellulose-to-glucose was achieved within 24 hours at enzyme loading of 15 FPU/g cellulose. This suggests that the lignin in the SPORL substrates is less inhibitive to enzymes. This is presumably because the partial sulfonation of lignin during the SPORL pretreatment increased the hydrophilicity of the lignin and therefore reduced the hydrophobic adsorption of enzymes on lignin.

## Conclusions

Woody biomass is an important feedstock for the future biobased economy. Effective pretreatment technologies to remove woody biomass recalcitrance are critical for efficient utilization of woody biomass for biofuel production. The goal of biofuel production is net energy output; therefore, an effective pretreatment process should be evaluated by both total sugar yield and pretreatment energy efficiency, that is, total sugar recovery divided by the total energy consumption for pretreatment. Wood size reduction to the level of fibers and/or fiber bundles for efficient enzymatic hydrolysis is energy-intensive. A post-chemical pretreatment wood size reduction approach is proposed to significantly reduce mechanical wood size reduction energy consumption. In addition to reducing pretreatment temperature, reducing L/W is critical to reduce thermal energy consumption in thermochemical pretreatment. Pretreatment processes that require fiberized materials and large L/W, such as IL, need to overcome the pretreatment energy barrier for woody biomass applications. SPORL pretreatment is an efficient process for woody biomass bioconversion. Finally, biomass recalcitrance can be quantitatively defined as the amount of energy consumed in pretreatment divided by the SED and the total sugar recovery.

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