Introduction

Lignocellulose, mostly existing in the form of plant materials, is one of the most abundant organic materials in natural world. It has been estimated that there is an annual worldwide production of 10–50 billion tons of dry lignocellulose, accounting for about half of the global biomass yield [1]. In recent years, due to the escalating energy crisis and environmental pollutions, more extensive attention has been paid to the production of biofuels and biochemicals from biomass via a “biorefinery” platform. According to National Renewable Energy Laboratory of the USA, biorefinery is defined as “a facility that integrates biomass conversion processes and equipment to produce fuels, power, and chemicals from biomass”, which is analogous to today's petroleum refineries [2]. It has been considered that lignocellulose biorefinery will most probably be pushed through with highest success among the potential industrial biorefineries, because that the raw material situation is optimal and the conversion products have a good position in the traditional petrochemical as well as in the future bio-based product market [3].

Pretreatment is a prerequisite step for enzymatic hydrolysis of cellulose for a subsequent bioconversion of the obtained fermentable sugar to fuels and chemicals. Various pretreatment techniques have been developed during last decades, which increase cellulose accessibility by modifying chemical compositions or cell wall structures [4]. Among these pretreatment techniques, organosolv pretreatment, which uses organic solvents or their aqueous solution systems with or without added catalysts to pretreat biomass in temperature range of 100–250°C, seems to be a promising approach for an integrated utilization of biomass compositions [5]. Generally, organosolv pretreatment yields three separate fractions: high-purity lignin, hemicellulosic syrup and a relatively pure cellulose fraction [6], all of which show promises for producing high value-added products. Therefore, organosolv pretreatment can be termed as a “fractionating” process to increase cellulose digestibility and obtain multiple products.

In the present paper, we have reported the recent progress in our group on the fractionating pretreatment of herbaceous biomass (sugarcane bagasse and wheat straw) to obtain cellulosic solid, hemicellulosic sugars and lignin products. Further utilization of these products for productions of biofuels (ethanol), chemicals (furfural) and materials (solid acid catalysts) also have been presented.

Results and discussion

Alkali/peracetic acid pretreatment for co-production of pulp, ethanol, microbial lipid and lignin

We have developed a biorefinery platform via alkali/peracetic acid delignification of lignocellulosic biomass as shown in Figure 1 [7]. Lignocellulosic biomass especially non-woody biomass (such as wheat straw and sugarcane bagasse), is of heterogeneity. Not all the part of the
Biomass can be used for pulp preparation. Only the long fibers can meet the demand of papermaking. Therefore, the short fibers, such as the pith, powder obtained during biomass preparation, and other part that cannot be used for pulp production, are used for fermentable sugar production. The long fibers, which has a relatively recalcitrant structure is delignified by alkali followed by peracetic acid (PAA) treatment. This two-stage process removed more than 90% of lignin and only about a half of the initial hemicelluloses. The obtained cellulosic pulp contained 78.5% cellulose, 98% holocellulose and 1.5% lignin. Further analysis indicated that alkali-PAA pulp had a high degree of polymerization and good mechanical properties. When this pulp was used for paper formation, the paper sheet showed a good mechanic strength [8]. The pulp also can be easily hydrolyzed by cellulase at a relatively low enzyme loading [9]. Simultaneous saccharification and fermentation (SSF) experiments demonstrated that the pulp could be well converted to ethanol with a final concentration of 40 g/L, and co-production of xylose with a concentration of about 40 g/L. This part of xylose can be converted to microbial lipid using oleaginous yeast, which has been successfully converted to biodiesel through transesterification with methanol as an acyl acceptor [10]. Two types of lignin, namely alkali lignin and PAA lignin were obtained during alkaline-PAA pretreatment. Characterization of the lignin products showed that alkaline lignin had a higher molecular weight and OCH3 group content, while PAA lignin had higher oxygen content because of oxidation [7].

Figure 1 Co-production of pulp, ethanol, microbial lipid and lignin via alkali/peracetic acid pretreatment

Alkali-PAA pretreatment pertains to be a mild fractionation process, in which most of lignin and a part of hemicelluloses are dissolved, remaining most of cellulose and about 50% of initial hemicelluloses recovered as a solid phase (pulp). The pulp can be used for either paper-making or sugar production by enzymatic hydrolysis. The hemicellulose, mainly consisting of xylan, is easy to hydrolyze in an enzymatic hydrolysis to produce xylose. This part of sugar can be converted to ethanol if genetically-modified yeasts or bacteria are used. Another approach is to convert xylose into microbial lipid using oleaginous microorganisms, and the lipid can be used as a feedstock for biodiesel production. Since no inhibitor is produced in enzymatic hydrolysis, the obtained xylose can be easily utilized by microorganisms without detoxification. The by-product lignin can be used as a
fuel for energy recovery, or modified for production of derivative products. It is easy to know that alkali-PAA process can be established by modifying an existing paper mill. Therefore, this process seems to be promising to convert a conventional paper mill to a biomass refinery for coproduction of paper, ethanol, lignin and microbial lipids.

**Fractionating pretreatment based on organic acid delignification**

We also have developed another fractionating pretreatment process using organic acid to dissolve lignin. Since lignin has a high solubility in organic acids, the pretreatment can be performed at a low liquid-to-solid ratio, or the spent liquor can be directly recycled for delignification. Energy consumption for solvent recovery can be greatly reduced when spent liquor is directly reused for delignification [11]. Another advantage for organic acid pretreatment is that most of hemicelluloses are hydrolyzed to form monosaccharides, and these sugars can be recovered as hemicellulosic syrup after lignin is precipitated. By this process, lignocellulosic biomass is fractionated into cellulosic solid, hemicellulosic syrup and high-purity lignin as shown in Figure 2. The cellulosic solid had cellulose content higher than 85% and lignin content less than 10%. However, cellulose was acylated (formylated or acetylated) during delignification process, which may limited the enzymatic hydrolysis of cellulose depending on the degree of acylation. The presence of acyl group may interfere the recognition of cellulase enzyme with cellulose substrate. Recently, we have found that substitution of cellulose –OH by acetyl group increases the hydrophobicity of the substrate, which increases the non-productive adsorption of enzyme and thus decreasing cellulose digestibility. Therefore, deacylation can well recover the enzymatic digestibility of the delignified solid [11, 12, 13]. Alkali and acid have been found to effectively remove acyl groups. Several processes have been developed based on the type of organic acids used for delignification and alkali or acid used for deacylation, including Formiline (formic acid for delignification and alkali for deacylation), Acetoline (acetic acid for delignification and alkali for deacetylation), Formicid (formic acid for delignification and mineral acid for deacylation) and Acetocid (acetic acid for delignification and mineral acid for deacetylation) processes.

![Figure 2 Fractionating pretreatment of lignocellulosic biomass based on organic acid delignification](image)

The pretreated cellulosic solid showed an excellent enzymatic digestibility and fermentability for ethanol production by SSF. For example, when Formiline pretreated sugarcane bagasse was used, the final ethanol concentrations were 55.4 and 80.1 g/L respectively at initial solid consistencies of 15%
and 20% \cite{11}. This increased digestibility is mainly attributed to the removal of lignin, thus exposing cellulose to cellulase enzymes. Another advantage of these pretreatment is the high cellulose content in the cellulosic solid. High glucose concentration can be obtained in the hydrolysis process, and product titer is increased accordingly.

The spent liquor must be evaporated to recover organic acid, where it becomes concentrated. Addition of water can precipitate lignin in the concentration spent liquor, remaining hemicellulosic sugar in the liquid phase. It has been found that xylose can be well converted to furfural under the catalysis of acetic acid at 180°C with furfural yield of 80% \cite{14}. Therefore, it is possible that the xylose obtained after lignin precipitation can be converted to furfural under the catalysis of the residual organic acids. The precipitated lignin is a good feedstock for production of carbon materials. In our group, we have prepared activated carbon and solid acid from lignin products as shown in Figure 3. The obtained solid acid catalyst has been successfully used for catalyzing the transesterification of oil feedstocks or esterification of fatty acid with methanol for biodiesel production. Biodiesel yield could be higher than 90% when 2% solid acid was used to catalyze the conversion of oleic acid to methyl oleate.

![Figure 3 Preparation of solid acid from lignin and its use for catalyzing biodiesel production](image)

**Novel kinetic models for delignification and polysaccharides dissolution by organic acids**

Delignification is important to increase cellulose digestibility by organic acid pretreatment. However, the simplest kinetic model, which assumes that all of the lignin in cell wall is reactive to be removed, cannot well describe the kinetic behavior of organic acid delignification. We have proposed a novel kinetic model by introducing the concept of “potential degree of delignification ($d_D$)” \cite{15}. In this model, it is assumed that only a part of lignin can be removed depending on the reaction severity due to the multi-layered structure of cell wall. This simple modification led to an excellent accordance of predicted data with experimental ones. It also has been found that the simplest model (Saeman’s model) \cite{16} showed a great deviation from our experimental data for dilute acid hydrolysis of sugarcane bagasse \cite{17} and polysaccharides dissolution during organic acid pretreatment. Similar parameter termed as “potential hydrolysis degree ($h_d$)” is thus introduced into the kinetic model. Experimental verification demonstrated that this novel kinetic model can be well applied to describe the dilute acid hydrolysis of sugarcane bagasse \cite{17} and polysaccharides dissolution during the sulfuric acid-catalyzed pretreatment of sugarcane bagasse \cite{18} (Figure 4).
Process simulation and optimization of fractionating pretreatment

To obtain general mass balance and energy balance data for a further economic evaluation of biomass refining via fractionating pretreatment, we have performed several process simulations and optimizations using Aspen Plus software as shown in Figure 5. According to the simulation results, the solvent recovery system is the most energy intensive, and direct recycling of spent liquor dramatically decreases energy consumption. When spent liquor is directly reused for delignification for 7 times (equaling that only 1/8 of spent liquor is evaporated for solvent recovery), the energy consumption for solvent recovery accounts for about 45% of the total. Further analysis results shows that refining to biomass to produce multi-products such as ethanol, lignin and furfural can bring more revenue than that of single product process.

Figure 5 A case of Aspen Plus process simulation for biomass refining based on fractionating pretreatment
Conclusions

Fractionating pretreatment has been found to greatly increase cellulose accessibility for enzymatic hydrolysis. Co-production of some other valuable products can be achieved by fractionating pretreatment. In the paper, we reported two biomass refinery platform respectively based on alkali-peracetic acid delignification and organic acid delignification. By these platforms, valuable products such as cellulosic pulp, ethanol, lignin, microbial oil (biodiesel) and furfural can be produced. Each of the product show promises for further application or conversion. More revenues can be obtained by this multi-product model.

Acknowledgement

These works are supported by National Natural Science Foundation of China (No. 21106081), National Basic Research Program of China (973 Program) (No. 2011CB707406), International Cooperation Project of the Ministry of Science and Technology of China (No. 2010DFB40170), and Tsinghua Research Foundation (No. 2012Z98148)

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