ROBUST AND EFFICIENT ENZYMATIC SACCHARIFICATION OF SOFTWOODS BY SPORL

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ABSTRACT

This study demonstrated Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose (SPORL) for robust conversion of softwood through enzymatic hydrolysis. At a sodium bisulfite charge around 9%, over 90% cellulose conversion could be achieved when spruce wood chips were pretreated at 180°C with pH near 2. For lodgepole pine, pretreatment liquor initial pH had no effect on enzymatic cellulose conversion in the tested range of 1.9 – 4.2 with a sodium bisulfite charge of 8%. The dissolution of glucan during pretreatment was only about 10%. Most of the hemicelluloses were recovered as monomeric fermentable sugars in the pretreatment spent liquor. SPORL was developed based on sulfite pulping, and therefore has a precedent for excellent scalability for future commercialization.

BACKGROUND

Wood is a sustainable feedstock and available in large quantities worldwide. About 368 million dry tons of woody biomass (or about 30% of the total biomass) can be sustainably produced annually in the United States alone based on the Billion Ton report [1]. The pulp and paper industry has long recognized the advantages of woody biomass over agricultural biomass for fiber and paper production. Other than its superior fiber properties over agricultural biomass, woody biomass has a high density that can significantly reduce transportation cost. Furthermore, woody biomass can be harvested almost any time on demand, which eliminates storage cost and deterioration. In addition, the productivity of woody biomass is equivalent to those of agricultural biomass but with lower labor intensity and fertilization requirements. These reduced inputs for woody biomass could reduce energy cost. More over woody biomass is the only feedstock which could be sustainably produced in large quantities in many regions of the world. Therefore, woody biomass will be a key part of the feedstock supply for the future biobased economy and to meet local and regional energy needs [1].

However, due to it's tough physical structure and high lignin content, forest biomass has much greater recalcitrance for biochemical conversion than does agricultural biomass. Most existing pretreatment technologies failed to efficiently remove the recalcitrance of forest biomass, especially of softwoods, despite decades of research and development in pretreatment. In this work, we present a novel process, SPORL (sulfite pretreatment to overcome recalcitrance of lignocellulose) [2, 3] which was developed based on sulfite pulping, for robust cellulose conversion of woody biomass.

It is well understood by the pulp and paper industry that sulfite pulping degrades hemicellulose as evidenced by the amount of hemicellulose sugars in the spent sulfite pulping liquor (SSL). Fermentation of the dissolved hemicellulose sugars in SSL has long been practiced by sulfite pulp mills to produce cellulosic ethanol. Sulfite pulping produces weaker pulp than does the Kraft pulping process due to the depolymerization of cellulose [4] and hemicellulose [5]. Sulfite pulping also causes lignin sulfonation and increases the hydrophilicity of lignin. The degrees of dissolution of hemicellulose, degradation of cellulose, and sulfonation and condensation of lignin are increased as reaction time and temperature increases, and pH decreases [6]. Therefore, it is conceivable to develop a pretreatment process by modifying the sulfite pulping to achieve maximum hemicellulose removal and cellulose depolymerization, while limiting lignin condensation in order to promote the enzyme process through lignin sulfonation. The objective of this study is to demonstrate such a pretreatment process, SPORL, for robust and efficient conversion of woody biomass through enzymatic saccharification.

EXPERIMENTAL

The present SPORL process consists of sulfite pretreatment of typical pulp mill wood chips. Spruce and Lodgepole pine wood chips were used in this study. Other wood species have also been tested in our laboratory [2, 3]. The pretreatments were conducted in two laboratory rotating batch type digesters with capacities of 100 g and 2000 g oven dry wood, respectively. Most of the studies used a liquor to wood ratio of 3 to reduce thermal energy consumption for pretreatment. Sodium bisulfite was used and mixed with acid for pH adjustment to produce the pretreatment solution. Magnesium and ammonia bisulfite were also found to be effective [2]. Typical pretreatments temperature ranges from 170-190°C. A temperature of 180°C was used in this study for a duration of about 30 minutes using a bisulfite charge of 3-9% and a sulfuric acid charge of 0-2.5%, based on oven dry wood. This resulted in a pretreatment liquor with an initial pH range of 2-4.5. After the pretreatment, the spent liquors, containing most of the hemicelluloses, were collected for future fermentation study. The wood chips were directly fed into

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either an 8 or 12 inch disk refiner (Andritz Sprout–Bauer Pressurized Refiner, Springfield, Ohio) to produce substrate. For the larger 2000 g batch runs, energy consumptions were recorded during size-reduction in the 12 inch disk refiner.

The obtained substrates were used for subsequent enzymatic hydrolysis to evaluate their digestibility. Enzymatic hydrolyses of the substrates were carried out at a substrate consistency of 2% (w/v) in 50-mL sodium acetate buffer (pH 4.8) at 50°C using a 200 rpm shaking incubator (Thermo Fisher Scientific, Model 4450, Waltham, Massachusetts) . A mixture of Celluclast 1.5 L, with an activity loading of 14.6 FPU/g substrate, and Novozym 188, with an activity loading of 22.5 CBU/g substrate, was used for enzymatic hydrolysis; this loading is equivalent to about 20 FPU/g and 30 CBU/g cellulose Excessive Novozym 188 was used to prevent cellobiose accumulation. Glucose concentration in the enzymatic hydrolysates were measured periodically by a commercial glucose analyzer (YSI 2700S, YSI Inc., Yellow Springs, Ohio).

Carbohydrate compositions of the original and pretreated wood samples were measured using a high-performance anion exchange chromatographic (ICS-3000, Dionex, Sunnyvale, California) method with pulsed amperometric detection (HPAEC-PAD) [7].

RESULTS AND DISCUSSION

Time-dependent Cellulose Conversion of Spruce

Spruce has long been used to produce sulfite pulp. In this study we would like to demonstrate the excellent bioconversion of spruce through SPORL. Figure 1 shows the time-dependent cellulose enzymatic conversion of spruce after SPORL pretreatment, at different sodium bisulfite charges on wood. The results indicate that over 90% cellulose conversion was achieved in 48 hours of hydrolysis when wood chips were pretreated by a 9% charge of sodium bisulfite . The results also indicate that the repeatability of the experiments was very good. Increasing the sodium bisulfite charge from 3% to 9% increases cellulose conversion. However, further increases of the bisulfite charge above 9% reduced cellulose conversion [2].

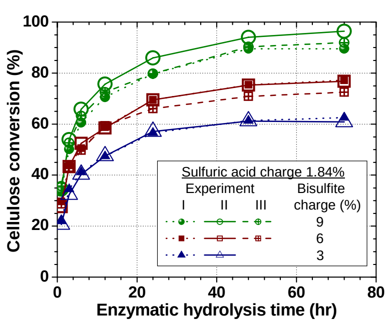

Figure 1: Effect of sodium bisulfite charge on time-dependent cellulose conversion of spruce at sulfuric acid charge of 1.84% on od wood

The effect of pretreatment pH on enzymatic glucose yield of spruce is shown in Fig. 2. The pH values shown in the figure were the initial pH of the pretreatment liquor. Both hydrochloric and sulfuric acid were used. As can be seen there is an optimal pH at which glucose yields were maximized. It should be pointed out that cellulose content of original spruce wood was 43%. Therefore, the enzymatic glucose yield of over 36% wt wood, achieved with a pH of about 2.0, is excellent. Examining the chemical composition of the pretreated substrate indicates that only the small amount of about 10% glucan was lost (dissolved) in the pretreatment liquor.

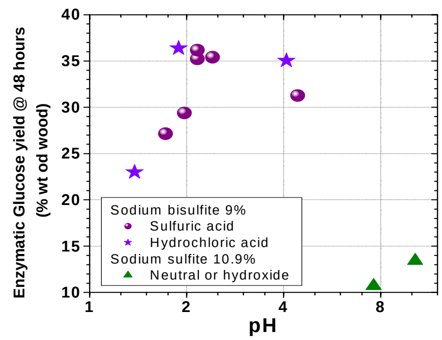

Figure 2: Effect of pretreatment initial pH on enzymatic hydrolysis glucose yield from Spruce.

Substrate Morphology

Figure 3 shows a typical SEM image of a substrate (Spruce) produced through SPORL pretreatment of wood chips followed by disk milling. The pretreatment conditions for the substrate shown were an initial pH of 2.16 and sodium bisulfite charge of 9% at 180^{o}C. The pretreatment significantly altered wood physical and chemical structure. The energy consumption in disk milling was only about 20 Wh/kg od wood [2, 8], or a reduction in milling energy consumption by about a factor of 20 compared to about 400 Wh/kg od wood for untreated spruce wood. The SEM images shows some of the fibers, or at least portions of the fibers, remained intact while some fibers were peeled and destroyed, breaking into fibrils or fibril bundles. This form of destruction increases the accessibility of enzyme to cellulose fibrils.

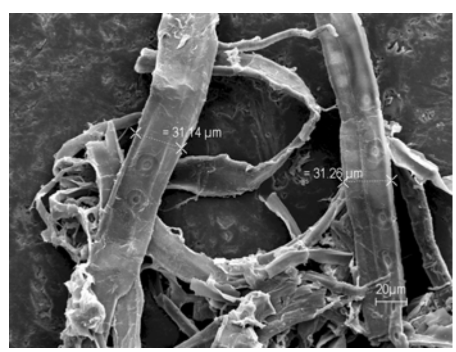

Figure 3: SEM image of the substrate obtained with 9% sodium bisulfite charge and pH = 2.16.

Cellulose Conversion of Lodgepole Pine

Millions of acres of Lodgepole pine trees are infested with mountain pine beetles in North America. Use of these trees for biofuel production is a potential avenue to reduce fuel loading in forests and therefore reduce the risk of catastrophic fires. As a softwood, lodgepole pine has a very strong recalcitrance to enzyme attack. Fig. 4 shows SPORL is very effective at removing the barriers to enzymes for efficient cellulose hydrolysis of lodgepole pine. Over 90% cellulose conversion by enzymatic hydrolysis was achieved with an 8% sodium sulfite charge. Furthermore, pH has little effect on cellulose conversion for lodgepole pine. Fig. 4 also shows a dilute acid pretreatment, at an initial pH of 1.1, only produced about 40% cellulose conversion.

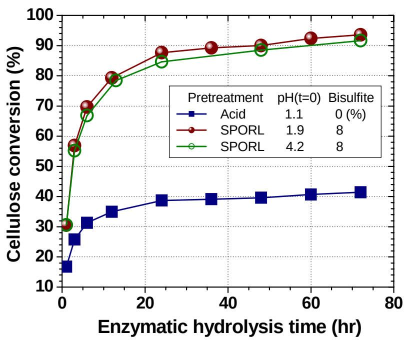

Figure 4: Time-dependent enzymatic cellulose conversion of Lodgepole pine after SPORL and acid pretreatment.

Hemicellulose Sugar Recovery

Preliminary analysis of the spent liquor from pretreated spruce, at a sodium bisulfite charge of 9% and initial pH of 2.16, suggests excellent hemicellulose sugar recovery as monomeric fermentable sugars [2]. ; the. xylose and mannose recovery were 76 and 88%, respectively. Likewise, 92% of the cellulose dissolved was recovered as glucose.

CONCLUSIONS

This research demonstrated SPORL provides robust conversion of softwoods via enzymatic hydrolysis, using commercial enzyme at about 20 FPU/g cellulose, in 48 hours. SPORL has excellent scalability as it's is development is based on sulfite pulping. It can use the capital equipment and infrastructure existing in the pulp and paper industry for immediate commercialization. The excellent hemicellulose sugar recovery and good fermentability of spent liquor, using existing sulfite pulping liquor fermentation technology, makes SPORL more economical than the organosolv process.

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