암모니아수를 이용한 식물계 바이오매스의 분별 및 당화

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Fractionation and Saccharification of Herbaceous Biomass by Aqueous Ammonia

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Introduction

Corn stover has emerged as one of the most promising renewable feedstocks for biological conversion to fuels and chemicals. Pretreatment is an essential element in the bioconversion of lignocellulosic substrates. Ammonia has a number of desirable characteristics as a pretreatment reagent. It is an effective swelling reagent for lignocellulosic materials. It has high selectivity for reactions with lignin over those with carbohydrates. It is one of the most widely used commodity chemicals with about one-fourth the cost of sulfuric acid on molar basis. Its high volatility makes it easy to recover and reuse. It is a non-polluting and non-corrosive chemical. One of the known reactions of aqueous ammonia with lignin is the cleavage of C-O-C bonding in lignin as well as ether and ester bonding in lignin carbohydrate complex (LCC). Indications are that use of ammonia in the pretreatment would be effective in selectively reducing the lignin content in the biomass. There are many advantages of removing lignin at the early phase of the conversion process before it is subjected to the biological processing. Lignin is believed to be one of the major hindering factors in the enzymatic reaction. Lignin and its derivatives are toxic to microorganism and inhibitory to the enzymatic hydrolysis. The low-lignin in the substrates can therefore improve the microbial activity and the overall enzyme efficiency, eventually lowering the enzyme requirement. Complete delignification of biomass, however, is difficult for a number of reasons including its location within biomass structure existing in deep cell wall, hydrophobicity, physical stiffness, strong poly-ring bonds of C-O-C, C-C, and the tendency of recondensation after delignification. A number of factors other than lignin have also been suggested in relation with enzymatic hydrolysis. They include the crystallinity of biomass, degree of polymerization, particle size, surface area, and pore size. We have previously investigated on various pretreatment processes using a flow-through (percolation) reactor system in our laboratory. Among them is the ammonia recycled percolation (ARP) process that we have studied for pretreatment of various lignocellulosic biomass feedstocks including hardwood herbaceous biomass, and pulp mill sludges. Modification of this process has also been attempted combining the process with treatment with additional reagent. The primary purpose of this investigation is to assess the effectiveness of the ARP treatment as a pretreatment process specifically for corn stover. We were interested in verifying the changes in chemical composition and physical characteristics of biomass brought about by the pretreatment and how those factors affect the enzymatic digestibility.

Materials and methods

Materials; Corn stover was supplied from National Renewable Energy Laboratory (NREL, Golden, CO). It was ground and screened. The fraction collected between of 2mm~35mesh was used in all experiments. The initial compositions of corn stover was determined to be: 40.19% glucan, 21.71% xylan, 2.61% arabinan, 0.29% mannan, 0.68% galactan, 18.53% Klason lignin, 2.30% acid soluble lignin, 7.08% ash, 2.20% acetyl group, 2.90% protein, and 1.51% unaccounted for. The α-cellulose was purchased from Sigma (Cat. No. C-8200, Lot No. 11K0246). The cellulase enzyme, Spezyme CP, Lot 301-00348-257, was obtained from Genencor International Inc. (Paulo Alto, CA). The average activities of the enzymes as determined by NREL are 31.2 FPU/mL. The β-glucosidase was purchased from Sigma (Cat. No. G-0395).

Experimental setup and operation of ARP; Overall layout of the ARP apparatus is described in Fig. 1. The system consists of a stock solution reservoir, pump, temperature-programmable oven, SS-316 column reactor (9/10 in ID × 10 in L, internal volume of 101.9cm^3), and liquid holding tank. The reactor was operated in a flow-through mode, the liquid flowing through the reactor column packed with biomass. The reactor system was pressurized by nitrogen at 325 psig to prevent vaporization. In a typical ARP experiment, 15g of biomass sample were packed into the reactor, soaked with ammonia solution and left overnight. The reactor temperature was controlled in a forced-air convection oven. About 15 minutes of preheating time was necessary to reach the desired temperature. The reaction time was counted after the desired temperature was attained.

Reaction Conditions; 50°C, pH 4.8 (0.05M sodium citrate buffer), 150 rpm in a temperature controlled shake flask (New Brunswick Scientific, Model Innova 4080). The digestibility is defined as % of theoretical glucose released after 72 h of incubation with cellulase enzyme. Enzyme loading: 10-60 FPU of Spezyme CP/g-glucan of biomass supplemented with 37 IU of β-glucosidase (Sigma, G-0395).

Analytical methods; The corn stover samples were analyzed for sugar, Klason lignin, and acid-soluble lignin following the procedures of NREL Chemical Analysis and Testing Standard Procedures No. 001~004. The moisture content was measured by automatic infrared moisture analyzer (Denver Instrument Company, IR-30). Sugars were determined by HPLC using a Bio-Rad Aminex HPX-87P column for carbohydrate analysis of solids. For enzymatic digestibility, the glucose content was measured by HPX-87H column.

Results and Discussion

Effect of ARP treatment on Composition of Corn Stover; On the basis of our previous investigation and the results of preliminary experiments of this work, 170°C and 15wt% of ammonia concentration was chosen for the ARP operation of corn stover. The most significant change of the composition is in the lignin. The ARP process removed 70-85 % of the total lignin of the corn stover feedstock. The delignification reaction is rapid to the extent that 70% of lignin is removed within 10 minutes of treatment. About half of xylan (main component of hemicellulose) is also solubilized. The glucan content, however, remains relatively intact. The overall reduction of solid mass (expressed as solid remaining, S.R.) by the ARP is slightly less than half (53.6-61.4%). As indicated in the table by the total of glucan and xylan (amount of carbohydrates in the solid plus that in liquid), the accountability of sugars is above 95% for glucan and near 100% for xylan. The carbohydrates in the biomass are thus well preserved in the ARP process, a very important benefit as a pretreatment process.

Compositional Changes and the Enzymatic Digestibility; The digestibilities were measured with two different loadings of cellulase: 60 FPU/g-glucan and 10 FPU/g-glucan. Regardless of the treatment conditions, the digestibility of the pretreated biomass has significantly improved from that of the control (untreated biomass). The lowest digestibility of the treated biomass is 84% @ 10 FPU/g-glucan which occurred with the sample treated by the ARP for 10 minutes. The digestibility of the control is 14.3% @10 FPU/g-glucan. The digestibilities increase with treatment time and with enzyme loading. With 60 FPU/g-glucan enzyme loading, the digestibilities were all above 90%, the highest being near quantitative, 99.6%, which is observed with biomass treated for 90 minutes. The difference of digestibility with regard to the composition is more discernible with low enzyme loading (10FPU/g glucan) than with high enzyme loading (60 FPU/g-glucan). The digestibilities of the ARP treated biomass are substantially higher than those of α-cellulose that stand at 71.7% and 93.4% with enzyme loadings of 10 and 60 FPU/g-glucan respectively. The hemicellulose fraction of corn stover exists in amorphous form. The lignin is closely associated with cellulose fibers and acts as a binding reagent of fiber bundles. They both exist in non-crystalline zone of the biomass. Removal of these materials would therefore increase the surface area and porosity within the biomass, thus providing easier access to cellulose and more reactive sites for the enzymes. In theory of catalytic reaction, the rate of enzymatic hydrolysis is proportional to the surface area. However, Yoon et al. (1995) found that the BET surface area increases by about 50% by the ARP pretreatment, which does not provide sufficient ground to explain 7-10 fold increase in digestibility. Burns et al. (1989) provides a partial explanation for it; for substrates with pores too small to accommodate the macromolecules of cellulase enzyme (20-40Å), the reaction takes place on the external surface. It appears that the mechanism of cellulase reaction as it relates to the porosity and surface area is quite complex and not fully understood. Since both lignin and xylan are reduced with the ARP treatment, it is unclear which of the two factors and to what extent is affecting the enzymatic hydrolysis. To further verify this point, we have conducted digestibility tests on corn stover samples treated differently. For these samples the treatment was done with dilute acid (0.07% H_2SO_4, 180^oC) instead of aqueous ammonia. In comparison to the ARP, the dilute-acid treatment removes most of the xylan, but only 20% of the total lignin in the biomass. The dilute-acid treated biomass is therefore basically xylan-free whereas the ARP treated biomass is lignin-free. The enzymatic digestibilities of ARP sample (lignin-free) are 99.6% at 60FPU/g glucan of enzyme loading and 92.2% at 10FPU/g glucan loading, whereas those of dilute acid treated samples (xylan-free) are 89.9% with 60FPU/g glucan loading and 82.8% with 10FPU/g glucan loading. The hydrolysis rate of ARP treated samples is also much higher than dilute-acid treated samples. The yield of 60FPU loading reached 97% in 6 hour and the yield of 10FPU loading reached 90% in 24hr. The lignin as a factor hindering the enzymatic hydrolysis of lignocellulose is well documented. Delignifed refiner mechanical pulp was hydrolyzed more completely by cellulase than untreated substrate and suggested that steric hinderance from resiudual lignin may be one of the rate-limiting factors. The rate and extent of enzymatic correlated better with removal of alkali-insoluble lignin than with removal of xylan.

Lignin content has the greatest impact on biomass digestibility, whereas the acetyl content has a minor impact in their study of lime pretreatment . According to Converse (1993), it is possible to disrupt the lignin shield without removing the lignin. While this makes the cellulase more accessible, the enzyme adsorbs on the lignin making the enzyme ineffective. Our findings are in line with many of the previous studies in that lignin plays a larger role as a resistance to the enzyme reaction than hemicellulose. It also explains why lignin-free corn stover shows higher digestibility than xylan-free biomass.

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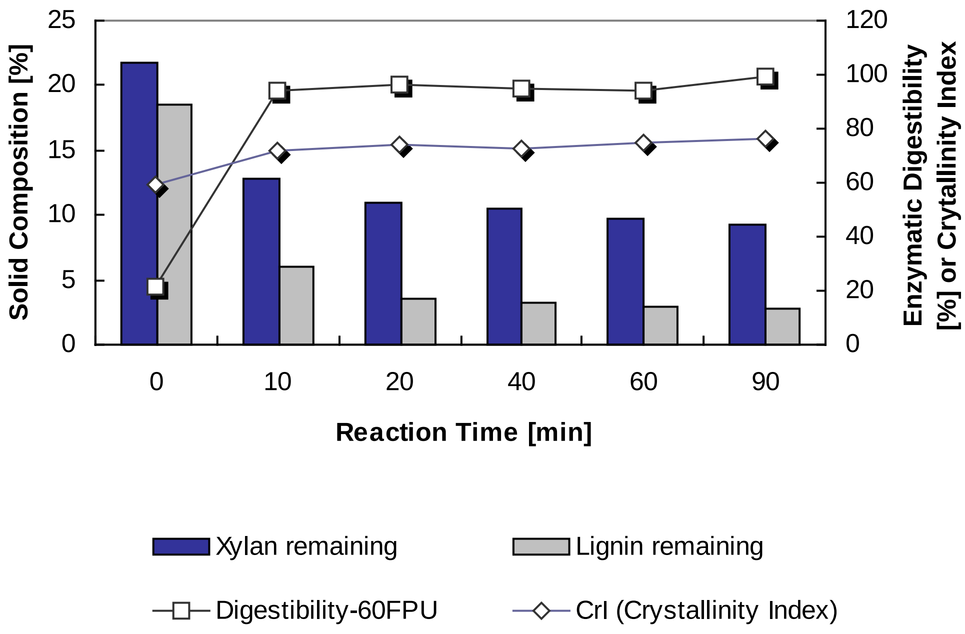

(b) Lignin-free vs Xylan-free sample