암모니아 전처리 및 동시당화발효 공정을 이용한 에탄올 생산

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Bioethanol Production from Ammonia Pretreated Waste Oak Wood by SSF

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INTRODUCTION

Oak wood is 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. In this study, treatment with aqueous ammonia is evaluated as a pretreatment method for enzymatic saccharification. Ammonia recycled percolation (ARP) process was invented as a pretreatment method for agriculture residue in this lab. Its high volatility makes it easy to recover and reuse. Ammonia is one of the most commonly used commodity chemicals. It is a non-polluting and non-corrosive chemical. There are many advantages in removing lignin at the early phase of the conversion process before it is subjected to the biological processing. Lignin is believed one of the major hindering factors in the enzymatic reaction. (Dulap et al., 1976, Mooney et al 1998, Dora et al., 1995) Lignin and its derivatives are toxic to microorganism and inhibitory to the enzymatic hydrolysis. The low-lignin in the solid substrates therefore improves the microbial activity and the overall enzyme efficiency, eventually lowering the enzyme dosage. We have developed an efficient delignification process with a packed-bed flow-through reactor. According to previous reports, this process allows high and adjustable degree of delignification of biomass feedstock. (Iyer et al., 1996, Kim et al., 1996, Kim et al., 2000, Yoon et al., 1995.) Oak wood consists of three major components, such as cellulose, hemicellulose, and lignin. Lignin is polyphenolic compound that mainly consist of coniferyl and synapyl alcohol based polymer. And the plant cell is lignified from the dead cell or against the fungi. In the cellulosic material, the middle lamella has the highest concentration of lignin but the S_2 layer (deep cell wall) has a most of total lignin due to its size. (Timmel, 1967) The complete delignification is difficult because of as follows.

1. Existence in deep cell wall (deep penetration of agent is difficult)
2. Characteristics of hydrophobic (non-water soluble) and stiffness.
3. Strong bonding of lignin (C-O-C, C-C are very strong bonds, its depolymerization, solubilization is difficult)
4. Original LCC (lignin carbohydrate complex) form, recondensation and repolymerization after lignin solubilization

Ammonia, very effective swelling agent, can resolve the penetration problem because of its small molecular size. This is also well known chemical for cleavage of C-O-C bonding in lignin as well as ether and ester bonding in LCC. The recondensation and repolymerization of lignin can be minimized by choice of percolation reactor. In the long run, the pretreatment is to contribute for improving

enzymatic hydrolysis followed by fermentation at the end step. Many former researchers have suggested various factors affecting to enzymatic hydrolysis. Crystallinity, degree of polymerization, particle size, surface area, pore size, and lignin content. (Dulap et al., 1976, Mooney et al 1998, Polcin et al., 1977, Converse 1993) Crystallinity index (CrI) has been used for investigating the relationship between enzymatic hydrolysis and composition. Corn stover is very heterogeneous form of cellulose, hemicellulose, and lignin. It is considered that lignin and hemicellulose are present as an amorphous state in the plant. As lignin and hemicellulose, i.e. amorphous region, are removed, the relative CrI value is expected to increase. There also must exist a linkage between lignin and hemi- or cellulose. The hindering role of lignin in enzymatic hydrolysis plays physically as well as chemically limiting of enzyme accessibility. When it comes to surface area, pore size, and relative crystallinity index, they are related to lignin removal. During removing of original lignin, the accessibility of enzyme is likely to be increased. In conclusion, lignin removal is very effective pretreatment method to enhance an enzymatic hydrolysis.

MATERIALS AND METHODS

Experimental setup and operation of ARP

The system consists of a stock solution reservoir, pump, temperature-programmable GC oven, SS316 reactor (dimension of 9/10 in id × 10 in L, volume; 101.9cm3), and liquid holding tank, which also served as a back-pressure vessel pressurized by nitrogen cylinder at 325 psig, preventing evaporation of ammonia. In an ARP experiment, 15g of biomass sample were packed into the reactor, soaked with ammonia solution and left during overnight. From 16 to 17 min of preheating time is necessary to reach at the desired temperature.

Digestibility test

Enzymatic hydrolysis of pretreated substrate was performed at 50°C and pH 4.8 (0.05M sodium citrate buffer) on a shaker bath agitated at 150 rpm with enzyme loadings of 60~10FPU/g glucan. The initial glucan concentration was 1% (w/v) based on 100mL of total volume. 250mL screw caped Erlenmeyer flasks containing the samples were placed in an incubator shaker (New Brunswick Scientific, Edison, NJ). β-glucosidase was supplemented as much as ~30 IU/g glucan (Sigma, Cat No. G-0395, activity=12.5 IU/g). Total glucose content after 72 h of hydrolysis was taken to calculate the enzymatic digestibility. Untreated corn stover and α-cellulose were subjected to the same digestibility test as a control and as a reference.

Analytical methods

The solid samples were analyzed for sugar, Klason lignin, and acid-soluble lignin following the procedures of NREL Chemical Analysis and Testing Standard Procedures. For the moisture analyzing, automatic infrared moisture analyzer (Denver Instrument company, IR-30) or convection oven method was used. Sugars were determined by HPLC using a Bio-Rad Aminex HPX-87P column and HPX-87H column.

RESULTS AND DISCUSSION

Effect of ARP treatment on Enzymatic hydrolysis

In previous reports (Iyer et al., 1996, Yoon et al., 1995.), 170°C and 10~15wt% of ammonia concentration is very good reaction condition for delignification without significant loss of cellulose. In this study, ARP treatment was performed at 170°C and 15wt% of ammonia concentration.

The enzymatic digestibility was increased in both enzyme loadings (10 and 60FPU/g glucan), as treatment time increased. Xylan removal and lignin removal were also increased. The result shows that the decrease of lignin and hemicellulose content in solid be directly related to enzymatic hydrolysis. Especially at low enzyme loading of 10FPU/g glucan, substantially different results were found from

83.90% of 10min to 92.50% of 90min. At any pretreatment conditions, there was a drastic increasing in terms of enzymatic hydrolysis as compared with that of untreated substrate (14.3%). Despite the ARP treatment is a pretreatment method in order to remove the lignin only, the big quantity of xylan loss (41.13~57.3%) was inevitable during pretreatment, even for very short reaction time (10min). For reasons of hemicellulose removal in company with delignification, the finding the component which affect enzymatic activity is very difficult. The lignin and most of hemicellulose, however, can be considered as an amorphous region and the amorphous region can be easily accessible, solubilized and hydrolyzed than high crystalline region by agent. From the Figure 2, the removing of amorphous region has relation to enzymatic hydrolysis. As a supplementary method, relative CrI (crystallinity index) can be used for the purpose of the measure the ratio of non-crystalline part. It is reported by Burns et al. that crystallinity is accessible surface area. Nature cellulose is considered as a structure protected and linked with hemicellulose and lignin. The elimination of lignin and hemicellulose, therefore, can improve enzymatic reaction by physically and chemically. The higher crystallinity index indicates the higher crystalline structure in the sample. Most of cellulose exists in a crystalline structure in the plant. In the more pure cellulose samples, enzyme can easily access to the cellulose lattice and catalyze it. The decrease of amorphous portion (i.e. increase of CrI) explains the increase of enzymatic digestibility. Furthermore, fast hydrolysis rate (over 80% of digestibility at 10FPU/g glucan) was obtained by ARP treatment.

Lignin vs. hemicellulose

The dilute acid (0.07wt% of sulfuric acid) treatment was used for hemicellulose removal. The ARP treated samples have low lignin and high xylan content (Lignin-free sample). On the contrary, the dilute acid treated samples have high lignin and low xylan content (Xylan-free sample). Enzymatic digestibilities of ARP sample 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 are 89.9% with 60FPU/g glucan loading and 82.8% with 10FPU/g glucan loading. Lignin content in the biomass appears to be one of the critical factors affecting the enzymatic digestibility.

The hydrolysis rate of ARP treated sample are so fast that the yield of 60FPU loading reached to 97% in 6 hour and the yield of 10FPU loading reached to >90% in 24hr. That of acid treated sample, however, are relatively fast (75% in 6 hour for 60FPU loading) but maximum yield was stopped at 89.9% (60FPU) and 82.8% (10FPU). There is little difference in maximum digestibilities for two treated samples when enzyme loadings above 60 FPU are applied. However, the difference of hydrolysis rate became very significant. It is seen that the lignin play a roll as a bigger resistance of enzyme reactivity in the lignocellulosic structure than that of hemicellulose.

SEM and lignin staining

The lignin staining method was used to make out lignin quantity and distribution in the substrate tissue then the SEM was performed to study of visible change at the surface of substrate. There is significant change between untreated and treated samples in the fiber bundle. The untreated sample has a very compact and highly ordered fibrils but the fiber of ARP 90min treated sample is very distorted and altered. A collapse of the original structure at the end of reaction. Even for ARP 10min sample, lots of opening and cleavage of fiber was observed. ARP treatment results not only delignification but also modify bundle and its fibril.

The pictures of lignin distribution tell how much quantity of lignin exists and how lignin is distributed in the samples by institution. Photos of 7-a, b, and c are original color picture. Lignin stained a bright red color, which darkened to black with increasing quantities. For the gray scale picture, original pictures are filtered and selected by red color only by Adobe photoshop program. In the second row, only the red color channel was express as gray scale picture. The darken area is more lignin and whiten area is less lignin. It proved that lignin was removed as reaction time increased.

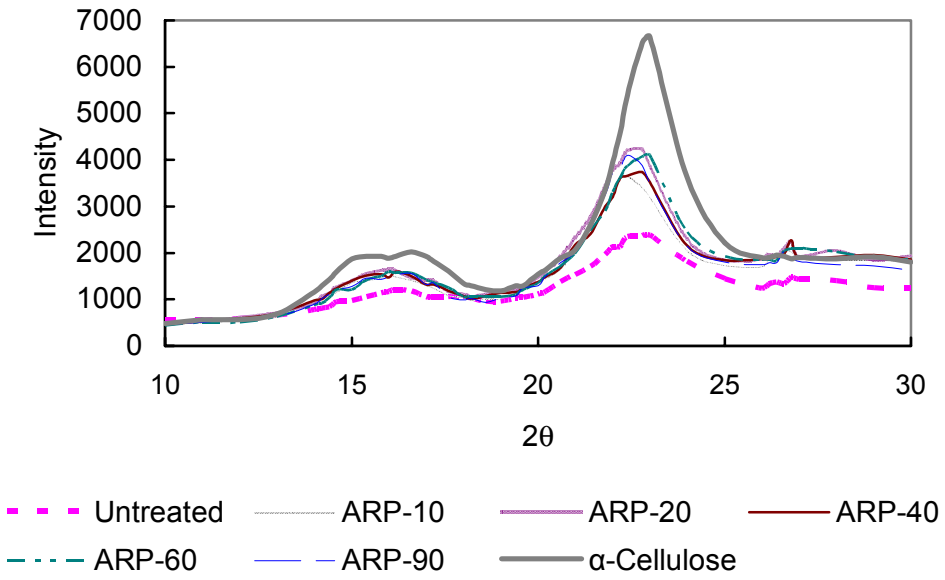

Figure. XRD diagram of ARP treated samples

(b) 10FPU/g glucan

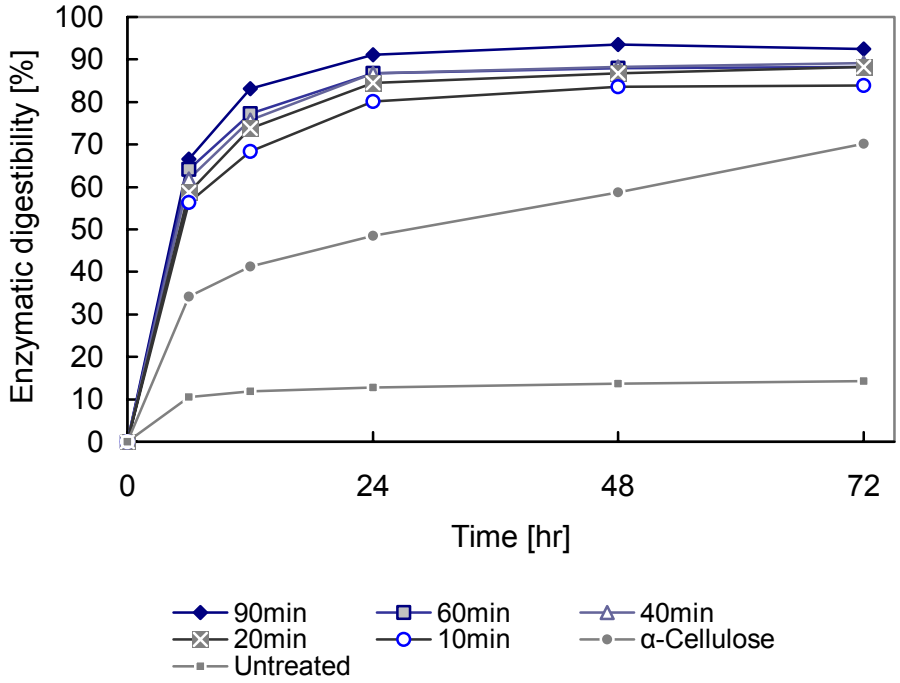

Figure. Enzymatic digestibility of ARP treated samples