암모니아 전처리를 한 폐참나무의 에탄올 생산을 위한 동시당화발효 공정

김남이, 김현준, 김준석*
경기대학교 화학공학과
(jskim84@kyonggi.ac.kr*)

Simultaneous Saccharification and Fermentation from Ammonia Pretreated Waste Oak Wood for Fuel Ethanol Production

Namyee Kim, Hyunjoon Kim and Junseok Kim*
Department of Chemical Engineering, Kyonggi University
(jskim@kyonggi.ac.kr*)

Introduction

Bioprocesses for converting lignocellulose to useful materials such as liquid fuels and chemicals have been receiving increasing attention. Pretreatment is an essential element in the bioconversion of lignocellulosic substrates. Oak wood is one of the most promising renewable feedstocks for biological conversion to fuels and chemicals. Waste oak wood have advantages in removing lignin better than oak wood. In our previous works, we used ammonia recycled percolation (ARP) process as a pretreatment method for agriculture residue. Aqueous ammonia is efficient to fraction carbohydrates to delignify in hard woods. Futher more, ammonia could be easily recovered and reused due to it's volatile nature. Ammonia recycled percolation (ARP) process are efficient in removing lignin at the early phase of the conversion process before it is subjected to the biological processing. Waste oak wood have composed various components like glucan, xylan, lignin, arabinan, mannan, etc. Lignin and its derivatives are toxic to microorganism and inhibitory to the enzymatic hydrolysis. Accordingly, lignin is considered as major interruptive factors in the enzymatic reaction. But ammonia is well known chemical for cleavage of C-O-C bonding in lignin as well as ether and ester bonding in LCC. And, it is very effective swelling agent. Also, ammonia can resolve the prnetration problem because of its small molecular size. In the long run, the pretreatment is to contribute for improving enzymatic hydrolysis followed by fermentation at the end step. The main goal of pretreatment exists in increasing the efficiency of hydrolysis by make structure of biomass alterative. The hindering role of lignin in enzymatic hydrolysis plays physically as well as chemically limiting of enzyme accessibility. While removing of lignin, the approchability of enzyme is likely to bo increased. Lignin remaval is very effective pretreatment method to enhance an enzymatic hydrolysis.

Materials and Methods

Materials

In this study, oak wood and waste oak wood, which was obtained from KIER, Korea, were used as biomess. Also, celluclast and Novozyme 188 were kindly supplied by Novo, Denmark.

Experimental setup and conditions of operated ARP

A schematic diagram of ARP system for ammonia pretreatment is shown in Fig.1. The system consists of a stock solution reservoir, temperature-programmable circulation drying oven, pump, reactor (dimension of 28.93 mm id x 190 mm L, volume; 124.9 cm3), and liquid holding tank, which also served as a back-pressure vessel pressurized by nitrogen cylinder at 200-300 psig, preventing evaporation of ammonia. In this experiment, 30g of biomass sample wee packed into the reactor, soaked with ammonia solution. From 18 to 20 min of preheating time is necessary to reach at the desired temperature.

Enzymatic digestibility test

Enzymatic hydrolysis of pretreated substrate was performed at pH 4.8 (0.05M sodium citrate buffer) and 50℃ on a shaking incubator agitated at 150 rpm with enzyme loadings of 60-10 FPU/g glucan. The initial glucan concentration was 1%(w/v) based on 250ml of total volme. Erlenmeyer flasks containing the samples were placed in a shaking incubator (K.M.C-8480SF, Vision Scientific). B-glucosidase was supplemented as much as ~35 IU/g glucan (Sigma, Cat No. G-0395,) with an activity of 12.5 IU/g. Total glucose content after 96 hr of hydrolysis was taken to calculate the enzymatic digestibility. Untreated oak wood and a-cellulose were subjected to the same digestibility test as a control and as a reference.

Analytical Procedures

To analyze the moisture we used the convection oven method or automatic infrared moisture analyzer (Sung Jin I&T company, FT/IR-430). The solid samples were amalyzed for sugar, K.lignin, and acid-soluble lignin following the procedures of National Renewable Energy Lab Chemical Alalysis and Testing Standard Procedures. Sugar were determined by HPLC (Hewlett Packard, Model No.1090) using a Bio-Rad Aminex7 HPX-87C column and Aminex7 HPX-87P column.

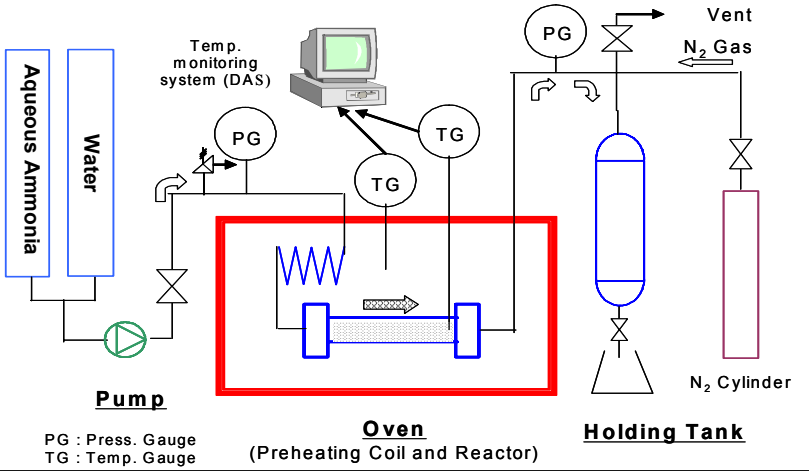

Fig 1. schematic diagram of ARP system

Results And Discussion

The enzymatic digestibility was increased in both enzyme loadings (15 and 60 FPU/g glucan), as treatment time increased. The enzymatic digestibility is shown in Fig 2. Also, lignin and xylan removal were increased. In this experimental, we have got to know that the decrease of lignin and hemicellulose content in solid be directly related to enzymatic hydrolysis. At any pretreatment conditions, there was a drastic increasing in terms of enzymatic hydrolysis as compared with that of untreated substrate. Unfortunately the ammonia recycled percolation treatment have a damage which loss the big quantity of xylan.

The elimination of lignin and hemicellulose can improve enzymatic reaction by physically and chemically. Fast hydrolysis rate (over 80 % of digestibility at 15FPU/g glucan) was obtained by ammonia recycled percolation treatment. The ammonia recycled percolation treated samples have low lignin and high xylan content. Enzymatic digestibilities of dilute acid treated samples and ammonia recycled percolation treated sample are 89.9% with 60FPU/g glucan loading and 99.6% at 60FPU/g glucan loading, respectively. The hydrolysis rate of ammonia recycled percolation treated sample are so fast that the yield of 60FPU loading reached to 97% in 6 hour.

The difference of hydrolysis rate became very significant. The lignin play a roll as a bigger resistance of enzyme reactivity in the lignocellulosic structure than that of hemicellulose. Between untreated and treated samples is significant change in the fibre bundle. The untreated sample has a very compact and highly ordered fibrils. The ammonia recycled percolation treated sample during 60min is very distorted and altered. Ammonia recyled percolation treatment results not only delignification but also moigy bundle and its fibril.

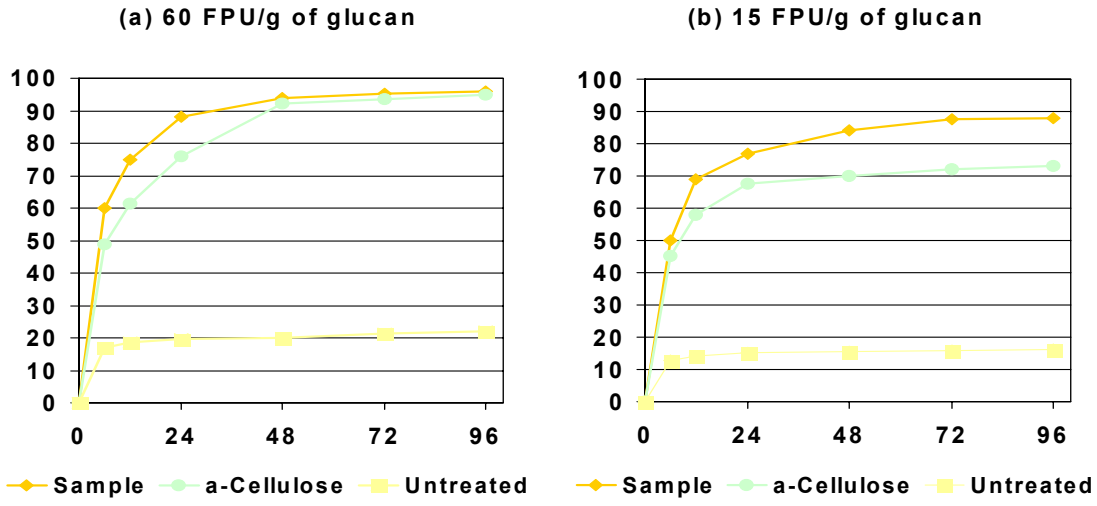

Fig 2. Enzymatic Digestibility of Solid Glucam in ARP-Treated Waste Oak Wood

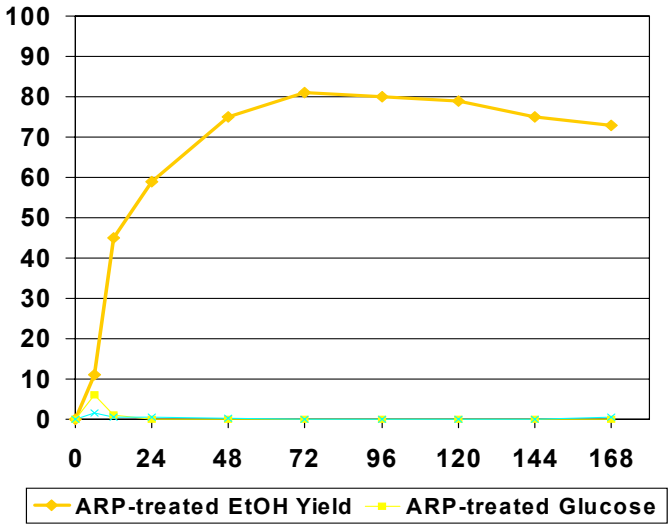

Fig 3. SSF of the ARP-treated Sample

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