Cellulose was dissolved in water under hydrothermal condition, and by cooling the solution, oversaturated cellulose aqueous solution was obtained. Hydrothermal treatment was conducted at 300-400°C and 25 MPa. Product of hydrothermal pretreatment process was further treated by 2 step homogeneous enzymatic hydrolysis, using cellobiosidase and β-glucosidase. Glucose yield was 0.24, obtained after enzymatic hydrolysis.

Bioethanol from lignocellulosics is expected to be a renewable fuel which does not compete with food production. Hydrothermal pretreatment, hydrolysis and fermentation are used for this purpose when hazardous chemicals are prohibited. Since cellulose is rigid crystal to which water and enzyme cannot easily access, the slow rate of hydrolysis is a problem. To solve this problem, cellulose dissolution is one possibility. Cellulose has low solubility in water and this technology has not been developed yet, but water under hydrothermal condition can dissolve cellulose, and this dissolved cellulose can be hydrolyzed by enzyme quickly\textsuperscript{1,2}. However, fundamental characteristics for this technology have not been elucidated. Thus, the purpose of this study is to determine the reaction characteristics of dissolved cellulose.

1. Introduction

Bioethanol from lignocellulosics is expected to be a renewable fuel which does not compete with food production. Hydrothermal pretreatment, hydrolysis and fermentation are used for this purpose when hazardous chemicals are prohibited. Since cellulose is rigid crystal to which water and enzyme cannot easily access, the slow rate of hydrolysis is a problem. To solve this problem, cellulose dissolution is one possibility. Cellulose has low solubility in water and this technology has not been developed yet, but water under hydrothermal condition can dissolve cellulose, and this dissolved cellulose can be hydrolyzed by enzyme quickly\textsuperscript{1,2}. However, fundamental characteristics for this technology have not been elucidated. Thus, the purpose of this study is to determine the reaction characteristics of dissolved cellulose.

2. Experimental

Fig. 1 shows the experimental apparatus for hydrothermal pretreatment. The reactor is made of SS316 steel tubing (ID: 1 mm, OD: 1.59 mm, length: 27 m). Cellulose slurry is fed to the reactor under hydrothermal conditions, and the effluent was rapidly cooled to obtain oversaturated cellulose solution.

Cellulose slurry was produced from cellulose powder (particle size 20 µm) purchased from Sigma Aldrich. Then, hydrothermally pretreated liquid product was analyzed by total organic carbon (TOC) analyzer and high performance liquid chromatography (HPLC) for carbon and sugar content. Hydrothermal pretreatment was conducted at temperature and pressure of 300, 350, 400 °C, and 25 MPa, respectively. The flow rate was 40, 60, and 100 cm\textsuperscript{3}/min. Cellulose concentration of 0.1, 0.5, and 1wt% were employed. Enzymatic hydrolysis was conducted in 2 steps which used hydrothermal pretreatment product as feedstock. Cellobiosidase and β-glucosidase were applied, respectively. Both enzymes were granted from Novozenes Co., Ltd., Denmark. All enzymatic hydrolysis steps were conducted at 50°C, pH 5.0, incubator speed of 250 rpm. Acetic acid and sodium hydroxide buffer fluid was used. The samples were collected after 0, 2, 4, 8, 12, 24 h after starting each enzymatic hydrolysis step.

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3. Results and discussions

3.1 Hydrothermal pretreatment

Fig. 2 shows the TOC of the hydrothermal pretreatment product. The obtained solubility of organic compound was 400mg/L. Higher temperature resulted in higher solubility. It was constant for 4 d, indicating the solution is rather stable. Fig. 3 shows the effect of flow rate on carbon yield in liquid product of hydrothermal pretreatment. From graph shows that flow rate does not affect solubility yield, maximum cellulose yield of 0.95 was obtained.

3.2 Enzymatic hydrolysis

Change of glucose concentration during the enzymatic hydrolysis is shown in Fig. 4. During the first step of cellobiosidase treatment, glucose concentration does not change, but sudden increase of glucose concentration is observed in the second step of β-glucosidase treatment. The reaction is completed in as short time as 4 h. Since β-glucosidase is an enzyme to hydrolyse cellobiose into glucose, cellobiose should have been produced before the second step. Glucose yield after enzymatic hydrolysis was 0.24, which corresponds to 0.0015 mol/L concentration.

4. Conclusion

Hydrothermal pretreatment of cellulose to obtain dissolved cellulose followed by enzymatic hydrolysis was conducted, expecting quick hydrolysis. Cellobiosidase and β-glucosidase was applied. Glucose yield of 0.24 was obtained in 4 h after β-glucosidase was added.

References