Biomass Pretreatment with Hydrothermal Explosion for Bioethanol Production

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For highly effective pretreatment of biomass for the production of ethanol, a hydrothermal explosion system was developed. Eucalyptus was selected as a sample hardwood biomass. Temperature and holding time dependence were studied to determine optimum conditions. Hydrothermal explosion and hydrothermal slow cooling were compared to confirm the explosion effect. Hydrothermal explosion showed higher sugar yield than the hydrothermal slow-cooling process, and sugar yield was 70% under optimized conditions. Dependence on enzyme concentration was also examined.

Key Words
Hydrothermal explosion, Pretreatment for ethanol production, Lignocellulosic biomass

1. Introduction

For bioethanol production from lignocellulosic biomass, it is important to develop highly effective pretreatment processes because lignocellulosic biomass has rigid structure compared to food biomass and requires pretreatment before the saccharization process. Many types of pretreatment technologies exist, such as milling, steam explosion, ammonia fiber explosion (AFEX), CO₂ explosion, ozonolysis, acid hydrolysis, alkaline hydrolysis, and oxidative delignification 1). Pretreatment must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis, (2) avoid the degradation or loss of carbohydrate, (3) avoid the formation of by-products inhibitory to the subsequent hydrolysis and fermentation processes, and (4) be cost-effective.

There are many reports about hydrothermal processes regarding biomass. According to hydrothermal experiments using cellobiose and glucose 3 - 6 or xylan and xylose 7 - 9 which are basic building blocks of biomass, it was found that glucose was degraded at over 200 °C, xylose was degraded at 180 °C, and the products were triose, tetrose, or acid. Concerning lignin, Ando et al. 7) reported that it started to decompose at around 180 °C. Regarding hydrothermal treatment of lignocellulosic biomass, Laser et al. 8) compared the pretreatment effects of liquid hot water and steam treatment of sugar cane bagasse and concluded that liquid hot water generated higher sugar yield.

We developed a hydrothermal explosion system for the pretreatment of biomass (Fig. 1) that enables both hydrothermal treatment and mechanical decomposition of the biomass. In the hydrothermal explosion, biomass was prepared via rough grinding into cm-order size pieces and then added to a high-pressure column. Preheated water was fed into the column and maintained for the optimized time, then exploded with a quick valve opening. Hydrothermal explosion has the following merits for pretreatment: it does not require fine grinding; it saves energy, compared to steam explosion; it contains 30-fold larger expansion energy than steam explosion; it is more effective for hydrolysis with enzymes, it enables treatment...
of high biomass concentrations; it decomposes lignin structure; and it decomposes cellulose crystalline structure.

In this study, we examined sugar yield and fermentation inhibitor yield of the hydrothermal explosion technology as a pretreatment for biomass. Temperature and holding time dependence were investigated to determine optimum conditions. Hydrothermal explosion and hydrothermal slow cooling were compared to confirm the explosion effect. Dependence on enzyme amount was also examined.

2. Experimental Procedures

2.1 Materials

As an experimental biomass sample, eucalyptus was cut to 5-10 mm (Fig. 2). The chemical composition of the eucalyptus (dry base) is summarized in Table 1, which was analyzed by TORAY TECHNO CO., LTD, showing a water content of 8 wt%. For saccharification, Cellic Ctec2 and Htec2 were provided by Nobozym Japan.

2.2 Hydrothermal explosion and hydrothermal slow cooling

Fig. 3 shows the experimental apparatus used for the hydrothermal explosion. The hydrothermal explosion apparatus consists of a preheating coil, a reactor (23 cc), a back-pressure regulator, a cooling coil, and a ball valve. The measuring procedure is shown below. At first, a biomass sample (1.5 g) was loaded into the reactor. Then, preheated water was fed into the reactor. The reactor pressure was controlled by a back-pressure regulator and was set over vapor pressure at a given temperature. The temperature profile is shown in Fig. 4. The reactor was maintained at a given temperature and holding time, and then the ball valve, which was at the bottom of the reactor, was opened at once. For hydrothermal slow cooling, the reactor heater was stopped after the holding time and a sample was collected at ambient temperature without explosion. Temperature was from 180 to 260 °C, and holding time was from 10 to 120 min.

2.3 Enzymatic saccharization

After hydrothermal pretreatment of the biomass, eluent and explosion samples were mixed and water was added for 500 ml total. The mixed sample was saccharized with Cellic Ctec2 (135 μl) and Htec2 (15 μl) at 50 °C in a shaking apparatus for 48 hours with 0.23 ml acetic acid and 0.77 g sodium acetate trihydrate for a pH buffer solution. The enzyme amount was varied to study the effect of enzyme amount on sugar yield.

2.4 Analysis

The samples before and after saccharization were analyzed for sugar yield and fermentation inhibitors, such as phenol derivatives or acids. Sugar yield was analyzed via HPLC with post-column fluorescence derivatization (column: Asahipak NH2P-50 4E 250 × 4.6 mm LD; eluent: acetonitrile/water/phosphoric acid (gradient); reaction solvent: arginine/boric acid; column oven: 40 °C; reaction temperature: 150 °C). In this system, several types of sugars from monosaccharaides to oligomers showed separate, sharp peaks. Phenol derivatives and furfural were analyzed using HPLC-UV (column: ODS Hypersil 250 × 4.6 mm; oven temperature: 30 °C, eluent: water/methanol/tetrabutylammonium hydrogensulfate = 714/245/0.6 weight base; DET: UV 280 nm), and organic acids were analyzed via HPLC-electric conductivity (column: Shodex IC NI-424).

Sugar yield and fermentation inhibitor yield were
3. Results and Discussion

3.1 Temperature and holding time effect

Fig. 5 shows the temperature dependence of sugar yield and fermentation inhibitor yield of hydrothermal explosion. Before saccharization, only xylose was found at 180-220 °C, though glucose was detected at 240 °C. After saccharization, sugar yield apparently increased, compared to before, and the maximum value of sugar yield was around 200 °C. Furfural or organic acids, which were decomposed material from sugar, increased as temperature increased and they had high concentrations, especially at 240 °C. Lignin decomposition materials, such as phenol derivatives (guaiacol, syringaldehyde, vanillin, and p-HBA), showed little temperature dependence. According to these results, hemicellulose started to decompose at 180 °C and cellulose started to decompose at 240 °C, but glucose also decomposed at 240 °C, which almost corresponds with previous reports. Fig. 6 shows the holding time effect on sugar yield and fermentation inhibitor yield for hydrothermal explosion at 200 °C. Sugar yield after saccharization increased, according to holding time increasing, and almost all the sugar was collected at 90 min. Fermentation inhibitor yield showed little dependence on holding time. Table 2 presents the sugar yield relation to temperature and holding time. Maximum sugar yield can be achieved at lower temperature, with long holding time. Fig. 7 shows the analysis result of the explosion materials.

![Diagram](image1)

**Fig. 3** Hydrothermal explosion apparatus

**Fig. 4** Temperature trend of hydrothermal treatment

![Diagram](image2)

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Fig. 5 Hydrothermal explosion results for temperature dependence (holding time: 30 min; enzyme amount 90 mg/g biomass)

Fig. 6 Hydrothermal explosion results for holding time dependence (reactor temp.: 200 °C; enzyme amount 90 mg/g biomass)
and eluent separately. Most of the glucose was found in the explosion materials, and xylose and lignin derivatives were in the eluent.

Romani et al.\textsuperscript{10,11} reported on the steam explosion of eucalyptus, and scanning electronic microscopy (SEM) images of the treated biomass showed that the structure was completely open at 210 °C and 30 min, though the fibrillar structure was not completely disrupted at 180 °C and 30 min. In our results, the exploded material at 200 °C and 90 min was slurry-like and did not include the wood portion, whereas with shorter holding time or cooler conditions, a small portion of wood remained in the exploded material. Castro et al.\textsuperscript{12} reported an optimum condition of steam explosion with dilute acid, with maximum sugar yield at 200 °C, 0.75% acid soak, and 15 min holding time, thus, small additive may improve the holding time.

3.2 Comparison between hydrothermal explosion and hydrothermal slow cooling

Fig. 8 shows the comparison data between hydrothermal explosion and hydrothermal slow cooling.

Each condition was carried out three times to confirm reproducibility. The sugar yield of the hydrothermal explosion was around 70%, whereas that of hydrothermal slow cooling was 40%. This difference shows that the explosion had an effect on the lignocellulose decomposition. With the explosion, biomass changes to small portion which may be easy to be attacked from enzyme. Fujimoto et al.\textsuperscript{13} reported that glucose yield was 77% and xylose yield 21% at 200 °C and 30 min of hydrothermal pretreatment with enzyme saccharization and also reported that hydrothermal with mechanochemical pretreatment improved the glucose yield to 77% and xylose yield to 54% at 160 °C and 30 min of hydrothermal pretreatment plus 40 min of ball milling pretreatment. In our study, the explosion process may have played a role as mechanochemical treatment, which takes a little time.

3.3 Enzyme amount effect

Fig. 9 shows the effect of enzyme amount on saccharization at optimized conditions (200 °C, 90 min). Sugar yield decreased with decreasing enzyme amount.

| Table 2 Sugar yield [wt%] relation to temperature and holding time |
|--------------------------|----------------|----------------|----------------|----------------|----------------|
| Holding time [min]       | 10  | 30  | 60  | 90  | 120 |
| Temperature [°C]         | 280 | 16.0|     |     |     |
|                         | 260 | 16.3|     |     |     |
|                         | 240 | 46.1| 53.0| 48.8| 41.4| 38.7|
|                         | 220 | 45.1| 53.9| 58.7| 60.3| 59.9|
|                         | 200 | 22.8| 55.8| 58.6| 69.8| 68.0|
|                         | 180 |     |     |     | 194 |     |
Nonaka et al.\textsuperscript{10} reported the adsorption of enzymes on lignin, and our results might be related to this enzyme adsorption. In the “Innovative Bioethanol Technology Research Union,” which was founded by several Japanese corporations to develop production technology to supply 40 yen/liter bioethanol, the target amount of enzyme is 1 mg/g glucose. Pretreatment technologies for biomass and enzyme ability need further development to achieve this target amount.

4. Required Energy for Hydrothermal Explosion Process

Fig. 10 shows the heat balance of the hydrothermal explosion process. In this process, water mass was determined using the bulk density of biomass, and water eluent was not considered. The required energy for the explosion was the enthalpy for heating water, and the results show around 11% compared to the Higher Heating

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Fig. 8 Comparison of pretreatment methods of eucalyptus with hydrothermal explosion (200 °C and 90 min) and hydrothermal slow cooling (200 °C and 90 min) (90 mg enzyme/g biomass)

Fig. 9 Effect of enzyme amount on saccharization (hydrothermal explosion at 200 °C and 90 min)

Fig. 10 Heat balance of the hydrothermal explosion process
Value (HHV) of the biomass

\[ Q_1 = (H(220°C, 3\text{ MPa}) - H(80°C, 3\text{ MPa})) \times 3.66 \text{ kg} = 2.23 \text{ MJ/kg}_\text{biomass} \]  

\[ \text{Energy ratio} = \frac{Q_1}{\text{HHV of Biomass}} = \frac{2.23 \text{ MJ/kg}_\text{biomass}}{20 \text{ MJ/kg}_\text{biomass}} = 0.11 \]  

5. Conclusion

Hydrothermal explosion of eucalyptus was examined at temperatures from 180 to 260°C and holding times from 10 to 120 min. At 200°C and 90 min, the sugar yield of the hydrothermal explosion was 70% and larger than that of hydrothermal slow cooling (40%). Most of the glucose was in the explosion materials, and xylose and lignin derivatives were in the eluent. The required energy for hydrothermal explosion was around 11%, compared to the HHV of the biomass.

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