Hot-press Pretreatment of Rice Straw for Enzymatic Hydrolysis and Volume Reduction

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(Received February 13, 2015)

This study investigated the effects of hot-press pretreatment on sugar recovery from rice straw. Hot-pressed rice straw was evaluated using enzymatic hydrolysis. After hot-press pretreatment with 30 % moisture at 180 °C, 16 MPa, the glucose yield of hot-pressed rice straw was 73 % and the density of it was 1.3 g-wet/cm³. In addition, the sugar recovery increased to 92 % when the wet milling process was added before hot-pressing. Rice straw after the hot-press pretreatment can be stored for more than six months, without changing the sugar recovery rate. These results indicate that the hot-press pretreatment eliminates the need for drying the raw materials, improves the storability, reduces the volume of the rice straw, and has pretreatment effect for enzymatic hydrolysis.

Key Words
Rice straw, Hot-press pretreatment, Volume reduction, Storability, Enzymatic hydrolysis

1. Introduction
Lignocellulosic biomass such as agricultural, hard wood, and softwood residues are attracting global attention as a potential solution for energy and environmental problems. They are abundant, renewable, and environmentally friendly. Moreover, they do not compete against food and feed resources. Lignocellulose can be converted into biofuels as an efficient alternative for transportation fuels. In Japan, rice straw is abundant and is the most attractive lignocellulosic biomass. However, rice straw in Japan has some problems regarding its efficient use. Firstly, rice straw contains 30-60 % moisture during the harvest season, while, less than 15 % moisture content is needed to prevent its decay. However, it is difficult to use energy for drying; therefore, drying must depend on the sun. If drying in the field is insufficient due to bad weather at harvest time, a significant amount of rice straw decomposes and is not available for energy production. In fact, rice straw decayed and could not be gathered due to bad weather after harvesting at the ethanol-production demonstration project in the Sea of Japan side that target rice straw. Secondly, the volume is a limiting factor because of its large bulk, which leads to inefficient transportation. Machine-baled rice straw has a density of only 0.15 g/cm³. That low density is a factor, which pushes up the raw material cost of rice straw.

Another problem is related to ethanol conversion. Rice straw consists of three main components including cellulose, hemicellulose, and lignin. The complexity of these components leads to the stability of its biomass and results in the difficulty of its enzymatic disassembly. Therefore, adequate pretreatment is necessary for enzymatic hydrolysis. Hydrothermal pretreatment is generally known as an enzymatic hydrolysis pretreatment of lignocellulosic biomass. The objective of the hydrothermal pretreatment is
to solubilize the hemicellulose and lignin partially, to make
the cellulose more accessible, and to enhance the enzymatic
hydrolysis of pretreated materials. Hideno et al. performed
hydrothermal pretreatment for 30 g rice straw and 300 mL water at 180 °C for 30 min. They reported
that the glucose yield after enzymatic hydrolysis was 85 %.
Hydrothermal pretreatment is generally performed at
more than 90 % moisture content, but the amount of water
required for the hydrothermal reaction is extremely small
in theory. Even at low moisture contents, if there is enough
water around the substrate by high-pressure pressing
to biomass itself, the treatment should be effective as a
pretreatment for enzymatic hydrolysis.

If the treatment process that can eliminate the need
for drying of raw materials, improve the storability, as
well as reduce the volume of rice straw has pretreatment
effect for enzymatic hydrolysis at the same time, it is very
significant from the point of view of practical application.
Therefore, this study investigated the effect of the hot-
press pretreatment condition on the sugar recovery of rice
straw. In addition, the effect of the volume reduction of
rice straw by hot-press pretreatment was investigated. In
addition, hydrothermal pretreatment for rice straw was also
performed, and the effect on sugar recovery was compared
with the effect of hot-press pretreatment.

2. Methods

2.1 Materials

Koshihikari rice straw was obtained in Minami-Boso
city, Chiba, Japan, in 2010. Rice straw was crushed using
a cutter mill equipped with a 3 mm screen. The glucan
content of the rice straw was analyzed by the method
recommended by National Renewable Energy Laboratory
(NREL). Briefly, 0.3 mL of 72 % sulfuric acid (H₂SO₄) was
added to approximately 30 mg of dried rice straw and the
mixture was incubated at 30 °C for 1 h. Next, the mixture
was hydrolyzed with 8.4 mL of water at 121 °C for 1 h. The
glucose concentration in the hydrolysate was measured by
the mutarotase-GOD method using a Glucose CII-test kit
(Wako Pure Chemical Industries, Ltd., Japan). The glucan
content in the rice straw was 34 %. The moisture content
was measured using a Moisture Analyzer MX-50 (A&D Co.,
Ltd., Tokyo, Japan).

2.2 Hot-press pretreatment

Hot-press pretreatment was carried out using a
reaction mold (φ 50 mm, Imoto Machinery Co.) and hot-
pressing apparatus (IMC-1A3F, Imoto Machinery Co.) in
Fig. 1. The clearance between the piston and the cylinder
of the reaction mold was around 0.5 mm. Before hot-press
pretreatment, pure water was added to the rice straw to
obtain a 30 % moisture content. This was assumed the
moisture content by natural drying in unfavorable weather.
Reaction diagram of hot-press pretreatment is shown in
Fig. 2. Fifty grams of the wet rice straw was stuffed
in the reaction mold and the mold was set in the hot-
pressing apparatus. The samples were pressed at the stated
pressure, and then heated under the pressure from room
temperature by two heating plates set on the top and the
bottom of the mold and a heating jacket surrounding the
mold. The reaction conditions are shown in Table 1 and the
holding time was 15 min. Since the pressure decreased as
the temperature rose, the pressure was kept by controlling
the hydraulic jack. The glucan content in each sample was
analyzed by the same method as described in section 2.1.

Fig. 1 (a) Reaction mold (φ 50 mm, Imoto Machinery Co.), (b) Hot-pressing apparatus (IMC-1A3F, Imoto Machinery Co.)
2.3 Hydrothermal pretreatment

Hydrothermal pretreatment was performed using the thermal pretreatment apparatus. The air in the vessel was replaced by nitrogen gas after 30 g rice straw and 300 mL water were placed in the vessel. The vessel was then heated to 180 °C with a holding time of 15 min. The sample was mechanically stirred. Moreover, the reaction temperature was measured directly using a thermocouple inserted into the vessel. After the reaction, the rice straw was removed for enzymatic hydrolysis.

2.4 Enzymatic hydrolysis

Hot-pressed rice straw samples were cut to less than 1 mm using a milling machine (IFM-800DG, Iwatani Co.). Enzymatic hydrolysis was performed using Celluclast 1.5 L cellulase (Sigma-Aldrich Japan K.K., Chiba, Japan), Novozyyme 188 β-glucosidase (Sigma-Aldrich Japan K.K.), and Optimash BG hemicellulase (Danisco Japan Ltd., Tokyo, Japan). The sample containing 100 mg of glucan, 8.3 mL of water, and 1.0 mL of 0.5 M acetate buffer (pH 5.0) was mixed in 25 mL bottle. Then 0.6 mL of enzyme cocktail (Celluclast, 20 mg protein/g-glucan; Novozyyme 188, 20 mg protein/g-glucan; Optimash BG, 200 μL/g-glucan), 0.1 mL of antibiotic solution containing tetracycline (60 μg/mL, final concentration) and cyclohexamide (80 μg/mL, final concentration) were added to the sample and the reaction mixture was incubated at 50 °C for 72 h. The amount of each sample was calculated based on its glucan content. The glucose yield was determined by high performance liquid chromatography (HPLC) using a Shimadzu LC-20 system (Shimadzu Co., Ltd., Tokyo, Japan) equipped with a sugar SP0810 column (Showa Denko K.K., Tokyo, Japan) and a refractive index detector RID-10A (Shimadzu). The mobile phase was water (H2O) applied at a flow rate of 1.0 mL/min, and the column temperature was set at 80 °C. Sugar recovery was calculated by dividing the glucose yield of each hot-pressed substrate divided by its glucose content.

2.5 Density of the hot-pressed rice straw sample

The density of the rice straw sample pressed at 180 °C under the pressure of 16 MPa was measured. The weight and height of the rice straw sample was measured after hot-press pretreatment. The volume was calculated by multiplying the area of each sample by its height and the density was calculated by dividing the weight of the sample by its volume.

3. Results and discussion

3.1 Hot-press pretreatment

Table 2 shows the moisture content and glucan content in the hot-pressed rice straw samples. The clearance between the piston and the cylinder of the reaction mold was sealed with black substance in the case that the moisture content was kept over 20 %, while the clearance...
was not sealed in the case that the water in the sample was lost. Hemicellulose component such as xylan was solubilized by hot compressed water treatment above 160 °C. So the black substance could be considered to be composed of solubilized hemicellulose in the rice straw sample. Under the reaction pressure of 1.6 MPa the solubilized hemicelluloses did not move sufficiently to seal the clearance before the water in the sample was lost. On the other hand, the heating rate was not the same among the different temperature condition in this study. Heating rate was higher in higher reaction temperature. Thus the water in the sample was lost at the reaction temperature of 200 °C and 220 °C before the clearance was sealed with solubilized hemicellulose. The glucan content in the sample increased as the reaction pressure or the reaction temperature rose. Severe reaction condition brought about more solubilization of hemicellulose, resulting in higher glucan content. This is similar to the results of hot compressed water treatment.

### 3.2 Enzymatic hydrolysis

Fig. 3 shows the relative sugar recovery of rice straw pretreated at different pressures. The reaction temperature was fixed at 180 °C. In the case that moisture content was kept over 20% after hot-press pretreatment, sugar recovery improved. The pretreatment effect at 1.6 MPa could not be investigated due to lack of water in the rice straw sample. Higher sugar recovery was obtained at higher pressure. Therefore, the sugar recovery of low water content rice straw could be improved by hot-press pretreatment at high pressure. Namely, a hydrothermal reaction could occur at high pressure even if there were low amounts of water throughout the pretreatment process.

Fig. 4 shows the relative sugar recovery of rice straw pretreated at different temperatures. The reaction pressure was fixed at 16 MPa. The maximum sugar recovery was 73% and was obtained at 180 °C. At temperatures higher than 200 °C, the pretreatment effect could not be investigated due to lack of water in the rice straw samples. Therefore, a certain amount of water was considered significant to improve sugar recovery of rice straw through hot-press pretreatment. In the case that the water in the rice straw sample was lost during hot-press pretreatment, the sugar recovery was very low compared to those of hot compressed water treatment at the same temperature. Hydrothermal reaction could not occur sufficiently due to lack of water in the rice straw sample during hot-press pretreatment. Generally drying inhibit the rate of enzymatic hydrolysis. Thus drying the rice straw samples during hot-press pretreatment could also cause the low sugar yield. Further research using sealed reaction mold to prevent water evaporation is needed to investigate the effect of hot-press pretreatment.

The sugar recovery of rice straw after hydrothermal pretreatment was 53%. Therefore, hot-press pretreatment was more effective as enzymatic hydrolysis pretreatment than hydrothermal pretreatment. However, Hideno et al. (2008) reported that sugar recovery of rice straw achieved 85% after hydrothermal pretreatment (180 °C, 30 min). This is thought to be due to the differences of substrate, enzyme loading, and holding time.

To obtain higher sugar recovery, the wet milling process was added before the hot-press pretreatment and the effect was investigated. Water was added to the rice straw to obtain a 60% moisture content, which was the assumed moisture content of raw rice straw immediately after harvesting. The 60% moisture content rice straw
was ground using an ultracentrifuge mill (ZM200, Retsch) at 6000 rpm. The particle size of rice straw after wet milling was less than 0.5 mm. The moisture content of wet-milled rice straw was around 55%. After wet milling, the rice straw was pretreated by hot-pressing at 180 °C and 16 MPa for 20 min. The enzyme loading was the same as the previous described conditions. A 92% sugar recovery was achieved after enzymatic hydrolysis.

This study defined sugar recovery as the ratio of the yield of glucose in enzymatic hydrolysate to the glucose content of each hot-pressed substrate. This is because xylose could not be recovered well by hot-press pretreatment at temperatures higher than 180 °C, which is the same problem, experienced in previous hydrothermal pretreatments. To solve this problem, more accurate temperature control or adding some catalysts is needed to be investigated.

3.3 Volume reduction

The density of the rice straw sample after hot-press pretreatment at 180 °C and 16 MPa was 1.3 g-wet/cm3. This density was significantly larger than machine-baled rice straw that is 0.15 g-wet/cm3. Therefore, hot-press pretreatment has possibilities to decrease the transportation cost of rice straw. Hot-press pretreatment also improved the storage stability of rice straw. Hot-pressed rice straw could be stored at room temperature for six months, and the sugar recovery could be kept for six months. That means hot-press pretreatment could contribute to the leveling of the operation rate of the biomass plant.

4. Conclusions

This study investigated the effects of the hot-press pretreatment condition on the sugar recovery of rice straw. It demonstrated that a sugar recovery of 73% was achieved for rice straw with a moisture content of 30% by hot-press pretreatment (180 °C, 16 MPa). That means the pretreatment effect for enzymatic hydrolysis could be obtained at high pressure even at low moisture content. By adding the wet milling process, a 92% sugar recovery was achieved. The density of the rice straw after hot-press pretreatment at 180 °C and 16 MPa was 1.3 g-wet/cm3. Rice straw after hot-press pretreatment could be stored more than six months without changing the sugar recovery rate. These results indicate that the hot-press pretreatment can eliminate the need for drying of raw materials, improve the storability, reduce the volume of the rice straw, and at the same time, has the pretreatment effect for enzymatic hydrolysis.

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