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Glucose content in the liquid hydrolysate after dilute acid pretreatment is affected by the starch content in rice straw

Hiroshi Teramura [a], Tomoko Oshima [a], Fumio Matsuda [b], Kengo Sasaki [c], Chiaki Ogino [a], Masanori Yamasaki [d], Akihiko Kondo [a,e,f,*]

[a] *Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University, 1-1 Rokkodaicho, Nada-ku, Kobe, Hyogo 657-8501, Japan*
[b] *Department of Bioinformatic Engineering, Graduate School of Information Science and Technology, Osaka University, 1-5 Yamadaoka, Suita, Osaka 565-0871, Japan*
[c] *Organization of Advanced Science and Technology, Kobe University, 1-1 Rokkodaicho, Nada-ku, Kobe, Hyogo 657-8501, Japan*
[d] *Food Resources Education and Research Center, Graduate School of Agricultural Science, Kobe University, 1348 Uzurano, Kasai, Hyogo 675-2103, Japan*
[e] *Biomass Engineering Program, RIKEN, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan*
[f] *Department of Food Bioscience and Technology, College of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Republic of Korea*

HIGHLIGHTS

- The biomass composition of 13 rice cultivars was analyzed.
- Starch content was correlated with glucose content in the liquid hydrolysate.
- Starch content was negatively correlated with acid-insoluble residue weight.
- Starch content in rice straw is a good indicator of the glucose distribution.
- Above relationship would be a universal characteristic among herbaceous biomass.

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ABSTRACT

Lignocellulosic biomass, such as rice straw, is often utilized as a bioresource after being hydrolyzed using dilute acid and separated into liquid hydrolysate and acid-insoluble residue. However, the biomass component that determines the distribution between liquid hydrolysate and acid-insoluble residue has not yet been clarified. In this study, the glucose content in the liquid hydrolysate and weight of acid-insoluble residue of 13 rice cultivars were analyzed. Starch content was positively correlated with glucose content in the liquid hydrolysate, and negatively correlated with acid-insoluble residue weight. These results indicate that the glucose in the liquid hydrolysate is mainly liberated from starch rather than cellulose in the rice straw. These observations suggest that starch content is a good indicator of the glucose distribution between the liquid hydrolysate and insoluble residue.

1. Introduction

Utilization of biofuels is required to curtail our dependence on fossil resources and to reduce net CO_2 emissions. Recently, starch-containing biomass, such as maize and barley grains, has been used in biofuel production. However, utilization of these biomasses has resulted in undesirable direct competition with global food production. Utilization of abundant inedible plant material such as lignocellulosic biomass could address this problem. In terms of total world production, rice (*Oryza sativa*) is an important grain crop, in addition to wheat and maize (Binod et al., 2010). Therefore, effective technologies to obtain fuels from rice straw are being developed.

Rice straw is mainly composed of cell wall components, such as cellulose, hemicellulose and lignin (Hou et al., 2012). In addition to these cell wall components, rice straw contains non-cell wall carbohydrates, such as starch (Arai–Sanoh et al., 2011). These polysaccharides need to be hydrolyzed to fermentable sugars in order to be utilized as a resource for microorganisms (Redding et al., 2011). However, cellulose and hemicellulose are densely packed by layers of lignin, and cellulose forms a highly crystalline structure (Mosier et al., 2005), which provide resistance to hydrolysis. On the other hand, the starch in rice straw has a relatively simplified structure and is not packed by layers of lignin (Park et al., 2011).

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* Corresponding author. Tel./fax: +81 8 803 6196.
E-mail address: akondo@kobe-u.ac.jp (A. Kondo).

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For effective biomass utilization, pretreatment is required to break down the biomass structure. Among the various pretreatment methods, dilute acid pretreatment has been widely studied because it is generally inexpensive, convenient and effective for a broad range of lignocellulosic biomass (herbaceous, softwood, and hardwood) (Lynd et al., 1999; Zheng et al., 2009). A slurry is obtained after dilute acid pretreatment, and can be separated into acid-insoluble residue and liquid hydrolysate (Supplementary Fig. S1). The acid-insoluble residue is mainly composed of cellulose and acid-insoluble lignin (Wei et al., 2012), while the liquid hydrolysate contains glucose, xylose, and a various byproducts (Redding et al., 2011). Different methods are utilized to obtain biofuels from the acid-insoluble residue (i.e., enzymatic saccharification and fermentation) and the liquid hydrolysate (i.e., neutralization and fermentation) (Hasunuma et al., 2011). Consequently, the sugar content of the liquid hydrolysate and the acid-insoluble residue weight are important factors in choosing the appropriate method for optimum biofuel production. It has been reported that the liquid hydrolysate glucose content showed large variation among rice cultivars (Matsuda et al., 2011). In addition, a previous study reported that the composition of polysaccharides in the biomass differed among rice cultivars (cellulose: 29.7%–41.1%, hemicellulose: 14.8%–21.7%, starch: 2.3%–21.6%) (Arai–Sanoh et al., 2011). However, it is unclear which biomass component determines the glucose distribution between liquid hydrolysate and insoluble residue.

The aim of this study was to investigate the relationship between biomass components, the sugar content of the liquid hydrolysate, and the insoluble residue weight, focusing specifically on forage rice cultivars. Forage rice cultivars are relatively recent developments in Japan (Sakai et al., 2003). Forage rice cultivars are promising candidates for higher production of the above ground biomass (e.g., rice straw). Focusing on this trait, the glucose distribution of the straw of 12 forage rice cultivars was analyzed, as well as Nipponbare, grown in 2011 and 2012. These results suggest that the starch content of rice straw has a clear relationship with glucose content in the liquid hydrolysate and insoluble residue weight.

2. Methods

2.1. Plant material

The 13 rice (*Oryza sativa* L.) cultivars were grown in 2011 and 2012, according to the same procedure, in the experimental field located at the Food Resources Education and Research Center, Kobe University (Kasai City, Hyogo Prefecture, Japan). Whole plants were harvested during 45 to 50 days after flowering, and were dried in the sun for 3 days. The grains were removed and the straw was powdered using a blender (WB-1; TGK, Hachioji, Japan) fitted with a 2 mm screen.

2.2. Sugar analysis

Equal amounts (1.5 μL) of sample and 0.1% w/w ribitol were added to tubes and dried using a vacuum concentrator (7810010; Labconco, Kansas City, MO). The resultant residue was dissolved in 100 μL of a mixture of 20 mg/mL of methoxyamine hydrochloride in pyridine and incubated at 30 °C for 90 min. After incubation, 50 μL of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was added to the sample solution and incubated at 37 °C for 30 min. Aliquots of sample solutions (10 μL) were subjected to gas chromatography–mass spectrometry (GCMS-2010 Plus; Shimadzu, Kyoto, Japan) under the following conditions: Column, Agilent CP-Sil 8CB-MS (30 m × 0.25 mm); carrier gas, helium; injection temperature, 230 °C; oven temperature, 80 °C at $t = 0$ to 2 min, then to 330 °C at 15 °C min^{-1} (Matsuda et al., 2011).

2.3. Dilute acid pretreatment

Dilute acid pretreatment was performed in a laboratory scale thermostirrer (100 ml total volume; HHE-19G-U; Koike Precision Instruments, Kawasaki, Japan) with an electric heater and magnetic agitation. Temperature and agitation speed during the pretreatment were determined according to a previous report (Matsuda et al., 2011). The powdered straw (6 g) was suspended in 80 ml of 1% v/v sulfuric acid solution, and samples were treated at 180 °C for 45 min with agitation at 200 rpm. After pretreatment, the mixture was separated into liquid hydrolysate and acid-insoluble residue by filtration using filter paper (No.1; ADVANTEC, Tokyo, Japan). The insoluble residue was washed with deionized water and neutralized to pH 7.0. After washing, the insoluble residue was dried and weighed using an electric balance (XS105DU; METTLER TOLEDO, Greifensee, Switzerland). The liquid hydrolysate was neutralized to pH 5.0 by the addition of calcium hydroxide powder. The sugar content in the liquid hydrolysate was determined by GCMS under the conditions described above.

2.4. Compositional analysis of biomass

The composition of raw materials was determined using a standard analysis procedure for biomass composition, a modification of the National Renewable Energy Laboratory (NREL) analytical method. Following two step-acid hydrolysis, the polysaccharide composition was determined based on the monomer content. In the first step, 3 ml of 72% w/w sulfuric acid was added to 300 mg of dried raw biomass, and incubated at 30 °C for 2 h. In the second step, the reaction mixture was diluted to 4% w/w with deionized water and autoclaved at 121 °C for 1 h. The hydrolysis solution was neutralized to pH 5.0 with the addition of powdered calcium hydroxide. The sugar content of the liquid hydrolysate was determined by GCMS under the conditions described above.

To release starch from raw biomass, 750 μL of 0.5 M sodium hydroxide was added to 75 mg of dried raw biomass and incubated at room temperature for 20 min. After removal of the residue, the sample solution was neutralized to pH 5 by the addition of 0.5 M acetic acid, and diluted to 1/10 using deionized water. 100 μL of enzyme mixture (8 units α-amylase, 13 units glucoamylase, 0.5 M sodium acetate buffer) was added to 100 μL of sample solution and incubated at 50 °C for 1 h. Liberated sugars were determined by GCMS under the conditions described above.

3. Results and discussion

3.1. Relationship between glucose content in the liquid hydrolysate and acid-insoluble residue weight

The straw of 13 rice cultivars in 2011 was pretreated with dilute acid and each detailed composition (glucose or xylose contents in the liquid hydrolysate, acid-insoluble residue weight) was determined. Similar to a previous report (Matsuda et al., 2011), glucose and xylose contents in the liquid hydrolysate varied among 13 cultivars (Fig. 1a and Supplementary Fig. S2). Glucose content varied greatly (Relative Standard Deviation; RSD = 43.6%) among the 13 cultivars, whereas xylose content showed small variability (RSD = 19.3%). In addition, the acid-insoluble residue weight showed small variation (RSD = 9.0%) (Fig. 1b). Interestingly, an obvious negative relationship was observed between the glucose content in the liquid hydrolysate and the acid-insoluble residue weight ($r = -0.97$) (Fig. 1c).

With respect to the liquid hydrolysate, the greater variability in glucose content compared to xylose content observed in the 13 rice cultivars corresponded with a previous report by Matsuda et al.