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High solid concentrations during the hydrothermal pretreatment of eucalyptus accelerate hemicellulose decomposition and subsequent enzymatic glucose production

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ABSTRACT

To improve the cost and energy efficiencies of glucose production from eucalyptus, we investigated the effect of solid concentration on hemicellulose decomposition during hydrothermal pretreatment, and the subsequent enzymatic production of glucose. The effect of reduced water use during hydrothermal pretreatment prior to enzymatic hydrolysis was examined, which led to relatively concentrated reaction conditions. Pretreatments were conducted at 200 °C with various solid concentrations (9–50 wt%) and severity factors (*SF* = 4.22 and 4.52). Acetic acid generated through hemicellulose deacetylation increased with increasing solid concentration and *SF*. Higher levels of acetic acid promoted hemicellulose decomposition during hydrothermal pretreatment through hemicellulose autohydrolysis. The maximum glucose yield following enzymatic hydrolysis was 436.7 mg/g-Eu, which was observed at the highest solid concentration and *SF* values (50 wt% and 4.52, respectively). We ascribe the observed enhancement to efficient hemicellulose decomposition during pretreatment, which improves enzyme access to the cellulose surface during enzymatic hydrolysis.

1. Introduction

In the context of global climate change, significant effort has been devoted to developing biorefineries that produce renewable low-carbon products from biomass as alternatives to traditional refineries that consume non-renewable resources, such as fossil fuels, and often damage the environment. Sugars have been important intermediate materials in biorefineries, as they can be used as raw materials for the production of a variety of chemicals and fuels. The production of sugars by the hydrolysis of cellulose and hemicellulose, as the main components of biomass, can be formally represented by the following reaction formulas:

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_6 \quad (1)$$

$$(C_5H_8O_4)_n + nH_2O \rightarrow nC_5H_{10}O_5 \quad (2)$$

where $(C_6H_{10}O_5)_n$ and $(C_5H_8O_4)_n$ are cellulose and hemicellulose, respectively.

Although enzymatic hydrolysis is a promising method for the production of sugars from biomass, its progress is impeded by the highly stable and complicated hybrid structures of cellulose, hemicelluloses, and lignin present in the biomass. Therefore, thermochemical (Grohmann and Bothast, 1997; Imman et al., 2015; Lee et al., 2017a; Mou et al., 2013; Negro et al., 2003; Romaní et al., 2010; Teramoto et al., 2008) or mechanical (Lee et al., 2017b; Lin et al., 2010) pretreatment, or a combination of the two (Barakat et al., 2014; Hideno et al., 2012; Inoue et al., 2008; Ishiguro and Endo, 2014; Jones et al., 2013; Kumagai et al., 2015), is generally required prior to enzymatic hydrolysis to effectively separate and destabilize the components.

In the hydrothermal pretreatment using subcritical water, as previously described by us (Fujimoto et al., 2008), the water is considered to work as an acid catalyst because the ionic product of water (*Kw*) increases from 10^{-14} to 10^{-11} with increasing temperature until approximately 250 °C (Akizuki et al., 2011; Cantero et al., 2015; Kruse and Einjus, 2007; Tapah et al., 2014). Therefore, the pretreatment does not require any harmful chemicals, such as acids and bases (Ishiguro and Endo, 2014), or expensive non-corrosive metals, making it an environmentally friendly alternative to other pretreatment methods.

Generally, hydrothermal pretreatment is conducted under dilute conditions with a low concentration of solid; typically, solid-to-liquid ratios of 1:5–1:10 (w/w) have been used (Mou et al., 2013; Negro et al., 2003; Romaní et al., 2010). The specific heat of water is 4.217 $J\,g^{-1}\,K^{-1}$, which is almost three times that of wood

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($1.554\ J\ g^{-1}\ K^{-1}$) (Fujimoto et al., 2006). Consequently, large amounts of energy are necessary to heat the surplus water rather than the wood sample. Although this heat can be recovered using a heat exchanger, its use increases the equipment cost of the overall process. In addition, the highly dilute product solution requires concentration and/or dehydration steps during the isolation of the product.

Simple consideration of these issues quickly provides a possible solution: energy efficiency should be improved by reducing the amount of water used and by increasing the solid concentration during hydrothermal pretreatment. Minowa et al. (1989) examined the effect of solid concentration on the energy consumption ratio (ECR: energy consumed/heating value of the generated oil) during the direct hydrothermal liquefaction of wood chips at high pressures and temperatures. They reported that the use of a relatively high solid concentration (40 wt%) during the liquefaction step led to a lower ECR and a higher oil yield when compared with the use of a low solid concentration (20–30 wt%). However, the effect of a much higher solid concentration (~50 wt%) during hydrothermal pretreatment is unknown, and the effect of solid concentration on the subsequent enzymatic hydrolysis has also not been sufficiently investigated.

In this study, we evaluate the effect of high solid concentrations during the hydrothermal pretreatment of eucalyptus under subcritical state of water, as a typical woody bioresource (Ishiguro and Endo, 2014), on the efficiency of the subsequent enzymatic hydrolysis and product yield. We show that a high solid concentration (50 wt%) during hydrothermal pretreatment facilitates effective enzymatic hydrolysis and improves the yield of glucose to 436.7 mg/g-Eu, comparable to that observed using a hydrothermal-mechanochemical procedure under highly alkaline conditions (20% NaOH) (Ishiguro and Endo, 2014).

2. Materials and methods

2.1. Samples

Eucalyptus globulus chips were purchased from Oji Holdings Co. and used as raw materials. The chips were milled using a cutting mill (P-15, Fritsch Japan Co., Ltd., Kanagawa, Japan), and the < 3 mm fraction was separated by sieving. The sample was then dried *in vacuo* at 40 °C overnight. The initial chemical content of the eucalyptus sample after drying was determined using an MT-6 CHN Corder organic microelemental analyzer (Yanaco New Science Inc., Japan); the sample composition was found to be 45.49% carbon, 6.02% hydrogen, and 0.14% nitrogen, with no sulfur detected. The oxygen content was determined to be 48.35% by subtraction. The composition of the monomeric sugars in the eucalyptus sample was determined by the following modified NREL laboratory analytical procedure (LAP) (Sluiter et al., 2008). Aqueous sulfuric acid (72 wt%; 0.6 mL) was first added to 50 mg of vacuum-dried eucalyptus in a glass tube. The mixture was stirred in an incubator at 30 °C for 90 min, diluted with 16.8 mL of water, and then heated in an autoclave at 120 °C for 120 min. The mixture was immediately cooled in an ice-water bath, and the volume was adjusted to 20 mL through the addition of water. The supernatant was filtered through a guard filter (Dionex OnGuard II A, Thermo Fisher Scientific K.K., Kanagawa, Japan) to remove sulfuric acid. The monomeric sugars in the filtered supernatant were analyzed by high-performance liquid chromatography (HPLC). The monomeric-sugar composition is shown in Table 1. The main components were glucose and xylose, at 500.4 mg/g-Eu and 101.1 mg/g-Eu, respectively.

Table 1
The monomeric-sugar composition of eucalyptus.

	Glucose (mg/g-Eu)	Xylose (mg/g-Eu)	Galactose (mg/g-Eu)	Arabinose (mg/g-Eu)
Eucalyptus	500.42	101.14	12.45	2.42

Fig. 1. Tube reactors used for hydrothermal pretreatment. Approximate dimensions: outer diameter, 25.4 mm (1 in.); wall thickness, 1.65 mm; length, 100 mm; and inner volume, 38.0 mL.

2.2. Hydrothermal pretreatment

As shown in Fig. 1, hydrothermal pretreatment was performed in a metal tube reactor with an outer diameter of 25.4 mm (1 in.), wall thickness of 1.65 mm, length of 100 mm, and inner volume of 38.0 mL (approximate dimensions). The reactor was made airtight by fitting a cap (Swagelok Japan Co., Hyogo, Japan) to its end. The inner temperature was measured with a type-K thermocouple fixed to one end and fitted with a coupling (Swagelok Japan Co., Hyogo, Japan). To evaluate the effect of solid concentration during hydrothermal pretreatment on the subsequent enzymatic hydrolysis and product yield, various amounts of water, ranging from 3.0 to 30.0 g, were placed in the reactor with a fixed amount of eucalyptus (3.0 g). At solid concentrations above 20 wt%, the water was entirely absorbed by the eucalyptus chips; hence, there was no apparent liquid phase. In contrast, at solid concentrations below 20 wt%, some of the water added was present as a liquid phase. Initially, the inside of the reactor was at atmospheric pressure. The reactor was placed in a preheated electric furnace (FO810, Yamato Scientific Co., Ltd., Tokyo, Japan), heated to 200 °C, and maintained at this temperature for the time required to achieve severity factors (*SFs*) of 4.22 and 4.52, as defined by the following equations (Overend et al., 1987):

$$R_0 = \int_0^t \exp\left(\frac{T - 373.15}{14.75}\right) dt \tag{3}$$

$$SF = \log(R_0) \tag{4}$$

where T is the temperature (K) and t is the duration (min) of the hydrothermal pretreatment.

In order to determine the *SF* as the temperature was elevated, the temperature in the reactor was measured by a thermocouple and recorded every second, beginning at room temperature. When the *SF* reached the target value, the reactor was removed from the furnace and quickly placed in an ice-water bath to quench the hydrothermal pretreatment process. After cooling, the reactor was opened to recover both the solid and liquid phases, and the inside of the reactor was rinsed with water (10.0 mL). The samples and the rinse water were combined in a vial and the volume was adjusted to 45.0 mL by the addition of water. For product analysis, a 1.0 mL aliquot was removed from the dilute suspension while it was uniformly stirred. The solid phase was filtered through a 0.2 μm filter and the liquid phase was analyzed by HPLC.

2.3. Ball mill pretreatment

A fixed amount of eucalyptus (20.0 g) was placed in a planetary ball mill (P-5, Fritsch, Kanagawa, Japan) with 25 20-mm-diameter zirconia