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Evaluation of hot compressed water pretreatment and enzymatic saccharification of tulip tree sawdust using severity factors

Dae Sung Kim, Aye Aye Myint, Hun Wook Lee, Junho Yoon, Youn-Woo Lee *

School of Chemical and Biological Engineering and Institute of Chemical Processes, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151–744, Republic of Korea

HIGHLIGHTS

- Severity factors affect the properties of tulip tree treated by hot compressed water.
- Glucose yields were strongly related with pretreatment severity.
- Hemicellulose removal yield was a dominant factor for enzymatic digestibility.

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ABSTRACT

Tulip tree sawdust was pretreated using hot compressed water with different pretreatment severities ($\text{Log}R_0$, 3.05–5.01) by varying reaction temperatures (180–220 °C) and residence time (1–30 min). It is found that the chemical composition and physicochemical properties of the pretreated products can be characterized and correlated with severity. Removal of most of the xylan and other hemicellulosic sugars from the raw material was observed at a severity of 4.5. Thus, the residual solids were recovered with increased cellulose and lignin contents. Nearly complete glucan conversion was achieved after 48 h of hydrolysis with 10 FPU/g of wet residual solid obtained above a severity of 4.8. The characteristics of the pretreated solids according to the pretreatment severity were strongly related with the glucose yield. The removal of structural barriers to the enzyme attack was the dominant factor affecting enzyme accessibility to the substrate.

1. Introduction

Over the past few decades, environmental problems due to global warming, skyrocketing crude oil prices, fossil fuel depletion, restrictions due to environmental regulations, and national security issues have contributed to an increase in research on renewable resources such as biomass (Mosier et al., 2005). Various bio-based fuels and chemicals can be produced from biomass resources such as agricultural and forest residues. In particular, lignocellulosic biomasses are considered to be a promising source of fermentable sugars for bioethanol as well as numerous platform chemicals due to its abundance, renewability, and sustainability (Liu, 2010).

Lignocellulosic biomass primarily consists of three components (cellulose, hemicellulose, and lignin) with minor components (i.e., proteins, chlorophyll, and ash). Cellulose can be converted to glucose by hydrolysis through biological treatment and be further used as a source of ethanol or other useful chemicals. However, the presence of lignin, hemicellulose, and extractives, which serve as a protective barrier against enzyme and microbial degradation, has been a hurdle for the efficient utilization of lignocellulosic biomass on the commercial scale (Santos et al., 2012). A pretreatment in the biorefinery process can be defined as the process that transforms native lignocellulosic biomass into a form which is amenable to the enzymatic hydrolysis reaction (Lynd et al., 2002). Therefore, pretreatment of lignocellulose is essential for ensuring the economic feasibility of the biorefinery process.

Various pretreatment approaches, including mechanical, physicochemical, biological methods, and a combination of these methods, have been investigated. Among the known pretreatment technologies, hot compressed water (HCW) pretreatment (also known as autohydrolysis, subcritical water pretreatment, and hydrothermal pretreatment) is a technically and economically viable process for the following reasons (Garrote et al., 1999): (a) organic solvents and catalysts are not required, thereby making it an environmentally friendly process; (b) mono- and/or oligosaccharides can be generated at high yields as a result of hemicellulose hydrolysis; (c) due to the mild pH of the reaction media, equipment corrosion can be minimized compared with that of acid pretreatment; (d) the entire procedure is simplified due to the non-use of acids and the lack of catalyst-recycling steps, thus reducing

* Corresponding author. Tel.: +82 2 880 1883; fax: +82 2 883 9124.
E-mail address: ywlee@snu.ac.kr (Y.-W. Lee).

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process cost; (e) the physicochemical changes in cellulose and lignin after the pretreatment promotes the further fractionation or processing of these portions; and (f) relatively low generation of toxic compounds that could affect the follow-up processes and not involving an expansion step compared with steam explosion pretreatment (Alvira et al.,2010).

In the case of biomass pretreatments that use water as a reaction medium (e.g., HCW treatment, steam explosion, and dilute acid hydrolysis), both pretreatment temperature and retention time are the key variables that affect the efficacy of pretreatment and enzymatic hydrolysis. Hence, these experimental results can be easily compared by using the severity factor, R_0, which describes the combined effect of temperature and time. The severity factor can be expressed by the following equation (Overend and Chornet, 1987):

$$R_0 = t * \exp(T - 100/14.75) \quad (1)$$

where t is the retention time in min and T is the pretreatment temperature in °C. The equation can be calculated based on the assumptions that: (a) the reaction occurs in a single step and is irreversible; (b) the reaction is a first-order reaction; (c) the reaction has an Arrhenius temperature dependence; and (d) the reference temperature is 100 °C. In most studies, including this one, the logarithmic values of R_0 have been used to evaluate the efficiency of the process and the characteristics of products.

Previous studies have reported the effects of severities in HCW processing for various lignocellulosic feedstocks such as rice husk (Vegas et al., 2008), wheat straw (Carvalheiro et al., 2009; Kabel et al., 2007), rice straw (Yu et al., 2010), eucalyptus (Romaní et al., 2010), and oil palm frond (Goh et al., 2012). However, most of them have peripherally considered the effects of the severities on the pretreatment results. Furthermore, the association between pretreatment severities and the enzymatic digestibility has rarely been suggested in spite of its considerable industrial importance. The effects of the severities and their interrelation to the various physicochemical features of pretreated products were generally considered using tulip tree (TL) as a feedstock in this study. It has also been attempted to suggest the proper pretreatment conditions for achieving high glucose yield.

2. Methods

2.1. Raw material

TL (*Liriodendron tulipifera)* sawdust was used as feedstock and was supplied by Nutrapharm Co., Ltd. (Gyeonggi-do, Korea). The feedstock was ground with a lab-scale grinder (HMK Co., Ltd., Korea) and screened to an average size of 0.25–0.42 mm. The sawdust was then extracted with a mixture of ethanol and benzene (1:2 v/v) for 12 h by using a Soxhlet extractor. This procedure removed nonstructural materials such as waxes or chlorophyll that might interfere with the post analytical steps (Sluiter et al., 2005). The extractive-free TL sawdust was dried in a vacuum oven at 45 °C until the dry matter content was greater than 95 wt% and was maintained in a desiccator at room temperature until use.

2.2. HCW pretreatment

TL sawdust was pretreated using HCW in a batch-type reactor system. The reactors were made of 316 SS and had a total volume of 26.7 mL. To heat the reactor, a molten salt bath with a mixture of $NaNO_3$, KNO_3, and $Ca(NO_3)_2$ was used. The salt bath temperature was automatically adjusted using a proportional integral derivative (PID) controller. A horizontally reciprocating shaker was used to provide a stirring effect for the reactor.

Approximately 1.3 g of TL sawdust and deionized water were loaded in the reactor with a solid to liquid ratio of 15. The sealed reactor was heated by immersion into the salt bath. It typically took 150 s to reach the desired temperature; therefore, this time was excluded when calculating the retention time. After the reaction, the reactor was removed from the salt bath and immersed in a cold-water bath for quenching. The reaction products were separated into liquid hydrolysates and solid residues by filtration with a G3 Pyrex glass filter (IWAKI, Japan) using a vacuum. The solid residues were further washed with hot water to neutralize pH and to remove any water-soluble degraded products. The wet solid residues were divided into two portions: one portion of wet solids was used to measure moisture contents, and another portion was directly used for enzymatic hydrolysis. Moisture content determination for wet solids was run in triplicate for each sample and the average values were approximately 60 wt%. Each value of the moisture content was considered for calculating the yield of glucose by enzymatic hydrolysis.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of pretreated solid residues was performed at 50 °C for 48 h (using 5 mM acetate buffer, pH 4.8) in 20 mL vials with an agitation speed of 150 rpm in a thermostatic incubator. The extractive-free TL sawdust was used as a negative control. A 2% concentration (based on oven dry weight, ODW) of substrate was used with a total volume of 10 mL in buffer solution. A mixture of 2 commercial enzymes, that is, cellulase (Celluclast 1.5 L from *Trichoderma reesei*) and β-glucosidase (Novozyme 188 from *Aspergillus niger*), was used. The enzyme activities were 339 ± 43.6 FPU/mL for cellulase and 241 ± 8.7 CBU/mL for β-glucosidase as determined using the methodology to assay cellulose activities (Ghose, 1987). The enzyme to substrate ratio was 10 FPU/g of wet substrate, and the cellulase to β-glucosidase ratio was 0.5 FPU/CBU. Prior to the addition of the enzymes, 0.1 mL of sodium azide solution with a concentration of 20 mg/mL was added as an antibiotic. Controls without substrate or without enzymes were also used simultaneously. After 48 h of enzyme hydrolysis, 0.5 mL aliquots were withdrawn from the broth and then filtered with a 0.2 μm syringe filter. The samples were analyzed by HPLC (Agilent 1200 series, Agilent Technologies, Inc., USA) by using a refractive index (RI) detector and an Aminex HPX-87P column (Bio-Rad Laboratories Inc., USA). In this study, only glucose was quantified as a product of enzymatic hydrolysis. The glucose yield (%) was calculated by the amount of glucose generated by enzymatic hydrolysis divided by the amount of glucose in the original substrate multiplied by 100%.

2.4. Analysis

2.4.1. Solid and liquid yields

The moisture in the solid residues obtained from the HCW pretreatment was evaporated at 105 °C for 24 h in a hot air oven. The dried solids were then cooled to room temperature in the desiccator to minimize moisture absorption during weighing. All the samples were run in triplicate, and the average value was used for compositional analysis. The solid yield (%) was calculated by ODW of HCW pretreated solid residue divided by ODW of raw TL multiplied by 100%. The liquid yield (%) can be simply calculated by subtracting the solid yield from 100%.

2.4.2. Chemical composition analysis

The chemical compositions of raw and HCW-pretreated samples were analyzed after quantitative acid hydrolysis according to a slightly modified laboratory analytical procedure (LAP) adapted from the National Renewable Energy Laboratory (NREL)

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