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Organosolv pretreatment with various catalysts for enhancing enzymatic hydrolysis of pitch pine (*Pinus rigida*)

Nahyun Park [a], Hye-Yun Kim [a], Bon-Wook Koo [a], Hwanmyeong Yeo [a,b], In-Gyu Choi [a,b,*]

[a] Department of Forest Sciences, College of Agriculture and Life Sciences, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-921, South Korea
[b] Research Institute for Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-921, South Korea

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ABSTRACT

Three different types of catalysts were evaluated for organosolv pretreatment with pitch pine (*Pinus rigida*). Sulfuric acid, magnesium chloride, and sodium hydroxide for acid, neutral and base catalysts, respectively, were used, and ethanol was the organic solvent. The pretreatment process was conducted at different temperatures and times. The enzymatic hydrolysis process followed to estimate the digestibility of the biomass. The digestibility of pitch pine by pretreatment process with 1% sulfuric acid at the optimal condition was approximately 55–60%, and that by 1% magnesium chloride was nearly 60%. The pretreatment with 1% sodium hydroxide had no effect on digestibility at 10%, but the digestibility improved by more than 80% when the concentration was increased to 2%. Theoretical ethanol yield was the highest at organosolv pretreatment with sulfuric acid at 70% and the lowest with sodium hydroxide at 45%.

1. Introduction

The history of ethanol as a fuel or a blend stock is comparatively long. Henry Ford developed several models including the Model-T, which could use ethanol. During the second World War, the US and Europe used alcohol fuels as gasoline extender or fuel (Thomas and Kwong, 2001). Today, ethanol is recognized as a replaceable and renewable fuel that can be used in various ways. It is generally used as a substitute oxygenated fuel additive, especially methyl tert-butyl ether (MTBE) (Wheals et al., 1999), or as replacement lead in gasoline (Thomas and Kwong, 2001). Mixing ethanol with gasoline can reduce gasoline used in cars by up to 10–20% (Galbe and Zacchi, 2002). The current Proalcool program in Brazil is a good example of this.

Ethanol production has been continuously studied with various kinds of biomass. Lignocellulosic biomass receives more attention because it does not compete as a food resource, and it can reduce carbon dioxide in the atmosphere by up to 75–100% (Fulton et al., 2004). Therefore, it is recognized as a long-term revenue fuel resource (Srinivasan, 2009). Especially, softwoods are the dominant lignocellulosic biomass in the northern hemisphere, and they are used in various countries (Galbe and Zacchi, 2002; Nguyen et al., 1999; Pan et al., 2005; Wu et al., 1999). However, they have high lignin and recalcitrant characteristics; thus, many studies have been worked on achieving the low efficiency of ethanol conversion (Clark et al., 1989; Galbe and Zacchi, 2002; Millett et al., 1976; Sun and Cheng, 2002).

Therefore, various pretreatment processes have been developed to enhance enzyme accessibility, and they have been evaluated using softwoods. Organosolv pretreatment is one of processes methods for lignocellulosic biomass. Aqueous ethanol pretreatment of hybrid poplar can improve the recovery of monomeric glucose by up to 85% after enzymatic hydrolysis (Pan et al., 2006). Due to its high efficiency, softwood has been evaluated by organosolv pretreatment. Pan et al. (2007) obtained cellulose-to-glucose conversion yield up to 93–97% with sulfuric acid–ethanol-treated lodgepole pine, while Araque et al. (2008) achieved 99.5% ethanol yield with sulfuric acid–acetone-treated radiate pine.

In the organosolv process, biomass is treated by an organic solvent and a catalyst. As catalysts, organic or inorganic acids, bases, and so on have been tested for lignocellulosic biomasses (Zhao et al., 2009).

Sulfuric acid, a strong mineral acid, is one of the most-used reagents for the pretreatment process due to its strong reactivity and high efficiency. (Araque et al., 2008; Pan et al., 2007). However, its toxic, corrosive, hazardous, and inhibitory characteristics limit its utilization. Magnesium chloride has not been fully studied for the pretreatment process, but several studies have employed it as a neutral catalyst (Chang and Paszner, 1982; Luis et al., 2009). Sodium hydroxide is a strong base, and it has been used in many industries due to its high dissolubility. It has also been evaluated

* Corresponding author at: Department of Forest Sciences, College of Agriculture and Life Sciences, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-921, South Korea. Tel.: +82 2 880 4785; fax: +82 2 873 2318.
E-mail address: cingyu@snu.ac.kr (I.-G. Choi).

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as a reagent for a pretreatment process, that is alkaline pretreatment (Fan et al., 1987; Millett et al., 1976), but is not fully enough for the organosolv pretreatment process.

Pretreatment of high lignin-containing lignocellulosic biomass such as softwoods have not been fully studied. Despite many developed techniques, these are not suitable for softwoods except for organosolv pretreatment (Zhu et al., 2009). Softwoods are not only sufficient but also excellent resources for bioethanol conversion. Therefore, more studies must be undertaken on adequate pretreatment methods.

This study focused on the pretreatment process of softwoods, especially pitch pine (*Pinus rigida*), to improve the enzymatic hydrolysis and fermentation processes. Primarily, pitch pine was treated with the organosolv pretreatment process, and three catalysts, acid, neutral, and base catalysts, which were evaluated for pretreatment reagents. The different catalysts on pretreatment variables then were analyzed and compared.

2. Methods

2.1. Materials

Logs of pitch pine (*P. rigida*) were obtained from Jangheung-kun, Jeollanam-do, South Korea. Logs were ground, sieved through 40-mesh screens, and dried to obtain moisture content less than 10%.

2.2. Organosolv pretreatment process

The organosolv pretreatment process was conducted in a mini-bomb (Bolted Closure Vessels, Hanul Autoclave, Co. Ltd.) made of stainless steel (SUS 316) with a capacity of 500 ml. The viton ring closure system was used to maintain high pressure, and an inner thermocouple was inserted to control and measure the internal temperature. The reactor was loaded with 20 g of wood powder and 200 ml of a 50:50% ethanol:water mixture (v/v) containing a catalyst. Three different catalysts were employed for the process. One percent of sulfuric acid (w/v), 1% magnesium chloride (w/v), and 1–2% sodium hydroxide (w/v) were selected as an acid, neutral, and alkaline catalyst, respectively.

Reaction temperature was from 150 to 210 °C at 10–20 °C intervals and was controlled electrically by an external controller. The reactor was heated for 50 min to achieve reaction temperature; residence time was counted from the moment the desired temperature was achieved. Reaction was carried out for 0–20 min. Afterwards, the reactor was placed in the ice chamber to cool down below room temperature. H-factor was employed to represent temperature and time including preheating time in a single parameter (Gullichsen et al., 2000), and severity factor was also used to represent them without considering preheating time (Galbe and Zacchi, 2007).

Materials were washed by distilled water to remove degradation products and solvents (Holtzapple and Humphrey, 1984). The pretreated mixtures were divided into solid fractions (residues) and liquid fraction (aqueous organo-soluble and aqueous organo-insoluble fraction) by a fritted glass-filtering crucible (2G2 Iwaki, Japan). All experiments were done in at least duplicate.

2.3. Enzymatic hydrolysis and fermentation

2.3.1. Determination of enzymatic digestibility

Enzymatic digestibility of pretreated materials was determined according to the National Renewable Energy Laboratory (NREL) Chemical Analysis and Testing Standard Procedure LAP-009 (NREL, 2005).

Cellulase from *Trichoderma reesei* ATCC 26921 (Celluclast 1.5 L, Novozymes, Demark) with an activity of 700 EGU/g, supplemented with β-glucosidase from *Aspergillus niger* (NS-50010, Novozymes, Denmark) with an activity of 250 CBU/g, was used for enzymatic hydrolysis.

Enzymatic hydrolysis was performed with 1 g of pretreated materials (solid fraction) in 100 ml of 50 mM acetate buffer (pH 5.0) containing enzymes. Mixtures were incubated and periodically sampled at 50 °C in a shaking incubator (New Brunswick Scientific, Innova-4080) at 150 rpm (Teramoto et al., 2008). The digestibility was defined as the percentage of enzymatically hydrolyzed biomass after 72 h of incubation.

2.3.2. Simultaneous saccharification and fermentation (SSF)

Saccharomyces cerevisiae ATTC® 26603 (NREL-D5A) was used to ferment microorganisms. Prior to SSF, *S. cerevisiae* was precultured in YP medium [10 g/l of yeast extracts (Sigma Cat. No. Y-0500), 20 g/l of peptone (Sigma Cat. No. P-6588), and 20 g/l glucose] at 30 °C in a shaking incubator (New Brunswick Scientific, Innova-4080) for 24 h. The culture broth was centrifuged at 5000 rpm at 4 °C for 10 min and was then washed with distilled water.

For the SSF procedure, Celluclast 1.5 L (Novo Co.) and NS-50010 (Novo Co.) were used for enzymatic hydrolysis. A 2 g/l of precultured *S. cerevisiae* was added for fermentation. A total of 5 g of pretreated materials was placed in 50 ml of working volume of liquid in a 250 ml Erlenmeyer flask. Incubation was carried out in a shaking incubator at 30 °C at 150 rpm. Supernatant of the mixtures was sampled periodically for ethanol yield determination.

2.3.3. Determining monomeric sugar and ethanol yield

The amount of glucose and ethanol content was analyzed by high-performance liquid chromatography (HPLC) (HP 1100, Hewlett Packard, USA) at 40 °C with 5 mM H_2SO_4 as eluant at a flow rate of 0.5 ml/min and an injection volume of 5 μl. A SugarPak™[1] column (Waters, 6.5 mm × 300 mm, 5 μm) was employed for sugar determination, while a BioRad (Hercules, CA) Aminex HPX-87H column (300 mm × 7.8 mm, 5 μm) was used for ethanol determination. A refractive index detector (HP 1100, Hewlett Packard, USA) was used to quantify the products.

Theoretical ethanol yield was calculated according to the NREL Chemical Analysis and Testing Standard Procedure LAP-008 (LAP et al., 1998).

2.4. Analytical procedure

2.4.1. Chemical composition

Klason lignin, acid-soluble lignin and structural sugar were analyzed in accordance with NREL Chemical Analysis and Testing Standard (NREL, 2005).

2.4.2. Surface morphology

Pretreatment effects on the surface of biomass were researched by field-emission scanning electron microscope (FE-SEM) (SUPRA 55VP, Carl Zeiss, Germany). Samples were mounted on aluminum stubs using carbon tape with conductive silver paint applied to the sides to reduce sample charging and sputter-coated with Pt–Pd by sputter coater. Images were taken at 3 kV of the beam voltages (Donohoe et al., 2008).

2.4.3. ^{13}C NMR analysis

AVANCE 600 High Resolution Nuclear Magnetic Resonance (NMR) spectrometer (Bruker, Germany) was used to obtain all spectra. A 100–150 mg sample was dissolved in 1 ml of dimethyl sulfoxide (DMSO) at 60 °C. The scan was performed for more than 12 h.