Biomass and Bioenergy 105 (2017) 190–196

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: http://www.elsevier.com/locate/biombioe

Research paper

Improved pretreatment of yellow poplar biomass using hot compressed water and enzymatically-generated peracetic acid

Hyeong Rae Lee [a], Hun Wook Lee [a], Youn-Woo Lee [a], Romas J. Kazlauskas [b, *], Tai Hyun Park [a, **]

[a] School of Chemical and Biological Engineering, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-744, Republic of Korea
[b] Department of Biochemistry, Molecular Biology & Biophysics and the Biotechnology Institute, University of Minnesota, Saint Paul, MN, 55108, USA

ARTICLE INFO

Article history:
Received 11 February 2017
Received in revised form
4 July 2017
Accepted 5 July 2017
Available online 16 July 2017

Keywords:
Yellow poplar
Hot compressed water
Peracetic acid
Pretreatment
Cellulase accessibility

ABSTRACT

Biomass forms a complex interwoven structure containing cellulose, hemicellulose and lignin that hinders enzymatic hydrolysis of cellulose. Enzymatic hydrolysis of the cellulose within yellow poplar (tulip tree) particles released only 9% of the total glucose in this study. To increase the accessibility of the cellulose component, wood particles were pretreated using hot compressed water and enzymatically-generated peracetic acid. The combined pretreatment started with hot compressed water (200 °C, 15 min), which selectively solubilized up to 90% of the xylan. The remaining solid was treated with peracetic acid (90 mM, 60 °C, 6 h), which solubilized up to 70% of the lignin. The remaining solid consisted of mainly glucan (~75%) and corresponds to 87% of the glucan initially present in the yellow poplar particles. Hydrolysis of the remaining solid using a low loading of cellulase/β-glucosidase for 72 h released 90% of the glucose. The removal of the xylan and lignin structural barriers dramatically increased the cellulase accessibility to cellulose. The structural characteristics (crystallinity, functional group changes, morphology) of combined pretreated solid residue changed in a manner consistent with increased enzymatic digestibility. The combined pretreatment with hot compressed water and peracetic acid was more effective than either single pretreatment and more effective than the sum of the single pretreatments to remove xylan and lignin, thus demonstrating a cooperative effect of the two pretreatments. In addition, the combined pretreatment enhanced the accessibility of cellulases to the cellulose resulting in more efficient cellulose hydrolysis.

1. Introduction

Replacement of fossil fuels with biomass-derived fuels is driven by the depletion of fossil fuels and by the emission of greenhouse gases from their combustion. Biomass is a complex material composed of intertwined cellulose, hemicellulose and lignin. The low cost, abundance and sustainability of biomass make it a potential feedstock for fuels and high-value chemicals [1]. Biomass may come from agricultural residues, energy crops, softwood or hardwood. The Korea Forest Service recommended yellow poplar (*Liriodendron tulipifera*, tulip tree) for lignocellulosic biomass [2]. Yellow poplars are not true poplars, but related to magnolias. They are fasting-growing (approximately 20 years harvest cycle) hardwoods indigenous to eastern North America, but planted worldwide because they acclimate to many environments and sequester larger amounts of carbon dioxide than other trees due to their extensive root system. Effective pretreatment of yellow poplar biomass is an important research goal [3,4].

Producing biofuels from lignocellulosic biomass requires, first, deconstruction and hydrolysis of the polysaccharides to sugars and, second, fermentation of the sugars to fuels. The deconstruction of lignocellulosic biomass to fermentable sugars is inefficient because its complex structure blocks access to the oligosaccharides and the hydrophobic lignin binds the hydrolytic enzymes. This inefficiency is an obstacle to produce biofuels because it increases their cost [5]. Pretreatment of biomass breaks up the complex structure and may remove hemicellulose and lignin, thereby leaving a disrupted structure where enzymes can more efficiently hydrolyze the

* Corresponding author.
** Corresponding author. T. H. Park, School of Chemical and Biological Engineering, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-744, Republic of Korea.
E-mail addresses: rjk@umn.edu (R.J. Kazlauskas), thpark@snu.ac.kr (T.H. Park).

http://dx.doi.org/10.1016/j.biombioe.2017.07.004
0961-9534/

oligosaccharides to sugars.

Hot compressed water (HCW), also called autohydrolysis or subcritical water, is a promising pretreatment method. HCW pretreatment uses only water, which does not need to be recycled unlike methods that use organic solvents or catalysts [6,7]. HCW pretreatment selectively hydrolyzes the hemicellulose component of biomass to monosaccharides yielding a cellulose- and lignin-rich solid residue [8]. The removed solution contains xylose and other five-carbon sugars, which can be used for fermentation [9].

One limitation of the HCW pretreatment is the limited removal of the lignin. The remaining lignin interferes with the cellulase-catalyzed hydrolysis by binding cellulases and by hindering their access to the cellulose [10]. Higher cellulase loading can overcome this interference by lignin, but higher loading increases the cost. Adding pretreatment steps to the HCW pretreatment to remove lignin could increase the yields of glucose without requiring high cellulase loading.

Peracetic acid (PAA), a strong oxidizing reagent, selectively oxidizes and removes lignin, while leaving the polysaccharide fraction intact [11]. An acidic mixture of acetic acid and hydrogen peroxide at 80 °C forms PAA in situ and also removes lignin [12]. Peracetic acid cleaves β-aryl ether bonds, which reduces the molecular weight of lignin, and introduces hydroxyl groups, which increases the water solubility of lignin and its fragments [13,14]. Peracetic acid is expensive [15] and concentrated forms are explosive and expensive to transport and store. An alternative is the in-situ generation of peracetic acid from hydrogen peroxide and acetate esters catalyzed by perhydrolase enzymes [16,17].

Previous research optimized time, temperature and concentrations for biomass pretreatment with HCW [18] and with PAA [17]. In this study, we combine optimal conditions of each one to yield a synergistic increase in effectiveness, Fig. 1. The first step hydrolyzes the xylan, while the second step removes most of the lignin. The effectiveness of combined-pretreatment was evaluated by measuring recovery yields, composition, enzymatic digestibility and changes in the structural characteristics of the pretreated solid residues.

2. Materials and methods

2.1. Materials

Yellow poplar (*Liriodendron tulipifera*) wood from 20-year-old trees, bark and foliage removed (NutraPharm Tech Co., Ltd., Korea) was ground with a laboratory-scale grinder (MHK Co. Ltd., Korea) and sieved to obtain 40/60 mesh fraction (average sizes of 0.25–0.42 mm). These particles were dried at 45 °C and stored in a desiccator at room temperature until use.

2.2. Hot compressed water (HCW) pretreatment of yellow poplar

HCW pretreatment was performed in batch reactor [18] made of stainless steel (SUS 316) with a total volume of 26.7 mL. Approximately 1.3 g of yellow poplar (YP) particles and deionized water (solid: liquid = 1:15, w/w) were placed in the reactor, tightly sealed and immersed in molten salt bath (a 24:46:30 mixture of $NaNO_3$, KNO_3, $Ca(NO_3)_2$) preheated to 200 °C with horizontal shaking of the reactor for 15 min (excluding warm-up time: 2 min 30 s). The temperature and reaction time were selected as optimal conditions for removal of xylan [18]. After heating the reactor was removed from the bath and immediately cooled to room temperature in a cold-water bath. The mixtures were filtered through DURAN P4 glass filter crucibles (DURAN, Germany) and the solid residues were washed with hot water to remove water-soluble degradation products until the filtrate was neutral. A portion of solid residue was dried at 105 °C for 24 h for characterization, while remaining solid residue was stored at 4 °C until next pretreatment and enzymatic hydrolysis.

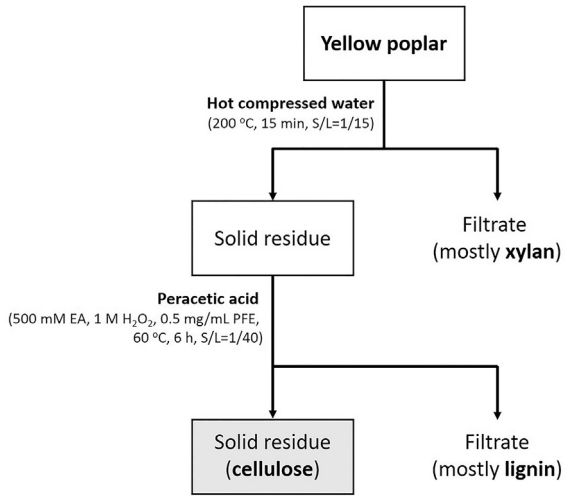

Fig. 1. Schematic of the combined pretreatment. The combined pretreatment of yellow poplar biomass uses hot compressed water to solubilize the xylan fraction followed by peracetic acid to solubilize the lignin fraction. S/L = solid to liquid ratio; EA = ethyl acetate; PFE = *Pseudomonas fluorescens* esterase.

2.3. Enzymatically-generated peracetic acid (PAA) pretreatment of yellow poplar

Pseudomonas fluorescens esterase (PFE) F162L gene containing a C-terminal $(His)_6$ tag (Yin et al., 2011) was transferred to pET-28b(+) expression vector (EMD Biosciences, USA). After transformation of *Escherichia coli* BL21 (DE3, Novagen, USA), cells were grown in lysogeny broth-kanamycin medium at 37 °C. The expression of PFE was induced when the cell density reached an O.D. of 0.6 by addition of isopropyl-β-D-thiogalactopyranoside (1 mM final concentration) and the cells were further incubated at 37 °C for 4 h. To isolate the protein, the culture was centrifuged at 8870 g and 4 °C for 20 min, the supernatant was discarded and the cell pellet was suspended in His-binding buffer (20 mM Tris, 500 mM NaCl, 20 mM imidazole, pH 8.0). The cells were disrupted by sonication at 25% amplitude and 5 s pulse on/off for 3 min. The cell lysate was centrifuged (13,680 g for 30 min) and the supernatant was filtered through a 0.45 μm membrane filter. The PFE F162L protein was purified using HisTrap HP column (GE healthcare, UK) with fast protein liquid chromatography (AKTA, GE healthcare), desalted in 20 mM Tris-HCl buffer (pH 8.0) using HisTrap Desalting column (GE healthcare), and stored at −70 °C until use. PAA was generated using purified PFE (0.5 mg/mL final concentration) in a solution of sodium phosphate buffer (100 mM, pH 7.2), hydrogen peroxide (1.0 M) and ethyl acetate (500 mM) [16] and incubated at 23 °C. Approximately 90 mM PAA was generated in 10 min, which was used directly to pretreat YP particles. The concentration of PAA was measured by the methyl tolyl sulfide assay [19]. Untreated or HCW-pretreated YP particles (1.0 g dry weight) were mixed with PAA solution (40 mL, 90 mM PAA plus enzyme, unreacted hydrogen peroxide and ethyl acetate) and incubated at 60 °C with shaking at 200 rpm for 6 h. The samples were filtered through DURAN P4 glass filter crucibles (DURAN) and washed with distilled water to remove PAA, hydrogen peroxide and ethyl acetate. A portion of the solid residue was dried in an oven at 105 °C for 24 h for characterization, while another portion was kept in 4 °C for enzymatic hydrolysis.