Biomass and Bioenergy 96 (2017) 96–102

Contents lists available at ScienceDirect

Biomass and Bioenergy

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

Research paper

Alkaline hydrogen peroxide (AHP) pretreatment of softwood: Enhanced enzymatic hydrolysability at low peroxide loadings

Carlos Alvarez-Vasco, Xiao Zhang*

Voiland School of Chemical Engineering and Bioengineering, Bioproducts, Science and Engineering Laboratory, Washington State University, Richland, WA 99354, United States

ARTICLE INFO

Article history:
Received 11 April 2016
Received in revised form
24 October 2016
Accepted 7 November 2016

Keywords:
Alkaline hydrogen peroxide
Softwood
Enzymatic hydrolysis
Glucomannan
Hydrogel
Low peroxide loading

ABSTRACT

This study investigated the specific effects of hydrogen peroxide (H_2O_2), alkali and temperature on substrate hydrolysability during alkaline hydrogen peroxide (AHP) pretreatment of Douglas fir. We demonstrated that the presence of a small amount of hydrogen peroxide is essential for effective pretreatment of Douglas fir by alkali. However contradictory to previous finding from others, we found that increasing H_2O_2 did not directly correlate to enhanced substrate hydrolysability, whereas the alkali charge played a more dominant role. The paper illustrated a strategy to apply AHP for softwood pretreatment with low peroxide loadings and achieve high cellulose-to-glucose yield (up to 95%). It was also found that glucomannan gelation (physicochemical change) occurred during AHP pretreatment of softwood which presents a newly identified recalcitrance factor to substrate hydrolysability. The resulting glucomannan derived hydrogel-like material has a high affinity toward cellulases and can cause a non-productive binding effect.

1. Introduction

Implementing cost effective softwood deconstruction processes to maximize fermentable sugars production remains a huge challenge [1,2]. Among a large selection of plant biomass pretreatment technologies developed to date, only a few of them, including acid or SO_2 pre-impregnated steam explosion [3], alkaline or sulfite pulping based pretreatments [4], wet explosion [5] and organosolv pretreatment [6], are able to deconstruct softwood for fermentable sugar production. However the cost associated with these processes is still formidable to justify for a biorefinery operation.

In recent years, alkaline hydrogen peroxide (AHP) treatment, a pulping and bleaching method employed in papermaking industry [7,8] has drawn attention for biomass pretreatment of hardwoods and grasses [9–11]. AHP pretreatment has shown to remove lignin while selectively retaining the cellulose [12]. In addition, AHP has a potential to simultaneously convert hemicelluloses to valuable organic acids (e.g. lactic, succinic and glycolic) products [13]. Results from most AHP studies suggest that increasing hydrogen peroxide (H_2O_2) loading is beneficial for improving substrate hydrolysability. Therefore, high optimum H_2O_2 loadings (mass fraction of H_2O_2 to biomass w_{H2O2}) have been proposed for treating most lignocellulosic biomasses including cashew apple bagasse ($w_{H2O2} = 0.86$) [14], rice hulls and straw ($w_{H2O2} = 0.80$) [15], and corn stover ($w_{H2O2} = 0.50$) [9]. Due to the high recalcitrance of softwood, the implementation of AHP for this biomass would require a high peroxide loading. which presents a significant cost factor of AHP treatment [16]. In this paper, we evaluated the use of different pretreatment conditions during AHP of softwood to with an objective to obtain high cellulose-to-glucose yields during enzymatic hydrolysis while maintaining a low peroxide loading ($w_{H2O2} < 0.10$).

It is generally recognized that AHP pretreatment is more effective under basic conditions at a pH around 11.6 [17]. However, this pH was identified based on optimum conditions for pulp bleaching. Applying AHP bleaching at mild temperatures (up to 90 °C) is another common practice during papermaking. A number of AHP pretreatment processes were conducted at mild temperatures (<50 °C) [9,15]. However, it has not been corroborated that conditions used during AHP for pulp and paper production are optimum for biomass pretreatment. It is conceivable that conditions aimed at obtaining strong (high crystallinity) and long fibers (low disruption of biomass matrix) for papermaking may not be ideal for biomass pretreatment for fermentable sugar production.

* Corresponding author.
E-mail address: x.zhang@wsu.edu (X. Zhang).

http://dx.doi.org/10.1016/j.biombioe.2016.11.005
0961-9534/

The objective of this study is to fill several knowledge gaps within AHP pretreatment of softwood. H_2O_2 is acidic in its native form. Alkali NaOH and Na_2CO_3 are commonly added to adjust and maintain the basic pH of the reaction solution. Therefore, an increase in H_2O_2 loading is always accompanied with an increase in alkaline concentration. The use of high H_2O_2 loadings on previous AHP pretreatment studies [9,14,15] was also associated with a high alkaline loading. A clear understanding of the individual effects of hydrogen peroxide (H_2O_2) and alkali (NaOH and Na_2CO_3) on substrate hydrolysability during softwood pretreatment is lacking. Glucomannan has been shown to be susceptible to alkaline degradation. However after pretreatment, an appreciable amount of glucomannan often remained in the residual substrate [13]. The properties of residual glucomannan and its effects on subsequent enzyme hydrolysis have not been investigated. It is conceivable that applying higher treatment temperature (180 °C) and pH (>11.6) may improve the AHP pretreatment of softwood for sugar production. However this effect has not been well understood. This study aims at providing new insights into identifying efficient AHP pretreatment method of softwood.

2. Materials and methods

2.1. Description and chemical composition of Douglas fir samples

Two different Douglas fir (*Pseudotsuga menziesii*) samples were used in this study, a saw mill residual pulp chips (FS01) with 1.4% bark collected on October 2011 from a wood chip pile on Southwest Washington (46° 6′ N and 122° 57′ W, located on Weyerhaeuser Longview Pull Mill) and a forest harvest residue (FS10) with 3.4% bark content collected on January 2013 from a road side pile on Southeast Oregon (43° 6′ N and 122° 9′ W, owned by Weyerhaeuser). Collected samples were chosen to represent typical forest residues of USA Pacific Northwest region as part of the Northwest Advance Renewable Alliance (NARA) project aimed to the production of fuels and chemicals from biomass. The used nomenclature for softwood residues states the order of their collection from a larger set of forest samples (FS) [18]. Forest samples were milled using a Peterson horizontal drum fixed-hammer grinder to obtain wood chips, and screened over a woven wire screen to remove fines (<3 mm). The wood chips were first air dried to less than 10% moisture before shipping for analysis to Washington State University, Bioproducts Science and Engineering Laboratory, Richland, WA.

Prior to analysis, the samples were milled to a specific size (mesh −40 to +60) and their cellulose, glucomannan, arabinoxylan and lignin compositions were determined following standard procedures [19,20].

2.2. Experimental conditions for alkaline hydrogen peroxide pretreatment

Alkaline hydrogen peroxide (AHP) pretreatment of Douglas fir was conducted in pressure tube reactors to allow processing a large number of treatments at identical conditions. Stainless steel pressure tube reactors were purchased from Swagelok Company (Solon, Ohio, U.S.A) with a volumetric capacity of 15 cm^3. AHP pretreatments of pulp wood chips (FS01) were carried out at different temperatures (25 °C, 90 °C, 140 °C, 180 °C and 200 °C), reaction times between 0 min and 90 min, peroxide loadings (mass fraction of H_2O_2 to biomass w_{H2O2}) from $w_{H2O2} = 0$ to $w_{H2O2} = 0.04$ (gram of peroxide per gram of biomass), and a solid concentration of 10% g/cm^3. Reaction time was recorded after the samples reached the desired temperature as previously reported [13]. The pH of the reaction solution was adjusted first by sodium carbonate to pH 10.6 (pH regulator), and then to pH 11.6 with the addition of sodium hydroxide. Due to the weak acidity of hydrogen peroxide, the amount of sodium carbonate and sodium hydroxide applied increased with the increase in H_2O_2 dosage.

After pretreatment, the solid fraction (water insoluble solids, WIS) and water soluble fraction (WSF) were separated and collected by filtration through a glass filtering crucible. The WIS was washed with water and then subjected to enzymatic hydrolysis to determine the resulting cellulose-to-glucose yields of pretreated Douglas fir samples.

2.3. Enzymatic hydrolysis of pretreated samples

Enzymatic hydrolysis of pretreated Douglas fir FS01 and FS10 were carried out at 2% substrate consistency (2 g odw biomass per 100 cm^3 solution) and 50 °C using Cellic® CTec2 (Ctec2) cellulase enzymes (Novozymes). Substrate and enzyme were mixed in sodium acetate buffer with a pH of 4.8. Sodium azide (0.02%) was also added to prevent microbial contamination according to NREL enzymatic saccharification procedure [21]. Enzyme dosages ranged from 20 FPU/g to 30 FPU/g (enzyme unit per gram of cellulose in the substrate) were tested from an initial enzyme stock with an activity of 119 FPU/cm^3 and 257 g/l protein concentration. Enzymatic hydrolysis experiments were performed at least in duplicates using 2 cm^3 Eppendorf tubes incubated in a molecular shaker (Eppendorf Thermomixer R). Hydrolysate samples were collected at different time intervals during 96 h. Glucose in the hydrolysate was measured by the glucose oxidase-peroxidase (GOPOD) assay kit (Megazyme, Ireland). Selected samples were also analyzed by HPLC to measure glucose, xylose, arabinose, galactose and mannose contents following NREL protocol [20].

Supplementing either mannanases NS-51023 (Novozymes) or xylanases (Pulpzyme HC 2500 and Novozymes Cellic® HTec2) to Novozymes Ctec2 enzyme for substrate hemicellulose hydrolysis was also tested. Three dosages of hemicellulolytic enzymes were evaluated, 0.3 g (low), 0.6 g (medium) and 1.2 g (high) of enzyme protein per 100 g of substrate. The enzyme protein concentration of mannanase NS-51023 (Mann), Pulpzyme HC 2500 (Pulp) and Novozymes Cellic® HTec2 (Htec2) stock solution were (77 ± 7) g/L, (72 ± 3) g/L and (364 ± 17) g/L, respectively. Enzyme protein content were determined using the Pierce® BCA protein assay kit (Thermo Fisher Scientific) following manufacturer's recommendations. In addition, control experiments using enzymes alone without the substrate were conducted to calculate background sugars in commercial enzymes mixtures.

2.4. Cellulase adsorption isotherm on model carbohydrates

Cellulose (Avicel® PH-101, Sigma-Aldrich), glucomannan (from Konjac, Megazyme) and xylan (from birch wood, Sigma-Aldrich) were used as model carbohydrate polymers to determine cellulase adsorption on specific biomass fractions. Celluclast (Novozymes) instead of Ctec2 was used for the adsorption isotherm experiments following a previous method [22]. Enzyme solutions with concentrations ranging from 0.05 g/l to 4 g/l were added to the buffer with substrates at 2% consistency. The mixture was incubated at 25 °C for 1 h to reach equilibrium [23], and after centrifugation the protein content in the supernatant was determined by Pierce® BCA protein assay kit using bovine serum albumin (BSA) as a standard. Samples were run in duplicates and the adsorbed protein was calculated based on the difference between the initial cellulase content and free cellulase content in the supernatant [22]. The Langmuir adsorption isotherm model was used to study cellulase adsorption on model biomass substrates. The surface concentration of adsorbed cellulases on substrate (Γ) as a function