Bioresource Technology 101 (2010) 7827–7833

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Can the same steam pretreatment conditions be used for most softwoods to achieve good, enzymatic hydrolysis and sugar yields?

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ARTICLE INFO

Article history:
Received 7 January 2010
Received in revised form 5 May 2010
Accepted 6 May 2010
Available online 31 May 2010

Keywords:
Douglas-fir
Steam pretreatment
Delignification
Softwoods
Enzymatic hydrolysis

ABSTRACT

Wood chips from six different Douglas-fir trees and a representative Lodgepole pine were steam pretreated at a single pretreatment condition (200 °C 4% SO_2 5 min) which had previously been shown to be effective for Spruce and Lodgepole pine chips. All of the softwood samples responded in a similar fashion with more than 60% of the cellulose hydrolysed after 72 h, at an enzyme loading of 20 FPU/g cellulose. However, when the enzyme loading was reduced to 5 FPU, less than 27% of the cellulose was hydrolysed. When the steam pretreated substrates were subsequently delignified they were almost completely hydrolysed, at both high, 20 FPU/g cellulose (less than 12 h) and low, 5 FPU/g (within 72 h) enzyme loadings. Although optimized steam pretreatment could result in greater than 90% glucose recovery, in order to obtain complete hydrolysis of the cellulosic component at reduced enzyme loadings a delignification step will likely be required.

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1. Introduction

Climate change and concerns of energy security have driven the recent global surge in research and development of technologies which convert lignocellulosic biomass to bioethanol (World Watch Institute, 2007). The bioconversion process to produce ethanol from lignocellulosics such as plant and woody biomass consists of three main steps including; pretreatment to increase the accessibility of the substrate to; enzymatic hydrolysis of the carbohydrate components to monomeric sugars which can subsequently be; fermented to ethanol (Chandra et al., 2007). Pretreatment has been shown to be one of the most critical steps in the biomass-to-ethanol process, especially when recalcitrant substrates such as softwoods are used, as it has a major influence on the overall process, particularly the subsequent ease of enzymatic hydrolysis and the fermentation of the resulting substrate (Chandra et al., 2007).

Of the various pretreatment processes that have been shown to be effective in processing a wide range of lignocellulosic substrates, steam pretreatment is recognized as one of the leading pretreatment strategies from both technical and economical points of view (Hendriks and Zeeman, 2009; Holtzapple et al., 1989). The process has been shown to be effective in providing a balance between the effective recovery of the water soluble hemicellulose sugars while increasing the enzyme digestibility of the water insoluble cellulosic fraction (Ramos et al., 1992a,b). The addition of an acid catalyst such as SO_2 or H_2SO_4 enhances the efficacy of the process by decreasing the required residence time and temperature of the process and by hydrolysing a good portion of the hemicellulose component thereby facilitating its recovery and use in the resulting water soluble fraction (Brownell and Saddler, 1987). Thus far, steam pretreatment has been shown to be effective in processing both agricultural and hardwood biomass (Ramos et al., 1992; Grous et al., 1986; Bura et al., 2002, 2009; Ohgren et al., 2007a; Schwald et al., 1988; Brownell and Saddler, 1984; DeBari et al., 2007). However, there have been mixed reports on how effective steam pretreatment is for processing softwoods (Schwald et al., 1989; Clark and Mackie, 1987; Maekawa, 1992; Mabee et al., 2006; Wu et al., 1999; Pan et al., 2004, 2005; Ewanick et al., 2007; Stenberg et al., 2000; Boussaid et al., 2000; Cullis et al., 2004; Monavari et al., 2009; Ewanick, 2006).

Softwoods are one of the major lignocellulosic resources available in geographical areas such as Scandinavia and the Pacific Northwest and they represent a potentially large source of biomass which may be utilized for bioconversion (Mabee et al., 2006). The ongoing outbreak of the mountain pine beetle (*Dendroctonus ponderosae*) in British Columbia and other parts of the Pacific North West has reduced the quality and value of affected wood at a rate beyond which the infected wood can be harvested and used for traditional uses such as timber and pulp and paper. This has lead to the availability of beetle-killed Lodgepole Pine at advanced

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0960-8524/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2010.05.023

stages of infection with limited value for these traditional ''structural'' applications (Kim et al., 2005; Pan et al.; 2008). Recent work has investigated the feasibility of utilizing beetle-killed Lodgepole pine as a biomass feedstock for bioconversion processes (Ewanick et al., 2007; Pan et al., 2008). Previously, Douglas-fir, (*Pseudotsuga menziesii*) another dominant softwood species in the Pacific Northwest, was studied extensively for its potential as a biomass source for bioconversion processes (Wu et al., 1999; Pan et al., 2004, 2005; Boussaid et al., 2000; Cullis et al., 2004; Yang et al., 2002). This earlier work had indicated that more severe pretreatment conditions and/or a subsequent delignification step were required before good sugar yields could be obtained with Douglas-fir substrates.

In subsequent work we compared the recalcitrance of a dominant North West America softwood (Lodgepole pine) with a dominant Scandinavian softwood (Spruce) and showed that similar pretreatment conditions (200 °C, 4% SO_2, 5 min) could result in reasonable hydrolysis yields (60–70%) for both species, albeit at relatively higher enzyme loadings (at 20 FPU/g of cellulose), without the need for a subsequent delignification step (Ewanick et al., 2007; Monavari et al., 2009; Ewanick; 2006). As these pretreatment conditions were significantly less severe than the conditions that had been used previously for the earlier Douglas-fir work, we wanted to determine if the previously observed recalcitrance of the Douglas-fir substrate might have been due to some unique features of that particular wood sample. It should also be noted that the Douglas-fir wood chips utilized in the previous studies (Cullis et al., 2004) were obtained from a log bolt derived from a single tree, which was more than 150 years old, and the log bolt had been stored in an air-dried condition for many months. Thus it was possible that recalcitrance observed with this particular sample may not have been representative of different aged or stored Douglas-fir wood chip samples and those fresh samples may in fact show optimized steam pretreatment conditions that were similar to those observed with other softwood species.

The work described here compared six Douglas-fir wood chip samples from six different trees with a representative Lodgepole pine sample, to assess their responsiveness to a single pretreatment condition (200 °C, 5 min, 4% SO_2) which had previously been shown to be effective for Lodgepole pine and Spruce in terms of both sugar recovery (hemicelluose and cellulose) and subsequent ease of cellulose hydrolysis. In addition, as mentioned previously, the majority of the studies on steam pretreated softwood have evaluated the hydrolysis of the substrate at a relatively high enzyme loading (20–80 FPU/g of cellulose) (Wu et al., 1999; Pan et al., 2004, 2005; Ewanick et al., 2007; Boussaid et al., 2000) which will likely not be economically feasible in a commercial bioconversion scheme (Donghai and Junshe, 2007; Sun and Cheng, 2002; Gregg and Saddler, 1996; Merino and Cherry, 2007). Consequently, we assessed the enzymatic hydrolysis of the steam pretreated softwood substrates at both relatively high (20 FPU/g cellulose) and low enzyme loadings (5 FPU/g cellulose) to determine whether the steam pretreated softwood substrates could be effectively hydrolysed at reduced enzyme loadings.

2. Methods

2.1. Preparation of the wood samples for the pretreatment

Six different Douglas-fir (*Pseudotsuga menziesii*) wood samples and one Lodgepole pine (*Pinus contorta*) sample were used in this study. The Douglas-fir logs were collected from six different trees ranging from 22–107 years old. Three were obtained from the interior of British Columbia (DF1, DF2, DF3) and the rest were obtained from coastal regions of British Columbia (DF4, DF5, DF6). Lodgepole pine wood samples from the same batch which had been used previously by Ewanick et al. (2007) (101 ± 20 years) were used for comparison. All of the wood logs were debarked, split, chipped and screened to an approximate size of $2 \times 2 \times 0.5$ cm^3. The moisture content of the wood chip samples was in the range of 7–11%.

2.2. Pretreatment

Prior to steam pretreatment, the wood chips were impregnated by adding a specified amount of SO_2 (4% wt/wt of the substrate) to sealable plastic bags containing 300 g dry weight of chips (Ewanick et al., 2007). Once the desired amount of SO_2 was added to the bags, the bags were immediately sealed and left for approximately 12 h before steam pretreatment was carried out. Steam pretreatment was conducted in a 2 L StakeTech steam gun at 200 °C for 5 min. After the pretreatment, the entire slurry was removed and the water soluble (WS) and insoluble (WI) fractions were separated by vacuum filtration. The WI fraction was washed with approximately 5 L water and vacuum filtered to a final moisture content of 76–82%. An aliquot of the wash water was also collected to account for the sugar loss during washing. For specified samples, a delignification treatment of the steam pretreated softwood substrates was used, according to Useful Method G.10U of Pulp and Paper Technical Association of Canada (PAPTAC).

2.3. Enzymatic hydrolysis

The solid fractions were enzymatically hydrolysed at 2% (w/wt) consistency in acetate buffer (50 mM, pH 4.8) at 50 °C and 150 rpm. In those experiments where a higher enzyme loading was employed, cellulases were added at 20 FPU/g of cellulose (Celluclast 1.5 L, Novozymes, Bagsvaerd, Denmark) and β-glucosidase (Novozymes 188, Bagsvaerd, Denmark) at 40 CBU per g of cellulose. Low enzyme loading refers to 5 FPU cellulase/g of cellulose and 10 CBU β-glucosidase/g of cellulose, respectively. The Filter paper activity of the cellulase preparation was 60.4 FPU/ml and the cellobiose activity of the cellulase was 0.2 CBU/ml while the cellobiase activity of the β-glucosidase was 360 CBU/ml. Tetracycline (40 μg/ml) and cycloheximide (30 μg/ml) were added to inhibit microbial contamination during the hydrolysis process. At a given enzyme loading, the enzymatic hydrolysis of all the steam pretreated substrates was conducted simultaneously. Sampling of 500 μl supernatant was done at 12, 24, 48 and 72 h from the reaction mixture. The enzyme activity was stopped during sampling by incubating the aliquots of the reaction mixture on a hot plate at 100 °C for 10 min and subsequently stored at −20 °C until sugar analysis was performed.

2.4. Analytical methods

All analysis was done in triplicate. The substrates were analyzed for acid insoluble lignin and carbohydrates using the Tappi-T-22 om-88 as previously described (TAPPI, 1994). The hydrolysate from this analysis was retained and analyzed for soluble lignin by reading the absorbance at 205 nm (Dence, 1992). Sugars were measured on Dionex (Sunnyvale, CA) HPLC (ICS-3000) equipped with an AS 50 auto sampler, ED50 electrochemical detector, GP 50 gradient pump and anion exchange column (Dionex CarboPac PA1). Acetone soluble extractives were estimated using Tappi T 204 om-88 with the following modifications. Briefly, 10 g of air-dried sample was extracted for 8 h with acetone with 6 cycles/h. The acetone in the round-bottomed flask is then evaporated in the fume hood and then dried in the oven at 100 °C overnight to determine the weight of extractives present in the sample flasks.

To assess the substrate accessibility, a modified version of the Simons' staining (SS) procedure (Chandra et al., 2008) was used. Briefly, Direct Blue (DB) (Pontamine Fast Sky Blue 6BX) and Direct

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