Bioresource Technology 179 (2015) 50–57

Contents lists available at ScienceDirect

Bioresource Technology

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

Simultaneous pretreatment and saccharification: Green technology for enhanced sugar yields from biomass using a fungal consortium

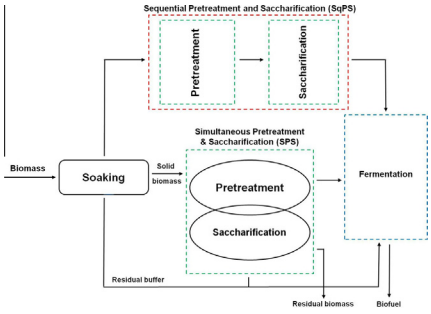
Saurabh Sudha Dhiman [a,b], Jung-Rim Haw [b], Dayanand Kalyani [a], Vipin C. Kalia [c], Yun Chan Kang [d], Jung-Kul Lee [a,b,*]

[a] Department of Chemical Engineering, Konkuk University, 1 Hwayang-Dong, Gwangjin-Gu, Seoul 143-701, Republic of Korea
[b] Institute of SK-KU Biomaterials, Konkuk University, 1 Hwayang-Dong, Gwangjin-Gu, Seoul 143-701, Republic of Korea
[c] Microbial Biotechnology and Genomics, CSIR-Institute of Genomics and Integrative Biology, Mall Road, Delhi 110007, India
[d] Department of Materials Science and Engineering, Korea University, Anam-Dong, Seongbuk-Gu, Seoul 136-713, Republic of Korea

HIGHLIGHTS

- It reports a novel simultaneous pretreatment and saccharification (SPS) methodology.
- High level production of lignocellulases using fungal consortium is achieved.
- Efficient detoxification of biomass in SPS enhances final saccharification yield.
- High yield of biofuel production is achieved from reducing sugars obtained by SPS.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 11 September 2014
Received in revised form 12 November 2014
Accepted 13 November 2014
Available online 20 November 2014

Keywords:
Ethanol
Fungal consortia
Pretreatment
Saccharification
Reducing sugars

ABSTRACT

Two different biomasses were subjected to simultaneous pretreatment and saccharification (SPS) using a cocktail of hydrolytic and oxidizing enzymes. Application of a novel laccase as a detoxifying agent caused the removal of 49.8% and 32.6% of phenolic contents from the soaked rice straw and *willow*, respectively. Hydrolysis of soaked substrates using a newly developed fungal consortium resulted in saccharification yield of up to 74.2% and 63.6% for rice straw and *willow*, respectively. A high saccharification yield was obtained with soaked rice straw and *willow* without using any hazardous chemicals. The efficiency of each step related to SPS was confirmed by atomic force microscopy. The suitability of the developed SPS process was further confirmed by converting the hydrolysate from the process into bioethanol with 72.4% sugar conversion efficiency. To the best of our knowledge, this is the first report on the development of a less tedious, single-pot, and eco-friendly SPS methodology.

1. Introduction

Currently, two separate processes are conducted for the pretreatment and hydrolysis of the biomass (Jeya et al., 2012). Energy is consumed during the pretreatment processes of activation or surface modification of the biomass that are performed to yield substrates readily available for subsequent hydrolysis (Jiang et al., 2012). The amount of energy consumed also correlates to the efficiency of the specific pretreatment process selected (Gomez et al., 2008). Numerous chemical and energy intensive pretreatment processes are currently used for a variety of biomasses. Various pretreatment techniques include ammonia fiber explosion, chemical treatment, biological treatment, and steam explosion,

* Corresponding author at: Department of Chemical Engineering, Konkuk University, 1 Hwayang-Dong, Gwangjin-Gu, Seoul 143-701, Republic of Korea. Tel.: +82 2 450 3505; fax: +82 2 458 0879.
E-mail address: jkrhee@konkuk.ac.kr (J.-K. Lee).

http://dx.doi.org/10.1016/j.biortech.2014.11.059
0960-8524/

which alter the structure of cellulosic biomass to make cellulose more accessible (Lee et al., 2012). A major drawback associated with the use of chemical strategies is the release of bulk phenolic compounds and ecotoxic effluent (Kalyani et al., 2012). Therefore, a process based on enzymatic pretreatment and saccharification is regarded today as the most promising alternative for the conversion of carbohydrates in lignocellulosic materials into useful products with high yields and low production costs (Galbe and Zacchi, 2002).

To date, little emphasis has been placed on research to address the challenge of finding an optimal substrate for saccharification that possesses inherently favorable biomass characteristics for sugar release (Wooley et al., 1999). Several plant species have been investigated for their suitability as dedicated biomass crops, such as switchgrass (*Panicum virgatum*), miscanthus (*Miscanthus giganteus*), and poplar (*Populus* sp.), or for food crop waste and dedicated perennial (non-food) crops, such as fast growing grasses and trees (Jagtap et al., 2012). However, few studies have investigated the optimization of saccharification process and the quality of short rotation coppice (SRC) *willow* (*Salix koreensis*) for this end use. Of the crop feed stocks available, there is considerable potential for the SRC *willow* to be used as a dedicated bioenergy crop for lignocellulosic saccharification and biofuel production (Brereton et al., 2011). However, little work has been done to realistically meet the intended targets for future global demand. As a result of these concerns, interest on sustainable production of biofuels has increased (Sassner et al., 2006).

A high substrate concentration is necessary to lower overall energy utilization and water consumption, as well as to improve the cost-effectiveness of this process. However, the use of high dry matter content leads to an increased concentration of inhibitory compounds, which, at a certain level, results in reduced sugar yield. Therefore, an efficient and ecofriendly strategy that can be performed in a much simpler manner than the biphasic system of pretreatment followed by hydrolysis is required. This may be accomplished by integrating the two major steps of pretreatment and hydrolysis simultaneously within a single pot to develop a monophasic simultaneous pretreatment and saccharification (SPS) process. This SPS process would also alleviate end-product inhibition of the enzymes, and would be less expensive than sequential pretreatment and hydrolysis (SqPS).

The primary objective of the present study was to develop a new integrated methodology for pretreatment and saccharification using activated biomasses by soaking method. Two different biomasses, SRC *willow* and agro-residual (rice straw) waste, were used to broaden the scope of the methodology. Comparative analysis was performed using the alkali pretreatment method, followed by hydrolysis with lab-scale-produced enzyme as a reference. To the best of our knowledge, this is the first report to describe the development of integrated pretreatment and saccharification methods using lab-scale-produced enzymes from a fungal consortium. In addition, Baker's yeast, *Saccharomyces cerevisiae* (Jastbolaget AB, Rotebro, Sweden), which ferments glucose and mannose, was used for fermenting the substrate consisting of slurries obtained from the SPS strategy.

2. Methods

2.1. Strain identification

Soil samples collected from Sorak Mountain, Republic of Korea, were diluted in sterile dilution solutions (0.9% saline) using a capillary tube method. Aliquots were spread onto agar plates containing 2% carboxymethyl cellulose (CMC) and then incubated for 3 days for screening of the fungal cultures. The isolated strain was identified based on the internal transcribed spacer of ribosomal DNA (ITS rDNA) sequence analysis. For the sequence analysis, the ITS1-5.8 S-ITS2 rDNA region of the fungi were amplified by polymerase chain reaction (PCR) using the following primer set: pITS1 (5′-TCCGTAGGTGAACCTGCCG-3′) and pITS4 (5′-TCCTCCGCTTATTGAT-ATGC-3′) (White et al., 1990). Two different amplicons of 780-bp and 592-bp were obtained, cloned, and sequenced. Similarly, a new strain for laccase production was isolated on agar plates containing 0.2% of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) as the substrate. The ITS1-5.8 S-ITS2 rDNA region was amplified using a set of specific primers. Pairwise evolutionary distances and a phylogenetic tree were constructed using the MegAlign software (DNA Star, Madison, WI, USA) for all the isolated strains (Clewley and Arnold, 1997).

2.2. Evaluation of fungal consortia

All the previous information related to enzyme production individually from *Pholiota adiposa* (Jagtap et al., 2013b) and *Armillaria gemina* (Dhiman et al., 2013) was taken into account for the optimized production of enzyme using a fungal consortium. Different nutritional and physical parameters that could potentially affect enzyme production were optimized using a 'one-variable-at-a-time' strategy. Ranges of physical parameters, including temperature, pH, and RPM, were selected based on previous studies (Jagtap et al., 2013a,b). Xylanase activity of the crude extract was assayed using birchwood xylan by the modified Bailey's method (Bailey et al., 1992) with a dinitrosalicylic acid (DNS) reagent (Miller, 1959). Similarly, endoglucanase (EG), β-glucosidase (BGL), and cellobiohydrolase (CBH) activities of the crude extract was determined using CMC, *p*-nitrophenyl glucoside (*p*NPG), and *p*-nitrophenyl cellobioside (*p*NPC) as substrates, respectively, according to standard procedures (Lee et al., 2012). Filter Paper activity Unit (FPU) can be defined as the amount of enzyme that released 1 μmol of glucose equivalents from Whatman No. 1 filter paper per min (Jagtap et al., 2013a,b; Lee et al., 2012). Bradford's method was used to estimate the protein content in all the enzyme samples (Bradford, 1976). All the experiments were performed independently in triplicates, and the results are presented as the mean of the three independent values.

2.3. Biomass

All the biomass samples were procured from Phygen Co. Ltd. (Daejeon, Korea). Two different lignocellulosic biomasses, *willow* (*S. koreensis*) and RS (*Oryza sativa* L.), were used as the substrates for hydrolysis. The shoots of *willow* were harvested and immediately chopped and placed into plastic bags. Both the substrates were stored at 4 °C until further processing. The dry matter content of the fresh substrate chips was approximately 46.8% and 86.7% for *willow* and RS, respectively. The moisture, ash, and structural carbohydrate/lignin content were determined according to the standardized methods of the National Renewable Energy Laboratory (NREL, Golden, CO, USA) protocols NREL/TP-510-42621, 42622, and 42618, respectively (Sluiter et al., 2008).

2.4. Front-end processing

The *willow* shoots were milled using a laboratory hammer mill. Milled material was further separated (size reduced to about 8–10 mm) using a portable sieve shaker. The *willow* chips were screened to remove all the particles greater than 15 mm and less than 6 mm in length to ensure smooth operation during disk