Bioresource Technology 241 (2017) 228–235

Contents lists available at ScienceDirect

Bioresource Technology

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

An integrated process to produce bio-ethanol and xylooligosaccharides rich in xylobiose and xylotriose from high ash content waste wheat straw

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HIGHLIGHTS

- An integrated process for bio-ethanol and XOS productions from straw pulping residue.
- Prewashing step greatly improved enzymatic glucose yield and XOS content.
- Highly similar ethanol yields were obtained from SSF at varied substrate loadings.
- Enzymatic post-hydrolysis significantly increased xylobiose and xylotriose.

ARTICLE INFO

Article history:
Received 22 March 2017
Received in revised form 16 May 2017
Accepted 17 May 2017
Available online 19 May 2017

Keywords:
Waste wheat straw
Bio-ethanol
Xylooligosaccharides
Prewashing
Liquid hot water pretreatment

ABSTRACT

A bio-refinery process of wheat straw pulping solid residue (waste wheat straw, WWS) was established by combining prewashing and liquid hot water pretreatment (LHWP). The results showed that employing a prewashing step prior to the LHWP remarkably improved enzymatic glucose yields from 39.7% to 76.6%. Moreover, after 96 h simultaneous saccharification and fermentation (SSF), identical ethanol yields of 0.41 g/g-cellulose were obtained despite varied solid loadings (5–30%). Beyond ethanol, enzymatic post-hydrolysis of the prehydrolyzate effectively increased xylobiose and xylotriose yields from 15 mg/g-WWS and 14 mg/g-WWS to 53 mg/g-WWS and 20 mg/g-WWS, respectively. For mass balance, about 10.9 tons raw WWS will be consumed to produce 1 ton ethanol, in addition to producing 614.8 kg xylooligosaccharides (XOS) containing 334.3 kg xylobiose and 124.8 kg xylotriose. The results demonstrated that the integrated process for the WWS bio-refinery is promising, based on value-adding co-production in addition to robust ethanol yields.

1. Introduction

The worldwide rapid growth of energy demand, the decreased reserve of fossil fuel, and the concern for global climate change have spurred us to search for renewable resources. Bio-ethanol based on lignocellulose is a promising alternative energy of fossil fuels (Liu and Chen, 2016). Lignocellulosic biomass, the most abundant and sustainable resource worldwide, is predominantly composed of the biopolymers cellulose, hemicellulose and lignin in different proportions. Cellulose and hemicellulose are polymers comprised of glucose and other carbohydrate residues. When these polymers are successfully hydrolyzed into their monomeric constituents, the resultant monosaccharides are capable of being further converted to second generation bio-fuels, e.g. bio-ethanol. The main steps during bio-ethanol production involve sequential pretreatment, enzymatic hydrolysis/saccharification, fermentation, and finally, distillation (Gu et al., 2012). Biomass pretreatment processes aim to break the solid matrix present within lignocellulosic materials, with intention to enhance enzymatic digestibility of the pretreated samples. An effective pretreatment method must meet the following requirements: (1) improving the enzymatic digestibility to decrease enzyme dosages; (2) increasing the cellulose recovery yield and concentrations of the target products while simultaneously limiting production of byproducts; (3) the pretreatment process should be classified as environmentally-friendly (Hendriks and Zeeman, 2009). Amongst the various pretreatment methods, liquid hot water pretreatment (LHWP) has come to focus as a "green technology", that is, without impregnation of chemical additives prior to the pretreatment process. The LHWP predominantly degrades hemicellulose without

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http://dx.doi.org/10.1016/j.biortech.2017.05.109
0960-8524/

causing significant degradation of cellulose and lignin (Kim et al., 2009; Narron et al., 2016; Weil et al., 1997). LHWP has been demonstrated as an effective pretreatment across a wide temperature range of 130–230 °C (Garrote and Parajó, 2002). The extent of degradation depends upon cooking severity (temperature and time). When biomass is subjected to LHWP at a minor severity, solubilized hemicellulose can be mainly recovered in the form of oligosaccharides that can be further purified for valuable prebiotic applications (Gullón et al., 2008). Finally, the remaining cellulose reserved within the pretreated solids can be hydrolyzed by enzymes to glucose and further fermented to ethanol.

Recently, identifying and securing a reliably-obtainable and low-cost feedstock has been a key obstacle towards successful industrialization of the bio-ethanol from lignocellulosic biomass. Waste wheat straw (WWS), the solid residue remaining as a by-product from the wheat straw pulping industry, has the potential to satisfy both aforementioned needs (availability and low cost). Generally, the WWS is separated from crude wheat straw by screening and is mainly composed of wheat leaves, ears, straw scraps and large amount of ash, which is not utilizable for pulp making. It is estimated that the annual generation of the WWS from a Chinese straw pulp mill can be as high as 2×10^5–4×10^5 tons per year. However, most of the residue is simply burned, representing cost-ineffective usage, in addition to causing potential environmental harm. The near-zero cost of the WWS as feedstock for a co-located bio-refinery within a straw pulp mill is a compelling potential economic advantage which merits further investigation.

A different goal for enabling the establishment of industrial-scale bio-refinery processes involves obtaining high titer ethanol concentrations in the fermentation broth to lower the energy cost of the necessary distillation operations (Cannella et al., 2014). Simultaneous saccharification and fermentation (SSF), which immediately converts the sugars to ethanol during the enzymatic hydrolysis process, is one of the more feasible ways of obtaining the desired high titer ethanol solutions from fermentation. Compared to separate hydrolysis and fermentation (SHF), SSF has been shown to effectively increase ethanol yields while eliminating end-product inhibition between glucose and cellulose degrading enzymes (Saha et al., 2011). In addition, SSF does not require any separation operations between enzymatic hydrolysis and fermentation. High solid loading SSF has recently become a tendency for producing bio-ethanol due to the high ethanol titer obtainable by SSF (Koppram and Olsson, 2014). However, mass and heat transfer limitations significantly hinder ethanol yields at high solid loadings (Pimenova and Hanley, 2003). An alternative solution to this obstacle is the employment of fed-batch protocols during enzymatic hydrolysis (Rudolf et al., 2005).

Compared to other lignocellulosic feedstocks, the WWS contains a significantly larger amount of ash. When the LHWP is applied to WWS without any de-ashing steps, the efficiency of both pretreatment and enzymatic hydrolysis is known to be very low (He et al., 2014). In the current work, we carried out a prewashing treatment prior to the LHWP to remove the WWS-borne ash. After pretreatment, the solid fraction was subjected to SSF for ethanol production, and the liquid fraction was treated with endo-β-1-4-xylanase to produce xylooligosaccharides rich in xylobiose and xylotriose. In addition, we sought to experiment upon further increasing the solid loadings for SSF and fed-batch fermentation.

2. Materials and methods

2.1. Raw material

The WWS was provided by a wheat straw pulp mill in Shandong province, China. The raw WWS was stored in a dark room for air drying before prewashing treatment and LHWP. The moisture content of the raw WWS after drying was 8.8%.

2.2. De-ashing treatment and liquid hot water pretreatment

The de-ashing treatment operation in this work has been previously described in great detail (Huang et al., 2016). The raw WWS was prewashed with different water dosages of 10, 50, 100, 300 and 500 times the weight of the raw WWS. 200 g raw WWS (dry-basis) was soaked in water for 10 min with agitation, and then collected by centrifugation. After that, the wet substrate was stored at 4 °C prior to the LHWP without any drying steps.

The LHWP was carried out in ten steel reaction vessels immersed in a hot oil bath. For each treatment, 50 g (dry-basis) raw or prewashed WWS was loaded into the reactors and then supplemented with the appropriate volume of deionized water (accounting for biomass moisture content) to set the final solid to liquid ratio at 1:10. After loading, the reactors were lowered into the oil bath, which was heated at the rate of 1 °C/min from room temperature to the target temperature (140–220 °C). Once target temperature was achieved, the vessels remained in the hot oil bath for 20–80 min. After pretreatment, the reactors were cooled by submersion into a cold water bath containing ice. After sufficient cooling, the solid and liquid fractions were separated utilizing cheesecloth, with the solid fraction next being subjected to PFI milling without any post-washing treatments.

The PFI milling was performed using a PFI refiner with a plate opening of 0.005 in. About 20 g (dry weight) pretreated sample was added to appropriate volume of water to set the final moisture content around 90%, and then subjected to 8000 revolutions of PFI milling. After that, the milled solid was collected by cheesecloth, air-dried, and finally stored at room temperature for future experiments.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was carried out in 150 mL flasks with a solid loading of 5% (w/v). 2.5 g (dry weight) of pretreated WWS was loaded into the flasks along with 2.5 mL 1 M citrate buffer to control the system pH around 4.8. An efficient cellulase (*Cellic® CTec2*) kindly provided by *Novozymes* (Franklinton, NC, USA) with filter paper activity of 250 FPU/mL (150 mg protein/mL) was then added into the enzymatic hydrolysis system at a dosage of 25 FPU/g-cellulose (15 mg protein/g-cellulose). Next, an additional volume of deionized water was added to set the volume 50 mL. Finally, the flasks were incubated at 50 °C and 150 rpm for 48 h. After enzymatic hydrolysis, the samples were taken and diluted for HPLC (high performance liquid chromatography) analysis of sugar products. All experiments were carried out in duplicate.

2.4. Microorganism and cultivation

The glucose fermenting strain *S. cerevisiae* was provided by Biochemical Engineering Research Institute of Nanjing Forestry University. The *S. cerevisiae* seed medium contained: glucose 20 g/L, peptone 5 g/L, and yeast extract 3 g/L. The strain was cultivated in flasks with 300 mL of seed medium for 24 h (30 °C, 150 rpm), and then transferred to another fresh seed medium for further cultivation. After 3 rounds of sufficient cultivation, the cells were collected by centrifugation (at 1705*g* for 10 min) and washed three times with deionized water to remove residual sugars. Then, 50 mL sterilized water was used to re-suspend the cells, and the number of the cells was counted visually under microscope after appropriate dilution.