Bioresource Technology 214 (2016) 30–36

Contents lists available at ScienceDirect

Bioresource Technology

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

Cellulosic bioethanol production from Jerusalem artichoke (*Helianthus tuberosus* L.) using hydrogen peroxide-acetic acid (HPAC) pretreatment

Younho Song [a], Seung Gon Wi [b], Ho Myeong Kim [a], Hyeun-Jong Bae [a,b,*]

[a] Department of Bioenergy Science and Technology, Chonnam National University, Gwangju 500-757, Republic of Korea
[b] Bio-energy Research Center, Chonnam National University, Gwangju 500-757, Republic of Korea

HIGHLIGHTS

- Jerusalem artichoke is a suitable candidate biomass crop for bioethanol production.
- Hydrogen peroxide-acetic acid (HPAC) pretreatment can effectively remove lignin.
- Optimized enzyme cocktails enhanced enzymatic hydrolysis of Jerusalem artichoke.
- Fermentation of the hydrolyzates with *S. cerevisiae* gave a fermentation yield of 84%.

ARTICLE INFO

Article history:
Received 11 February 2016
Received in revised form 14 April 2016
Accepted 15 April 2016
Available online 19 April 2016

Keywords:
Biomass feedstock
Bioethanol production
Enzyme optimization
Jerusalem artichoke
Pretreatment

ABSTRACT

Jerusalem artichoke (JA) is recognized as a suitable candidate biomass crop for bioethanol production because it has a rapid growth rate and high biomass productivity. In this study, hydrogen peroxide-acetic acid (HPAC) pretreatment was used to enhance the enzymatic hydrolysis and to effectively remove the lignin of JA. With optimized enzyme doses, synergy was observed from the combination of three different enzymes (RUT-C30, pectinase, and xylanase) which provided a conversion rate was approximately 30% higher than the rate with from treatment with RUT-C30 alone. Fermentation of the JA hydrolyzates by *Saccharomyces cerevisiae* produced a fermentation yield of approximately 84%. Therefore, Jerusalem artichoke has potential as a bioenergy crop for bioethanol production.

1. Introduction

Lignocellulosic biomass from dedicated bioenergy crops and agricultural wastes has been considered as a potentially cheap, abundant and renewable feedstock to produce energy and replace fossil fuels used for transportation (Sánchez and Cardona, 2008; Kootstra et al., 2009). Bioenergy production from lignocellulosic biomass is, however, more difficult to accomplish than starch- or molasses-based biomass feedstock because lignocellulosic biomass is more difficult to hydrolyze using cellulolytic enzymes (Fernandes et al., 2009; Alvira et al., 2010). The biomass of plant cell walls is composed of complex and compact materials, including cellulose, hemicellulose, and lignin (Zhao et al., 2012; Mood et al., 2013). This complexity inhibits the accessibility of enzymes and limits digestibility (Gupta et al., 2011).

To improve enzymatic hydrolysis, lignocellulosic biomass requires pretreatment to change the chemical composition and structure of the cell wall (Kumar et al., 2009; Yu et al., 2009; Gao et al., 2014). Generally, hemicellulose and lignin can be broken down during pretreatment. Enzymatic hydrolysis of cellulose to fermentable sugars is enhanced significantly by partial removal of lignin (Sun and Cheng, 2002; Kim et al., 2003; Nakagame et al., 2009). Hydrogen peroxide-acetic acid (HPAC) pretreatment is effective in removing lignin from biomass (Tan et al., 2010; Wi et al., 2015). The lignin content of HPAC-treated pine, oak wood, bagasse, and rice straw was reduced by approximately 98%, 97%, 97%, and 85%, respectively.

Many microorganisms such as fungi can produce and secrete cellulolytic enzymes to degrade plant cell walls (Wilson, 2011). Among them, *Trichoderma reesei* is known to secrete large amounts of cellulolytic enzymes (Ilmén et al., 1997; Saloheimo and Pakula, 2012). A mutant strain, *T. reesei* RUT-C30, produces more cellulase than the wild type. The hypercellulolytic RUT-C30 strain produces approximately 2.7 times the amount of extracellular protein, has

* Corresponding author at: Department of Bioenergy Science and Technology, Chonnam National University, Gwangju 500-757, Republic of Korea.
E-mail address: baehj@chonnam.ac.kr (H.-J. Bae).

http://dx.doi.org/10.1016/j.biortech.2016.04.065
0960-8524/

2.8 times the filter paper activity, and has twice the endoglucanase activity of the wild type (Peterson and Nevalainen, 2012).

Jerusalem artichoke (*Helianthus tuberosus* L.) is one of potential crop for bioenergy production from biomass. It is an herbaceous perennial plant species, related to sunflower (*Helianthus annuus* L). This plant has several advantages as a raw biomass material including fast growth, high production, minimal requirements for pesticide, fertilizer, or water, and it can be grown on marginal land (Long et al., 2014; Yang et al., 2015). In addition, it contains a large holocellulose component, containing approximately 50% cellulose and 10% hemicellulose (Zhou et al., 2013). Due to this advantage, JA for ethanol production has previously been reported (Bajpai and Margaritis, 1986). Bioethanol production of JA was reported to use dilute acid and alkali pretreatment (Kim and Kim, 2014). However, the hydrolysis of dilute acid and alkali treated JA have used a large amount of cellulase due to the low delignification efficiencies.

In this study, the Jerusalem artichoke stalk (JAS) was evaluated as a potential bioenergy feedstock to produce bioethanol. Although aggressive growth is expected, bioethanol production from JAS is still at the development stage. HPAC pretreatment and various extracellular enzyme cocktails were tested to enhance enzymatic hydrolysis. In addition, analysis of the anatomical features was also provided essential information required for the better understanding of the control and HPAC-pretreated JAS.

2. Materials and methods

2.1. Cultivation and preparation of Jerusalem artichoke

Jerusalem artichoke (JA) was planted on April 11, 2014 at Chonnam National University, Gwangju in South Korea. The average temperature for the growing season was 28 °C. Initial flowering began 22 weeks after planting, and the artichokes were harvested at maturity at the end of November. JA stalks were naturally air-dried for 3 days, and then chopped using a chopping machine.

2.2. Light, polarized, and fluorescence microscopy

Samples were soaked in water and then 20 μm thick sections were cut transversely at several heights on the stalks. Cross-sections were stained with safranin to microscopically observe tissues using a Zeiss microscope.

To compare the morphological differences before and after pretreatment, cross-sections (20 μm thick) were treated with a mixture of acetic acid and hydrogen peroxide for 2 h and then gently wash with distilled water. Unstained sections were mounted in water and observed through birefringence and auto-fluorescence. The auto-fluorescence was recorded using the UV filter cube (excitation filter band pass 330 nm to 385 nm and barrier filter 420 nm).

2.3. HPAC pretreatment of Jerusalem artichoke stalks

Jerusalem artichoke stalks was treated using HPAC pretreatment method described by Wi et al. (2015) and slightly modified in this experiment. Sample of 10% (w/v) chopped JA stalks were soaked in HPAC solution (hydroperoxide: acetic acid = 5:5, (v/v)) and then incubated at 80 °C for 3 h. HPAC-pretreated JA stalks were filtered to separate the HPAC solution from the solid residue, and the solid residue was washed with flowing tap water to remove the remaining HPAC solution. HPAC-treated JA stalks were dried using a lyophilizer at −45 °C for 5 days.

2.4. Chemical composition analysis of Jerusalem artichoke stalks by gas chromatography

The chemical composition (lignin, organic solvent extractives, and ash) of r raw and HPAC pretreated JA stalk sample was determined using TAPPI Standard Methods (1992). The Jerusalem artichoke stalks were analyzed for their neutral sugar content using gas chromatography (GC). Each raw and HPAC pretreated JA stalk sample was treated with 0.25 mL of 72% (v/v) sulfuric acid (H_2SO_4) for 45 min at 30 °C and was diluted with distilled water to 4% (v/v) H_2SO_4. The hydrolysis step was performed at 121 °C for 1 h, and a solution containing a known amount of myo-inositol was used as an internal standard and was neutralized with ammonia water. A sodium borohydride solution (1 mL) and 0.1 mL of glacial (anhydrous) acetic acid (18 M) were added in order to degrade the sodium tetrahydroborate. Next, 0.2 mL of methyl imidazole and 2 mL of anhydrous acetic acid were sequentially added, and finally, 5 mL of deionized water was added and extracted with 2 mL of dichloromethane. The samples were analyzed via GC (GC-2010; Shimadzu, Otsu, Japan) using a DB-225 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W; Agilent, Folsom, CA, USA) operated with helium. The operating conditions were as follows: injector temperature of 220 °C, flame ionization detector (FID) at 250 °C, and an oven temperature of 110 °C for 1.5 min with a constant increase of 10 °C/min to 220 °C.

2.5. Production of enzymes for the hydrolysis of Jerusalem artichoke stalk

T. reesei RUT-C30 was obtained from the Korean Collection for Type Cultures (KCTC 6968), it was grown in PDB media at 25 °C for 3 days. *T. reesei* mycelia was inoculated in Czapek–Dox medium with 1% (w/v) Avicel as a carbon source and an inducer, and incubated at 25 °C for 1 week. The culture medium was centrifuged at 3500 rpm at 4 °C for 10 min, and the cellulase activity of supernatant was the evaluated by the NREL method (Adney and Baker, 2008).

2.6. Enzymatic hydrolysis

2.6.1. Hydrolysis of Jerusalem artichoke stalk before and after pretreatment

The raw and pretreated JA stalks as 1% (w/v) substrate were soaked in 50 mM sodium citrate buffer (pH 5.0) supplemented with 0.01% (w/v) sodium azide. The loadings of RUT-C30 cellulase were 30 FPU per gram of glucan and all samples were completely suspended in a rotary shaker at 200 rpm at 37 °C. All enzymatic hydrolysis experiments were performed in triplicate.

2.6.2. Optimization of enzyme loading

To optimize the hydrolysis, 1% (w/v) pretreated JA stalks were hydrolyzed using three different enzymes RUT-C30, commercial pectinase (Pectinex Ultra SP-L; Novozymes, Denmark) and xylanase (endo-1, 4-β-xylanase from *Trichoderma longibrachiatum*, Sigma–Aldrich, USA) and evaluated for three different hydrolysis times at 37 °C. The pectinase and the xylanase were loaded from 0 to 10 in steps of 2.5 μg mg^{-1} substrate and 0 to 30 μg mg^{-1} substrate in steps of 7.5 μg mg^{-1} substrate, respectively. The concentration of the total released sugar was measured via the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959).

2.7. Separate hydrolysis and fermentation

Enzymatic saccharification was conducted in a 500 mL Erlenmeyer flask with a total working volume of 150 mL at a substrate concentration of 10% DM (w/v) with 0.1% (w/v) yeast extract,