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Evaluation of whole Jerusalem artichoke (*Helianthus tuberosus* L.) for consolidated bioprocessing ethanol production

Seonghun Kim [a,b,*], Chul Ho Kim [a]

[a] Jeonbuk Branch Institute, Korea Research Institute of Bioscience and Biotechnology, 181 Ipsin-gil, Jeongeup 580-185, Republic of Korea
[b] Synthetic Glycobiology Research Unit, Korea Research Institute of Bioscience and Biotechnology, 181 Ipsin-gil, Jeongeup 580-185, Republic of Korea

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ABSTRACT

For consolidated bioprocessing (CBP), components of Jerusalem artichoke (*Helianthus tuberosus* L.) tubers and stalks as a potential bioenergy crop were analyzed as carbon and nutrient sources, respectively. The effectiveness of chemical pretreatment with dilute acid or alkali was evaluated to develop a CBP method. Cellulose content, delignification, and enzymatic hydrolysis efficiency of the pretreated stalks were increased more effectively by NaOH treatment than dilute H_2SO_4 treatment. However, weight loss was greater during alkali pretreatment. Additionally, large volumes of water were required to wash the alkali-treated biomass. Therefore, CBP using the dilute acid-pretreated stalk and the ground tuber of *Kluyveromyces marxianus* were investigated. Fermentation of both pretreated stalks and tubers by *K. marxianus* with no nutrient supplementation proceeded acceptably. At 10% (w/v) stalk and 8% (w/v) tuber loading, *K. marxianus* produced 45.3 g/L ethanol after 30 h. The ethanol yield was 0.252 g ethanol per g dry biomass, or 0.32 g ethanol per g fermentable sugars, with a fermentable sugar conversion rate of 60%. These results suggest a cost-effective CBP strategy for bioethanol production from the whole Jerusalem artichoke plant.

1. Introduction

The Jerusalem artichoke (*Helianthus tuberosus* L.) is a tuberous plant belonging to the sunflower family. It accumulates inulin in its roots, tubers, and stalks [1]. This plant has been reported as a potential alternative sugar crop, obtaining a yield of more than 90 tons per hectare by fresh biomass weight, a carbohydrate yield of ~4–15 tons per hectare [2,3]. Moreover, the plant grows well on poor land and is resistant to harsh climatic conditions, such as frost or drought [2–4]. It can grow to a height of 2–4 m, has opposite or alternate leaves on the lower part of the stem, 3–20 cm long × 5–8 cm broad, has yellow flowering heads, and produces tubers that grow in the ground [5]. Jerusalem artichoke tubers consist of 75–79% water, 2–3% protein, and 15–16% carbohydrate [4]. The tuber contains inulin, a linear fructan, in which the polymer of fructose consists of β(2→1) linkages with glucose termination. It is an excellent biomass crop resource for renewable bioenergy production, such as bioethanol, methane from anaerobic digestion, and biogas from pyrolysis [6–8]. Bioethanol production using both the tuber and its extract has been investigated extensively [2,4,9–11]. Despite being the bulkiest part of the plant, use of the Jerusalem artichoke stalk, which contains lignocellulosic materials, in bioenergy production has been studied less intensively [4,6].

Recently, the development of cost-effective process technologies for bioethanol production from lignocellulosic biomass has been investigated actively [12]. Consolidated bioprocessing (CBP), in which biomass pretreatment, cellulose hydrolysis, and fermentation are performed in a single step, is a promising approach to low-cost hydrolysis and fermentation of lignocellulosic biomass [12]. Jerusalem artichoke contains a high inulin content in the whole plant; i.e., the tuber and stalk. Thus, it is considered a candidate for the development of CBP methods. Moreover, the whole Jerusalem artichoke may represent a carbohydrate and nitrogen source for ethanol fermentation with no need for addition of supplementary nutrients [4]. However, the suitability of the tuber as a nutrient and carbon source for ethanol production is unknown. The productivity of tubers and stalks is dependent on growth conditions, including climatic conditions, cropping techniques, and genetic factors [13].

Additionally, the xylem, phloem, and cambium in the stalk consist of a rigid cellulose structure, cross-linked with amorphous hemicellulose and a lignin. Pretreatment is necessary to convert

* Corresponding author. Jeonbuk Branch Institute, Korea Research Institute of Bioscience and Biotechnology, 181 Ipsin-gil, Jeongeup 580-185, Republic of Korea. Tel.: +82 63 570 5113; fax: +82 63 570 5109.
E-mail address: seonghun@kribb.re.kr (S. Kim).

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these complex lignocellulose structures into more digestible forms, because these rigid structures are too chemically complex and too resistant to enzymatic hydrolysis to be used in generation of fermentable sugars [14]. Acid or alkali pretreatments, combined with high temperature or high pressure, have been used as conventional chemical treatment processes. Acid and alkali pretreatments of lignocellulosic biomass are known to reduce hemicellulose and lignin contents, respectively [14,15]. Sequential acid + alkali pretreatments effectively eliminate the hemicellulose and lignin components, enhancing enzymatic digestibility [16–18]. Nevertheless, the pretreatment(s) most suitable for CBP ethanol production using the whole Jerusalem artichoke plant remain unknown.

In this study, we analyzed the components of Jerusalem artichoke stalk and tuber for use in whole-plant ethanol production. To develop a consolidated bioprocess, we compared acid and alkali stalk pretreatments to enhance the cellulose content and increase enzymatic digestibility. Furthermore, we evaluated the advantages and disadvantages of the chemical pretreatment of the biomass. Finally, we investigated consolidated bioprocessing of Jerusalem artichoke pretreated with dilute H_2SO_4 for ethanol production using the inulinase-harboring yeast strain *Kluyveromyces marxianus*.

2. Materials and methods

2.1. Materials

The regional clone Jerusalem artichoke (*H. tuberosus* L.) stalks and tubers were harvested from a field in Jeongeup, Jeonbuk, South Korea, in mid December. The stalks were chopped into 3–5 cm lengths for chemical pretreatment. The tubers were cleaned of soil and other particles with tap water and then dried completely at 60 °C for 96 h. The dried tubers were ground in a grinder to an average size of 2–4 mesh. Cellic CTec2 cellulase was provided by Novozymes Korea (Seoul, Republic of Korea). All chemicals and reagents used in this study were of analytical grade.

2.2. Chemical pretreatments of Jerusalem artichoke (JA) stalks

Based on our previous acid pretreatment results, the chopped dry stalks were soaked in 0.1–8.0% (v/v) H_2SO_4 and then autoclaved at 121 °C, 15 psi, for 60 min [18]. The thermal-acid treated JA stalks were then dried at 105 °C for 24 h. For alkali pretreatment, physically prepared stalks were soaked in 0.1–8 M (0.4–32% w/w) NaOH, and were then thermally treated as described above. The thermal-alkali treated stalks were then washed with flowing tap water to remove residual NaOH. The waste water generated in the washing step was collected to determine the volume required for alkali pretreatment. The washed, alkali-treated biomass was dried in a drying oven under conditions to those described above. All chemical pretreatments of JA stalks were performed independently in triplicate. The compositions of non-treated and chemically pretreated JA stalks were analyzed according to the protocols described in the National Renewable Energy Laboratory (NREL) chemical analysis and testing laboratory analytical procedures (LAPs) of the US Department of Energy (DOE).

2.3. Carbohydrate composition and ion analysis in Jerusalem artichoke tubers

To assess the carbohydrate composition of the Jerusalem artichoke tuber, dried tuber was ground into a powder. Specifically, 1 g of tuber powder was dissolved in 10-mL distilled water and then titrated with conc. H_2SO_4 to pH 1.0. JA tuber powder in dilute acid was autoclaved at 121 °C, 15 psi, for 60 min. After acid hydrolysis, the solution was passed through a 0.20-μm filter (DISMIC-13HP; Toyo Roshi Kaisha, Tokyo, Japan). The monosaccharides hydrolyzed from the tubers were then identified using a high-performance liquid chromatography (HPLC) system, as described below.

To assess their ion contents, harvested fresh Jerusalem artichoke tubers ($n = 10$) were cut into thin slices, within ~0.2 g. The slices were soaked in 1 mL of distilled water. To prepare extracts, tubers in distilled water were disrupted completely by sonication for 1 h. The extracts were then mixed by vortexing for 30 min. The homogenized tubers were centrifuged (13,000 rpm, 4 °C) and the supernatants were harvested and passed through a 0.20-μm filter for ion content analysis. Ion contents were determined using an ion chromatography (IC) system, as described below.

2.4. Enzymatic saccharification of the chemically pretreated Jerusalem artichoke stalks

Enzymatic hydrolysis of chemically pretreated Jerusalem artichoke (JA) stalks was performed in a 50-mL cap-tube filled with a 10-mL working volume using Cellic CTec2 cellulase. The pretreated biomass (10% w/v) was soaked in phosphate buffer (pH 6.0) and then cellulase was added at 20–80 FPU (filter paper unit) per g dry biomass. Enzymatic hydrolysis was carried out in a shaking incubator at 37 °C, 240 rpm, for 48 h. Samples were withdrawn at each time point and then centrifuged (13,000 rpm, 10 min). The supernatant was then subjected to HPLC for determination of the glucose and xylose sugars contents resulting from the enzymatic hydrolysis.

2.5. Microorganism and growth conditions

K. marxianus CBS1555 (KCTC7001) was used as the ethanol fermentation strain. The yeast strain was cultivated in YPD medium (1% Bacto yeast extract, 2% Bacto peptone, and 2% glucose) at 30 °C, 200 rpm, for 24 h. The 1% (v/v) seed culture was inoculated in distilled water containing 10% (w/v) dried tuber powder and then further cultivated at 30 °C, 240 rpm, for 24 h. Growth was monitored in terms of colony forming units (CFUs). An aliquot of the culture was withdrawn at each time point and spread on a YPD plate. Plates were incubated at 30 °C for 24 h and colonies counted.

2.6. Consolidated bioprocessing ethanol production

For *in situ* biomass pretreatment, a 5-L fermenter (R'ALF; Bioengineering AG, Wald, Switzerland) containing 10% (w/v) dried Jerusalem artichoke stalk in 1.5 L of 0.5% (v/v) H_2SO_4 was autoclaved at 121 °C, 15 psi, for 60 min. The acid hydrolyzate was neutralized to pH 6.0–6.5 using solid $Ca(OH)_2$. The $CaSO_4$ precipitate was not removed from the fermenter after neutralization. Dried tuber powder (8% w/v) was then added. A seed culture for ethanol fermentation of *K. marxianus* was inoculated in distilled water containing 10% (w/v) dried tuber powder as the sole carbon and nutrient source and then further cultured at 30 °C, 240 rpm, for 24 h. The pre-culture in tuber medium was then inoculated into a 5-L fermenter (~1.5 L working volume) containing 10% (w/v) acid-pretreated stalk and 8% (w/v) dried tuber powder, to which Cellic CTec2 cellulase was added at 30 FPU per g dry stalk. The fermenter was operated at 30 °C with stirring at 120 rpm. An overview of the procedure is shown in Fig. 1.

2.7. Analytical procedures

The sugar, organic acid, and ethanol yields of the fermentation were determined using a high-performance liquid chromatography