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Optimization of pretreatment, enzymatic hydrolysis and fermentation for more efficient ethanol production by Jerusalem artichoke stalk

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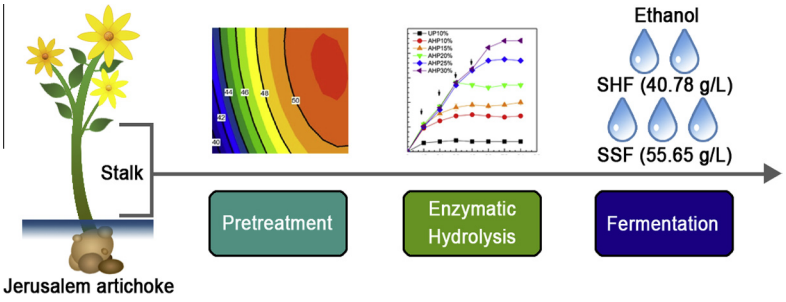
HIGHLIGHTS

- Non-food feedstock JAS suits ethanol production.
- Regression analysis optimized AHP pretreatment.
- Stepwise addition of biomass and enzyme benefited high mass loading.
- SSF performed better than SHF under high mass loading.

GRAPHICAL ABSTRACT



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ABSTRACT

Jerusalem artichoke (JA) is a potential energy crop for biorefinery due to its unique agronomic traits such as resistance to environmental stresses and high biomass yield in marginal lands. Although JA tubers have been explored for inulin extraction and biofuels production, there is little concern on its stalk (JAS). In this article, the pretreatment of JAS by alkaline hydrogen peroxide was optimized using the response surface methodology to improve sugars yield and reduce chemicals usage. Scanning electron microscopy, X-ray diffraction, and thermogravimetric analysis were applied to characterize the structures of the pretreated JAS to evaluate the effectiveness of the pretreatment. Furthermore, the feeding of the pretreated JAS and cellulase was performed for high solid uploading (up to 30%) to increase ethanol titer, and simultaneous saccharification and fermentation with 55.6 g/L ethanol produced, 36.5% more than that produced through separate hydrolysis and fermentation, was validated to be more efficient.

1. Introduction

At present, starch- and sugar-based biomass is still major resources for the production of biofuels and bio-based chemicals, which is not sustainable, particularly in developing countries. Therefore, non-food related feedstock has to be developed. Jerusalem artichoke (JA) is tolerant to environmental stresses such as drought, salinity and plant diseases and pests, which can thus grow well in marginal lands with high biomass yield, making it an alternative energy crop (Long et al., 2016). The major biomass of JA is from its tubers (JAT) and stalk (JAS). While JAT has been explored for inulin extraction (Li et al., 2012, 2015) and biofuel production as well (Matías et al., 2015; Sarchami and Rehmann, 2014; Gunnarsson et al., 2014), less concerns have been focused on how to utilize JAS to credit the JAT biorefinery.

Kim et al. (2013) and Kim and Kim (2014) converted the mixture of JAS and JAT into ethanol through simultaneous saccharification and fermentation (SSF) by the inulinase-producing yeast *Kluyveromyces marxianus*, but apparently such a process compromised the advantage for JAT to be used for producing value-added inulin and other products. Most recently, Khatun et al. (2015) explored lignocellulosic ethanol production solely

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from JAS by an engineered inulinase-producing yeast *Saccharomyces cerevisiae*. However, almost no research on the integration and optimization of the major unit operations has been reported.

Pretreatment is the first step of lignocellulose bioconversion, which aims to destroy the rigid structure of the feedstock and separate major components cellulose, hemicelluloses and lignin from each other for more efficient hydrolysis of the cellulose component. Among various pretreatment technologies, alkaline hydrogen peroxide (AHP) pretreatment could effectively remove lignin at moderate temperature (Banerjee et al., 2012; Correia et al., 2013; Mou et al., 2013), leading to high cellulose recovery and low inhibitor formation.

The second unit operation is the enzymatic hydrolysis of the cellulose component in the pretreated JAS to release glucose for microbial fermentation. Although the development of more efficient cellulase is essential, it is vital to explore the process engineering strategy to maximize the enzyme's potential. In order to meet the industrial requirement for more than 5% ethanol produced during the fermentation, glucose released from the enzymatic hydrolysis should be higher than 12%, resulting the uploading of the pretreatment biomass at least 20% (Chu et al., 2012). But the high solids uploading significantly deteriorates mixing and mass transfer performance of the hydrolysis and fermentation system, making it a necessities for optimizing the feeding of both the feedstock and enzyme (Chu et al., 2012; Yang et al., 2010; Olofsson et al., 2010; Liu et al., 2015; Unrean et al., 2015).

Fermentation can be coupled with the saccharification (simultaneous saccharification and fermentation, SSF) or separated from the unit operation (separate hydrolysis and fermentation, SHF). Compared to SHF, SSF enables the fermentation system to maintain at low sugar levels, which consequently alleviate substrate inhibition, particularly the inhibition of glucose in the cellulase activity, and in the meantime decrease the contamination risk for ethanol fermentation. However, temperature for ethanol fermentation by yeast is much lower than that for cellulase to efficiently hydrolyze the cellulose component. On the other hand, hydrolysate of the pretreated biomass presents various environmental stresses on yeast growth and ethanol fermentation, and the self-flocculating yeast exhibits better tolerance to these stresses (Liu et al., 2012).

In this study, AHP pretreatment was optimized by the response surface methodology (RSM) analysis, followed by the studies on uploading strategies for the pretreated biomass and enzyme and ethanol fermentation by the SSF or SHF processes using the self-flocculating *S. cerevisiae* SPSC01. At the end, the integrate process for ethanol production from JAS was assessed.

2. Materials and methods

2.1. Feedstock, strain and culture medium

JAS harvested from Dongying (Shandong Province), Yancheng (Jiangsu Province) and Yinchuan (Ningxia Province), China (Table 1), was dried naturally and milled to a size range of 1–10 mm and rinsed by water to remove dust and other impurities for reliable results, then dried at 50 °C for 48 h in an oven. The self-flocculating yeast *Saccharomyces cerevisiae* SPSC01 developed at the authors' laboratory and deposited at China General Microbiological Culture Collection Center (CGMCC) with the reference number of CGMCC No. 1602 was employed in this work. The YPD medium for seed culture consists of (g/L): glucose 30, yeast extract 4 and peptone 3.

Table 1
Composition of JAS from different regions.

Region	Yancheng	Dongying	Yinchuan
Geographic location	120.13W, 33.38N	118.49W, 37.46N	106.27W, 38.47N
Soil conditions	Alkali soil	Saline soil	Sierozem
Climate	Sub-tropical	Sub-tropical	Desert
Cellulose (%)	32.63 ± 0.38	37.06 ± 0.41	35.93 ± 0.19
Hemicelluloses (%)	19.03 ± 0.11	18.12 ± 0.17	22.21 ± 0.54
Lignin (%)	20.47 ± 1.19	18.10 ± 1.10	17.26 ± 0.52
Ash (%)	2.39 ± 0.15	2.17 ± 0.17	2.31 ± 0.50
Others (%)	23.65 ± 1.83	24.55 ± 1.17	22.38 ± 0.09

2.2. AHP pretreatment and regression optimization

JAS was pretreated by the mixture of NaOH (1%, 2% or 3%, w/w) and H_2O_2 (2%, 3% or 4%, v/v) in 5 L flasks at 10% (w/v) solid-liquid ratio and 121 °C for 90 min. The treated JAS was washed to neutral pH by water and dried at 45 °C for 48 h in an oven.

The central composite design was chosen to evaluate the effect of the pretreatment with NaOH and H_2O_2 on JAS by the software Design expert 8.0 (Statease, USA, MN). The model quality was estimated by variance analysis (ANOVA). As the response values (Y), the contents of cellulose, hemicelluloses, lignin and biomass recovery were fitted in the form of a quadratic polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^{k}\beta_i x_i + \sum_{i<j}^{k}\beta_{ij} x_i x_j + \sum_{i=1}^{k}\beta_{ii} x_i^2 \quad (1)$$

where, x_i and x_j are independent variables. β_0, β_i, β_{ij} and β_{ii} are interception effect, linear effect, linear-by-linear interaction and quadratic effect, respectively (Liu et al., 2014)

2.3. Chemical composition analysis

The chemical compositions of JAS including cellulose, hemicelluloses, lignin and ash were analyzed by the NREL laboratory analytical procedures with the two-step acid hydrolysis (Sluiter et al., 2008). Briefly, JAS was firstly hydrolyzed by concentrated acid (72% H_2SO_4 at 30 °C for 1 h), and then the mixture was diluted by deionized water and hydrolyzed in autoclave by diluted acid (4% H_2SO_4 at 121 °C for 1 h). Finally, solid residues collected through filtration were dried to determine the acid insoluble lignin and ash, and the filtrate was collected to analyze the chemical compositions of cellulose, hemicelluloses and acid soluble lignin.

The carbohydrates and metabolites in the supernatant were determined by HPLC (Waters 2695, Waters, Taunton, MA) with the column (Biored Aminex HPX-87H, 300 mm × 7.8 mm, Hercules, CA) and Waters 410 refractive detector. A flow rate of 0.6 mL/min was applied with 10 mmol/L H_2SO_4 as the mobile phase. The temperature of column and detector was 65 °C and 50 °C, respectively.

2.4. Hydrolysis analysis

The amount of 10 g JAS or AHP-JAS was enzymatic hydrolyzed by the commercial cellulase (Cellic CTec2, Novozyme, Bagsvaerd, Denmark) at the loading 20 FPU/g substrate in 100 mL citric acid-Na_2PO_4 buffer solution (0.1 mol/L sodium citrate and 0.2 mol/L Na_2HPO_4, pH4.8) at 50 °C. In order to elevate the impact of the mass loading on the enzymatic hydrolysis, fed-batch strategy was performed with 5 g biomass and corresponding cellulase supplemented into the reaction vessel every 12 h until reaching 30% solid-liquid ratio.

2.5. Morphological analysis

Scanning electron microscopy (SEM: Quanta 450, FEI, USA) was used to observe the morphology of JAS. The samples coated with gold under high vacuum conditions were fixed on the aluminum