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Optimizing enzymatic hydrolysis of inulin from Jerusalem artichoke tubers for fermentative butanol production

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ABSTRACT

In this study, a central composite design and response surface methodology were used to study the effect of various enzymatic hydrolysis variables (temperature, pH, substrate concentration and enzyme loading) on the enzymatic hydrolysis of Jerusalem artichoke-derived inulin. It was found that a quadratic model was able to predict inulin conversion as a function of all four investigated factors. The model was confirmed through additional experiments and via analysis of variance (ANOVA). Subsequently, numerical optimization was used to maximize the inulin conversion (94.5%) of Jerusalem artichoke powder within the experimental range (temperature of 48 °C, pH of 4.8, substrate concentration of 60 g l^{-1}, and enzyme loading of 10 units $g^{-1}_{substrate}$ for 24 h). The enzymatic hydrolyzate of Jerusalem artichoke was fermented via solventogenic clostridia to acetone–butanol–ethanol (ABE). An ABE yield of 0.33 $g_{Solvent}$ g^{-1}_{sugar} and an overall fermentation productivity of 0.25 g l^{-1} h^{-1} were obtained indicating the suitability of this feedstock for fermentative ABE production.

1. Introduction

In the current decade interest in research on the conversion of agricultural biomass into automotive fuels and chemicals has increased substantially, with a strong focus on ethanol [1,2]. Butanol contains two more methyl-groups as compared to ethanol, rendering it more hydrophobic, less volatile, higher in its energy density, and it is fully miscible with gasoline. Therefore, the fermentative production of butanol has received renewed attention in recent years [3].

One of the major obstacles to commercial acetone–butanol–ethanol (ABE) fermentation is the high cost and availability concerns of conventional substrates (corn, molasses) [4]. Substrate cost constitutes at least 50% of the total production cost during the ABE fermentation, and the process economics and feasibility largely depend on the availability of cost-effective raw materials [5–10]. To overcome this limitation lignocellulosic biomass such as corncob [8] and wastewater streams such as cheese whey [11], have been investigated and identified as alternative substrates for butanol production via ABE fermentation. Jerusalem

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artichokes (*Helianthus tuberosus* L.) as an alternative carbon source have potential as a renewable feedstock for solvent production when fermented by suitable microorganisms [12]. It is a low requirement crop with a high sugar production usually grown for its tubers. This plant is not only very resistant to frost and plant diseases but also can grow on poor land [13]. It has one of the highest carbohydrate yields ranging from 5 to 14 t per hectare [14] and therefore had been considered for butanol production in the past [15,16]. Jerusalem artichoke can be grown in various climate zones in North America, although the plant is better adapted to cooler climates [17]. It can potentially be grown in Ontario on lands traditionally used for tobacco production. Demand for tobacco is decreasing and the land requirements for the two crops are similar. Replacing tobacco fields with Jerusalem artichoke fields does not interfere with the current food production practices. Jerusalem artichoke tubers typically comprise about 80% water, 15–20% carbohydrates, 1–2% protein and virtually no fat [14]. The principal storage carbohydrate of Jerusalem artichoke is inulin; however, monomeric sucrose, glucose and fructose are also present. Inulin consists of linear chains of β (2 → 1) linked D-fructose units. Each chain is terminated by a D-glucose residue linked to fructose by α (1 → 2) bond [13]. Most organisms cannot directly ferment inulin, therefore inulin first needs to be hydrolyzed into fructose and glucose monomers. Hydrolysis can be achieved via an acid catalyst or enzymatically. Acid hydrolysis can lead to fermentation inhibiting byproducts, while enzymatic hydrolysis is dependent on potentially expensive enzymes, and therefore should be optimized. Among fungi one of the best inulinase yields can be obtained from *Aspergillus niger* (75 Unit ml^{-1}) [18], therefore many studies have been conducted using inulinase from this fungus for enzymatic hydrolysis of inulin [19,20]. However, information on the optimal condition of hydrolysis using inulinase from *A. niger* is limited in the literature.

The objective of this study is therefore twofold, the optimization of enzymatic hydrolysis of inulin to maximize its conversion to fermentable sugars, and the subsequent fermentation of hydrolyzate to butanol, an advance biofuel.

2. Materials and methods

2.1. Enzymatic hydrolysis

2.1.1. Preparation of Jerusalem artichoke flour

Jerusalem artichoke tubers, white flesh, were obtained from the Institute for Chemicals and Fuels from Alternative Resources (ICFAR), University of Western Ontario. The entire Jerusalem artichoke tubers were washed and sliced to approximately 2 cm cubes. The obtained slices were transferred directly to a drying oven and dried at 105 °C for 72 h, then ground to fine particles using a coffee grinder and passed through a 250 μm mesh. The prepared sample with approximately 3% moisture content was stored in a dry container at 4 °C for further use.

2.1.2. Inulin extraction

Inulin extraction was performed based on a method by Bekers et al. (2007). Extracts were obtained by adding 100 ml of water to 5 g of Jerusalem artichoke powder. The slurry was put into a water bath at 25 °C and agitated using a magnetic stirrer at 300 rpm for 1 h. The samples were then centrifuged for 20 min at 12,000 × *g* [21]. The supernatant was removed for HPLC analysis and enzymatic hydrolysis.

2.1.3. Enzymes

Inulinase from *A. niger* was purchased from Sigma–Aldrich with 286 units g^{-1} activity.

2.1.4. Experimental design

A central composite design (CCD) with four factors was selected to evaluate the response pattern and to determine the optimal combination of temperature, pH, substrate concentration and enzyme loading for maximizing inulin conversion to fermentable sugars (an initial full factorial design had shown significant curvature and confirmed the significance of all four parameters, data not shown). The un-coded values for each parameter were as follows [low star point, low central point, center point, high central point, high star point]: temperature in °C [35.9, 40, 50, 60, 64.1], pH [3.6, 4, 5, 6, 6.4], substrate concentration in g l^{-1} [11.7, 20, 40, 60, 68.3], and enzyme loading in units g^{-1} [0.34, 2, 6, 10, 11.66]. The experimental design was developed using Design Expert 8.0.7.1 (Statease, Inc., Minneapolis, MS, USA) and resulted in 26 conditions. All conditions were tested in triplicated, including 3 center points. The resulting 87 conditions (16 × 3 factorial + 10 × 3 augmented + 3 × 3 center points) were fully randomized.

2.1.5. Enzymatic hydrolysis of inulin

Batch enzyme reactions were performed for fructose production employing the selected experimental conditions. Enzymatic hydrolysis of extracted inulin was performed in 20 ml glass scintillation vials filled with a 10 ml working volume containing inulinase from *A. niger*. Each vial contained 5 ml of Jerusalem artichoke extract and 5 ml of 0.05 M sodium acetate buffer at the desired pH. Inulinase was mixed with inulin in the aforementioned buffer. All contents of the vials were at desired temperature prior to enzyme addition. The vials were hermetically covered with Parafilm and aluminum foil to avoid evaporative losses, and the mixture was incubated at the desired temperature for 24 h while shaking at 250 rpm.

2.1.6. Statistical analysis

Linear regression analysis was used to fit the experimental data with a second-order model as given in equation (1):

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \sum_{1 \le i \le j}^{4} \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

The experimental data was analyzed using Design Expert 8.0.7.1. The significance of each term was verified via analysis of the variance (ANOVA). The significance of each parameter, the interaction and quadratic effects were determined based on an α of 0.05 using the *F*-test. The fitted model was evaluated by normal probability plots, R^2 and adjusted R^2 and lack of fit coefficient for determining the adequacy. Numerical optimization via Design Expert 8.0.7.1 determined the optimal