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Jerusalem artichoke powder: A useful material in producing high-optical-purity L-lactate using an efficient sugar-utilizing thermophilic *Bacillus coagulans* strain

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HIGHLIGHTS

- Efficient high-optical L-lactate production from Jerusalem artichoke powder (JAP).
- Corn steep powder resulted in high L-lactate production.
- For the L-lactate fermentation based on JAP, SHF strategy is more efficient than SSF.
- High L-lactate production (134 g l^{-1}) was obtained.
- The final product optical purity was 99%.

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ABSTRACT

Jerusalem artichoke is a low-requirement crop, which does not interfere with food chain, and is a promising carbon source for industrial fermentation. Microbial conversion of such a renewable raw material to useful products, such as lactic acid, is an important objective in industrial biotechnology. In this study, high-optical-purity L-lactate was efficiently produced from the hydrolysates of Jerusalem artichoke powder by a thermophilic bacterium, *Bacillus coagulans* XZL4. High L-lactate production (134 g l^{-1}) was obtained using 267 g l^{-1} Jerusalem artichoke powder (total reducing sugars of 140 g l^{-1}) and 10 g l^{-1} of corn steep powder in fed-batch fermentation, with an average productivity of 2.5 g l^{-1} h^{-1} and a yield of 0.96 g g^{-1} reducing sugars. The final product optical purity is 99%, which meets the requirement of lactic acid polymerization. Our study represents a cost-effective and promising method for polymer-grade L-lactate production using a cheap raw bio-resource.

1. Introduction

Lactic acid is an important chemical that is widely used in food and non-food industries, including cosmetic, pharmaceutical, and chemical industries. However, the use in bio-based plastic is expected to outstrip its other applications. Polylactic acid (PLA) is renewable and biodegradable, and has shown to be a sustainable polyester. Due to the growing concern over the finite nature of fossil fuels and the environmental impact of greenhouse gases released into the atmosphere, PLA is gaining momentum as an alternative to petroleum-based plastics (Lee et al., 2011; Ou et al., 2011). As a precursor of PLA, the consumption of optically pure L-lactic acid is expected to increase rapidly in the near future. Although the demand for PLA has expanded, its current production capacity of approximately 500 million kg $year^{-1}$ is dwarfed by the 200 billion kg $year^{-1}$ of total plastics produced (Okano et al., 2010). One bottleneck of the industrial use of PLA is the high selling price, which must decrease by roughly half of its present price to compete with fossil-fuel-based plastics (Wee et al., 2006). And the lactic acid monomer production cost, mainly including the carbon and nitrogen sources, is the major concern of PLA production (Okano et al., 2010). As lactic acid has also been identified as one of the top 30 potential building-block chemicals from biomass (http://www.eere.energy.gov/biomass/pdfs/35523.pdf), the search for cheap raw materials is a critical objective that must be realized for the production of such low-value biocommodities.

In previous studies, considerable efforts have been made to reduce the microbial production cost of lactic acid. There are at least three critical areas of interest that can help reduce the production costs: find the means to lower the cost of sugars, use cheap and non-food sources as carbon resource (Ou et al., 2011), and reduce the cost of downstream processing by changing the neutralizers.

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Some cheap raw materials, such as molasses, rye, sweet sorghum, wheat, corn, cassava, potato, rice, barley, cellulose, corncob, waste paper, wood, and whey, have been used for L-lactic acid production (Wee et al., 2006; Wang et al., 2010a). The utilization of non-food sources and cellulosic materials for L-lactic acid production is considered a promising approach. Although lignocelluloses are inedible and represent one of the most abundant and inexpensive biomass sources in the world, their utilization is more difficult if compared with starchy materials (Okano et al., 2010). Cheap, non-food, and renewable sources need to be identified, and the appropriate fermentation strategies that can obtain high L-lactic acid yield and titer need to be developed. Thus, many challenges remain with regard to the development of efficient and sustainable L-lactic acid production.

Jerusalem artichoke is a low-requirement crop with high sugar content, and does not interfere with food chain (Matıas et al., 2011). It grows well in poor soil, and has a high tolerance to frost and various plant diseases (Ge et al., 2010). It grows particularly well in sand, and its widespread growth in such an environment can stop the expansion of the desert (Zhang et al., 2010). Jerusalem artichoke tubers typically comprise about 80% water, 15% carbohydrates, 1–2% protein and virtually no fat. Total sugars constitute about 75–85% of tuber dry weight (Matías et al., 2011). Therefore, the plant should be a useful energy crop for sustainable bio-based chemicals production. The principal storage carbohydrate of Jerusalem artichoke is inulin. Inulin consists of linear chains of β-2,1-linked D-fructofuranose molecules terminated by a glucose residue. Inulinase catalyze the removal of the terminal fructose residues from the non-reducing end of the inulin molecule, producing fructose as the main products and glucose as minor products (Zhang et al., 2010). Jerusalem artichoke tube is an excellent source of sugar and renewable raw material for the production of ethanol (Lim et al., 2011; Zhang et al., 2010), 2,3-butanediol (Li et al., 2010), fructose syrup (Ricca et al., 2009), single cell oil (Zhao et al., 2010b), fructo-oligosaccharides (FOSs) (Mughal et al., 2009) and lactic acid (Ge et al. 2009).

Many microorganisms, such as fungi, *Lactobacillus* species and various gene modified strains, have a proven ability to produce lactate. The potential utility of thermophilic *Bacillus* as an industrial strain is due to its remarkable capabilities of lactate production, a requirement for simple nutrition, compatibility with non-sterilization fermentation, and the simple maintenance of stock cultures (Qin et al., 2009). Furthermore, *Bacillus* species can ferment pentoses and hexoses in lignocellulosic biomass to L-lactic acid (Wang et al., 2010b). Thus, the *Bacillus* species is attracting increasing attentions as a potential bacterial biocatalyst for efficient L-lactate production.

Thermophilic *Bacillus coagulans* strain XZL4 is an efficient sugar-utilizing producer of important platform compounds (Su et al., 2011). In this study, we took optically pure L-lactate as an example for bulk chemical production from Jerusalem artichoke powder, a low-cost raw material. The efficiencies of various fermentation strategies, including simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF) were investigated. The present study is attempted to develop an efficient and cost-effective polymer-grade L-lactate fermentation process from the cheap resource, Jerusalem artichoke powder.

2. Methods

2.1. Chemicals

Jerusalem artichoke tubers were harvested at maturity from Nanjing Agricultural University, China at the end of November. Jerusalem artichoke tubers were air-dried, milled, and then passed through a 60 mesh sieve. Jerusalem artichoke powder contains 2.6% (w/w) of fructose, 10.3% (w/w) sucrose, 63.8% (w/w) of inulin, 1.5% (w/w) of nitrogen, 7.5% (w/w) of crude fiber, 5.8% (w/w) of ash, and 8.5% (w/w) of water. Commercially available inulinase (Fructozyme L^{TM} produced by Novozymes, EC 3.2.1.7) with an activity of 321 U g^{-1}, was purchased from Sigma Chemical Co. (MI, USA). Yeast extract (YE) and malt extract, with a nitrogen content of 9.8% (w/w) and 2% (w/w), respectively, were purchased from Aoboxing Biotech Company Ltd. (Beijing, China). Soybean meal, peanut meal, and corn steep powder, with nitrogen contents of 8% (w/w), 7.6% (w/w) and 4.02% (w/w), respectively, were purchased from Comwin Pharmculture Co., Ltd. (Beijing, China). All other chemicals were of analytical grade and commercially available.

2.2. Microorganisms and culture conditions

Bacillus coagulans 2–6, XZL4, and XZL9 were isolated in our research group and used in this study. They were homofermentative L-lactic acid producers (Qin et al., 2009; Wang et al., 2010b). The strains were maintained on De Man, Rogosa and Sharpe (MRS) agar slants. The pH was adjusted to 6.2–6.5. Following cultivation at 50 °C, the fully grown slant was stored at 4 °C.

The medium for inoculation contained glucose (70 g l^{-1}), YE (10 g l^{-1}), tryptone (5 g l^{-1}), and $CaCO_3$ (20 g l^{-1}). The seed cultures were prepared by incubating the strain in 100 ml of the above medium in 300-ml Erlenmeyer flasks for 24 h at 50 °C under static conditions. Then the seed cultures were inoculated into Erlenmeyer flasks or bioreactor for L-lactate production. The inoculum volume was 10% (v/v).

2.3. Enzymatic hydrolysis of Jerusalem artichoke powder

Jerusalem artichoke powder was hydrolyzed to reducing sugars (fructose and glucose) with inulinase. To study the optimal enzymatic hydrolysis condition, the effects of enzyme loading, reaction time, temperature, and pH were investigated. To study the amount of inulinase required for reducing sugars' complete release, 0, 10, 20, 30, 40, 50, 60, and 70 U inulinase per gram of Jerusalem artichoke powder were added. The pH value was adjusted to 5.0, and the mixture was maintained at 50 °C in a water bath and agitated intermittently for 12 h. To study the pH required for the complete release of reducing sugars, 30 U inulinase per gram of Jerusalem artichoke powder was used. The pH value was adjusted to 4.5, 5.0, 5.5, 6, 6.5, and 7. The mixture was maintained at 50 °C in a water bath and agitated intermittently for 12 h. To study the effect of temperature on the release of reducing sugars, 30 U inulinase per gram of Jerusalem artichoke powder was used at a pH value of 5.5. The mixture was maintained at 20 °C, 30 °C, 40 °C, 50 °C, 60 °C, and 70 °C in a water bath and agitated intermittently for 12 h. To study the effect of reaction time on the release of reducing sugars, 30 U inulinase per gram of Jerusalem artichoke powder was used at a pH value of 5.5. The mixture was maintained at 40 °C in a water bath with intermittent agitation for 0 h, 3 h, 6 h and 12 h.

The response surface methodology was also used to study the optimum condition for the release of reducing sugars. Design-Expert package (version 8.0, Stat-Ease Inc., USA) was used for all statistical analyses and for response surface plotting. The values of temperatures and pH levels used are presented in Table S1. Thirty U inulinase per gram of Jerusalem artichoke powder was used to release the reducing sugars, and the mixture was maintained in a water bath and agitated intermittently for 12 h.

The reactions were carried out in 100-ml Erlenmeyer flasks, each containing 30 ml of the mixed suspension.

2.4. Effects of various nitrogen sources on L-lactate production

The effects of different nitrogen sources on L-lactate fermentation were determined in medium containing 50 g l^{-1} Jerusalem artichoke powder and 25 g l^{-1} $CaCO_3$. According to the product