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Simultaneous saccharification and fermentation of *xylo*-oligosaccharides manufacturing waste residue for L-lactic acid production by *Rhizopus oryzae*

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ABSTRACT

High substrate cost and low lactic acid yield are the most pressing concerns in fermentative production of L-lactic acid by *Rhizopus oryzae*. In this study, waste residue from corncob after *xylo*-oligosaccharides (XOS) manufacturing was used as an alternative abundant, renewable, and inexpensive substrate for L-lactic acid production. After enzymatic hydrolysis, both glucose and xylose in the hydrolysate were converted to 34.0 g L^{-1} of L-lactic acid, equivalent to a yield of 0.34 g g^{-1} dry waste residue, by *R. oryzae* in separate hydrolysis and fermentation. In contrast, a higher L-lactic acid titer (60.3 g L^{-1}) and yield (0.60 g g^{-1} dry waste residue) were achieved in simultaneous saccharification and fermentation (SSF) with 10% (w/v) substrate loading at 40 °C, demonstrating, for the first time, the feasibility of L-lactic acid production from XOS manufacturing waste residues. The SSF process for L-lactic acid production from XOS waste residues was also demonstrated in a 5-L stirred-tank bioreactor, although further optimization would be necessary.

1. Introduction

Lactic acid is a commonly occurring organic acid that can be produced biologically from renewable carbohydrates, and is valuable due to its wide use in food and related industries [1]. In addition, a variety of useful chemicals, including plastics, fibers, solvents, and oxygenated chemicals, can be produced from lactic acid derived from renewable feedstocks by sustainable biotechnological routes [2]. More recently, bio-based L-lactic acid has attracted increasing attention for its use as a starting material in the synthesis of poly-lactic acid (PLA) polymers, which are biodegradable and biocompatible with wide applications that conventional petroleum-based plastics such as polyesters are not suitable or unfavorable due to environmental concerns [3]. Today, lactic acid produced in fermentation has become one of the most promising feedstock monomers in the chemical industry.

Current industrial production of lactic acid uses homolactic acid bacteria, mainly *Lactobacillus* spp., cultured in enriched (complex) media with glucose as substrate, which produce L(+)- or D(−)-form of lactic acid with high product yield and productivity, but usually suffer from high raw material and purification costs [1]. In contrast, the filamentous fungus *Rhizopus oryzae* can use relatively inexpensive polysaccharides (e.g., starch) in simple media with minimal nutrient supplementation and is easy to separate by filtration after fermentation, and is thus, advantageous for its potential to reduce lactic acid production cost [4–8]. It produces optically pure L-lactic acid, which is the desirable form for food and pharmaceutical applications, and can also use xylose [9], the main sugar component in hemicellulose, as substrate. Compared to bacteria, it can grow well at a wider temperature range (up to 40 °C) and pH range (from 4 to 9) [5]. Moreover, chitosan present in the fungal mycelia is a high-value product, and the fungal biomass and byproducts can be used in animal feeds to improve quality [4].

The current sugar and starch-based feedstock for lactic acid fermentation accounts for more than 30–40% of the total production cost [4]. Recent research on lactic acid production has thus focused on abundant, low-cost lignocellulosic feedstocks [4,10,11]. More than 20 million tons of corncobs are available annually in China [12]. Currently, a large amount of this is used to produce *xylo*-oligosaccharides (XOS) [13,14] from the hemicellulosic

Abbreviations: R. oryzae, Rhizopus oryzae; XOS, xylo-oligosaccharides; SHF, separate hydrolysis and fermentation; SSF, simultaneous saccharification and fermentation.

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materials, generating large quantities of waste residues, mainly cellulosic materials, which would cause major environmental, and economic problems if not properly treated or utilized. Because of its low cost, high enzymatic digestibility due to low lignin content, small particle size, and environmental benefits of the re-utilization of this industrial waste, XOS manufacturing waste residues can be considered as an attractive alternative substrate for L-lactic acid production. Compared to corncobs and other lignocellulosic biomass, XOS waste residues with most of xylan removed contain mainly glucose with a low content of xylose, and thus, would be better for lactic acid fermentation since most microorganisms cannot use xylose efficiently [4].

The goal of this study was to develop an economical fermentation process for L-lactic acid production from XOS waste residues by *R. oryzae*. Unlike the conventional separate hydrolysis and fermentation (SHF) process, simultaneous saccharification, and fermentation (SSF) can synchronize enzymatic hydrolysis and microbial fermentation in a single step, thus offering various advantages, including increased productivity and reduced processing time due to reduced product (glucose) inhibition on cellulose hydrolysis [11,15,16]. In this work, both SSF and SHF were studied and compared for lactic acid production from XOS waste residues. The hydrolysis and fermentation of XOS waste residues were studied at different solid loadings and temperatures, and the results are reported in this paper. To our knowledge, this is the first study demonstrating high-titer, high-yield, and cost-effective L-lactic acid production from lignocellulosic biomass in an SSF process.

2. Materials and methods

2.1. XOS waste residue

The XOS waste residue derived from alkali-pretreated corncobs was kindly provided by Jiangsu Kangwei Biologic Co., Ltd. Milled corncobs was stewed in 12 m^3 alkali extraction tank containing 7% (w/v) sodium hydroxide at 85–90 °C for 1 h. The liquid fraction, with a high content of hemicellulose was removed by vacuum filtration for further production of XOS; the solid waste residue was first soaked in water with a solid/liquid ratio of 1:10 (w/v), and then neutralized with 72% (w/w) sulfuric acid to adjust the pH to 5.0–5.5, followed by removing the water with vacuum filtration to obtain the solid XOS waste residue. The solid fraction was stored in plastic bags at 4 °C until use. Before treatments, the corncobs contained (%, dry weight basis) ~40% cellulose and ~31% hemicellulose. After treatments, the solid waste residues contained 65.5% cellulose and 22.2% hemicellulose or 72.8% glucose, 23.3% xylose, and 1.9% arabinose.

2.2. Microorganism and cultivation

R. oryzae NLX-M-1 was obtained from Institute of Biochemical Engineering, Nanjing Forestry University, Nanjing, China. The preculture medium consisted of (g L^{-1}): 50 glucose, 3 $(NH_4)_2SO_4$, 0.75 $MgSO_4{\cdot}7H_2O$, 0.20 $ZnSO_4{\cdot}7H_2O$, and 0.30 KH_2PO_4, which was found to be optimal with glucose as the carbon source. All media were sterilized by autoclaving at 121 °C, 15 psig for 30 min. The strain was first cultured on potato-dextrose agar slants at 30 °C for 3–5 days to generate spores. The preculture used to seed the fermentation was prepared in 250-mL Erlenmeyer flasks, each containing 50 mL preculture medium and 10 g L^{-1} $CaCO_3$, inoculated with a spore suspension containing 10^6 spores mL^{-1}, and incubated at 30 °C for 12 h in a rotary shaker agitated at 170 rpm.

2.3. SHF for lactic acid production

Cellic® CTec2 (Novozymes), a cellulase complex consisting of aggressive cellulases, β-glucosidases and hemicellulase, was used for the degradation of cellulose and hemicellulose to fermentable sugars. Enzymatic hydrolysis trials were performed with the substrate loadings at 5%, 10%, 15%, and 20% (w/v). Unless otherwise noted, the hydrolysis was carried out in a 250-mL Erlenmeyer flask with the enzyme dosage of 0.06 g g^{-1} biomass at pH 5.0–5.5, in a shaker controlled at 50 °C and 150 rpm, for 2–3 days. The cellulose (glucan) and hemicellulose (xylan) hydrolysis yields (%) were calculated as the percentages of obtained glucose and xylose in the hydrolysate to the total glucose and xylose present in the substrate, respectively. For complete hydrolysis, the theoretical sugar yields from cellulose and xylan are 1.11 g glucose g^{-1} glucan and 1.14 g xylose g^{-1} xylan, respectively [17]. All experiments were duplicated, and the average values are reported. Before use as substrate in fermentation, the enzymatic hydrolysate was centrifuged and filtered to remove solid residues, and then supplemented with minerals as follows (g L^{-1}): 1 $(NH_4)_2SO_4$, 0.38 $MgSO_4{\cdot}7H_2O$, 0.10 $ZnSO_4{\cdot}7H_2O$, 0.15 KH_2PO_4.

The fermentation was then studied in 250-mL Erlenmeyer flasks each containing 100 mL of the enzymatic hydrolysate of XOS waste residue. $CaCO_3$ was added at 50% of the theoretical amount of glucose derived from the dried material (w/w) to maintain the medium pH at >6.0 for good cell growth and L-lactic acid production in the fermentation. After autoclaving at 121 °C for 30 min, each flask was inoculated with the preculture at an inoculation size of 10% (v/v). Unless otherwise noted, the fermentation was performed at 40 °C in a rotary shaker at 170 rpm for 2–3 days or until glucose was depleted or lactic acid production ceased. Unless otherwise noted, each fermentation condition was studied in duplicate.

2.4. SSF for lactic acid production

The SSF process was studied in 250-mL Erlenmeyer flasks with a 100 mL working volume in a rotary shaker at 170 rpm and 40 °C, unless otherwise noted. The fermentation medium containing the same inorganic salts as in the SHF medium and XOS waste residue (5%, 10%, 15%, or 20% w/v), with pH of ~5.5, was sterilized by autoclaving at 121 °C for 30 min. After cooling, enzymes were added at the loading rate of 0.06 g g^{-1} biomass, and each flask was then inoculated with the preculture at 10% (v/v). $CaCO_3$ was added at 50% of the theoretical amount of glucose derived from the dried material (w/w) after 12 h of fermentation to keep the medium pH at >6.0. Unless otherwise noted, all batch fermentations were duplicated.

2.5. L-lactic acid production in bioreactor

The SSF process was also studied in a 5-L stirred tank bioreactor (Biostat B, B. Braun) with a rotating fibrous matrix, made of a cotton cloth (9 × 15 × 0.2 cm) fixed on the outer surface of a perforated stainless steel cup mounted on the impeller shaft, for cell immobilization [6]. The bioreactor with 3 L of the medium was sterilized at 121 °C for 30 min. After cooling, the bioreactor was inoculated with 10% (v/v) preculture and operated at 40 °C, with agitation at 200 rpm and aeration at 1.0 vvm. After 12 h, the reactor pH was maintained at >6.0 by adding $CaCO_3$ solution periodically. Antifoam 204 from Sigma (0.5 mL per L medium) was added to prevent foaming during fermentation.

2.6. Analytical methods

The spore concentration was determined by counting the spores on a haemocytometer under a microscope. Analysis of chemical composition in XOS waste residues was carried out according to the