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Lactic acid production from potato peel waste by anaerobic sequencing batch fermentation using undefined mixed culture

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ABSTRACT

Lactic acid (LA) is a necessary industrial feedstock for producing the bioplastic, polylactic acid (PLA), which is currently produced by pure culture fermentation of food carbohydrates. This work presents an alternative to produce LA from potato peel waste (PPW) by anaerobic fermentation in a sequencing batch reactor (SBR) inoculated with undefined mixed culture from a municipal wastewater treatment plant. A statistical design of experiments approach was employed using set of 0.8 L SBRs using gelatinized PPW at a solids content range from 30 to 50 g L^{-1}, solids retention time of 2–4 days for yield and productivity optimization. The maximum LA production yield of 0.25 g g^{-1} PPW and highest productivity of 125 mg g^{-1} d^{-1} were achieved. A scale-up SBR trial using neat gelatinized PPW (at 80 g L^{-1} solids content) at the 3 L scale was employed and the highest LA yield of 0.14 g g^{-1} PPW and a productivity of 138 mg g^{-1} d^{-1} were achieved with a 1 d SRT.

1. Introduction

Lactic acid (LA) is a useful organic acid that is widely used in food, pharmaceutical, cosmetic and industrial applications. The global market for LA is experiencing steady growth recently with its various industrial applications; moreover, rapid development and commercialization of polylactic acid is being driven by rising oil prices, strict government regulations, and consumer demand for green products. LA can be manufactured either by chemical synthesis from petrochemicals or carbohydrates fermentation with bacteria and fungi, in which the latter one is the most popular technology studied and currently nearly all LA production is based on carbohydrate fermentation in industries (Datta and Henry, 2006).

Glucose and other simple sugars are ideal substrates for LA production. However, the feedstock usually represents 30–40% of total operational cost for LA production (Zhang et al., 2007). Alternately, these substrates can be harvested from natural polysaccharides from agricultural crops such as wheat, corn and potato, which makes using inexpensive carbohydrate materials attractive (Alonso et al., 2010; Li et al., 2012; Sreenath et al., 2001; Zhao et al., 2009). In producing these natural substrates, operational restrictions such as the selection of inoculum, sterile condition, pH, temperature and nutrients are critical to fermentation process (Zhang et al., 2007), and the current research efforts are focused on looking at alternative fermentation technologies (Liang et al., 2014, 2015; Reddy et al., 2008).

Potatoes are the world's fourth most important crop behind corn, rice, and wheat, and have experienced steady worldwide growth in the last two decades as a stable food crop and major starch source (FAO, 2014). In 2012 in North America, more than 60% of potato production was processed for French fries, can foods, starch, and flour products in potato processing plants (NPC, 2012), which generated a significant amount of potato peel waste (PPW) during the peeling and cutting procedures (Liang and McDonald, 2014). This waste stream contains a large quantity of carbohydrate, especially starch, and can be easily digested by microorganisms for value-added bioproducts production (Liang et al., 2014, 2015).

Abbreviations: CCD, central composite design; COD, chemical oxygen demand; DOE, design of experiments; GC, gas chromatography; GCMS, gas chromatography mass spectrometry; HPLC, high performance liquid chromatography; LA, lactic acid; NMR, nuclear magnetic resonance; PPW, potato peel waste; RSM, response surface methodology; SBR, sequencing batch reactor; SC, solids content; SRT, solid retention time; VFAs, volatile fatty acids.

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In practice, most PPW from the potato processing industry is used for local livestock feed (Nelson, 2010). Parawira et al. (2004) evaluated the feasibility of volatile fatty acids (VFAs) production during anaerobic mesophilic digestion of PPW where 19 g L^{-1} total VFAs were obtained. Methane generation from PPW under mesophilic and thermophilic conditions was also studied by Parawira et al. (2007). The results showed that a higher methane yield of 0.49 L g^{-1} chemical oxygen demand (COD) was obtained under mesophilic conditions compared to thermophilic conditions (0.41 L g^{-1} COD) in methanogenic reactors. Arapoglou et al. (2010) studied ethanol production from high starch content PPW by enzymatic hydrolysis and fermentation, and a maximum of 7.58 g L^{-1} ethanol was obtained with two steps of versatile enzymatic hydrolysis and a follow-up anaerobic fermentation with *Saccharomyces cereviciae var. bayanus* at pH 5.0 and 32 °C for 2 days. The production of LA from PPW was also studied using single or mixed cultures such as *Lactobacillus var.* and *Rhizupus var.* (Afifi, 2011; Liu et al., 2005; Zhang et al., 2007), which has been regarded as one promising, efficient, and environmental friendly solution because starch can be converted into LA directly with no greenhouse gas CO_2 emission during the fermentation process. Recent studies also revealed that homogeneous LA producing bacteria can be formed during the undefined mixed culture fermentation process (Dreschke et al., 2015; Liang et al., 2015; Probst et al., 2015), which provided solid evidence of LA production from PPW and other waste stream such as sewage sludge.

The previous batch fermentation study has demonstrated that LA can be produced using undefined mixed cultures inoculated from a wastewater treatment plant under an optimal temperature of 35 °C without the assistance of enzymatic hydrolysis pretreatment (Liang et al., 2014). In this updated research, the continuous production of LA by anaerobic fermentation in sequencing batch reactors (SBR) was conducted at two scales (0.8 and 3 L) to further assess the potential of commercialization of this technology.

2. Materials and methods

2.1. Material

PPW (*Russet Burbank*) samples used in this study were collected from two different potato processing plants in Nampa and Caldwell, Idaho (JR Simplot Company) in February 2012 and March 2013, respectively. The PPW samples were each collected over a 2 h period, mixed thoroughly and stored frozen (−20 °C) in plastic containers before use. Freeze-dried samples were prepared for chemical composition analysis. Fresh mixed microbial cultures used for fermentation were collected from a local municipal wastewater treatment plant (Moscow, Idaho).

2.2. Reactor operation and experimental design

Two different sized fermenters were operated in this study. Small scale fermentations used working volume of 0.8 L glass bottles fitted with an air-lock, and 40 mL mixed culture was added to the reactors at the start of operation. The reactors were operated as sequencing batch reactors, magnetically stirred (500 rpm) and temperature controlled at 35 °C with a heated water-jacket. Weighted certain amount of PPW sample (according to the experimental design) from the Nampa plant and gelatinized at 100 °C for 30 min and cooled down prior to use.

A statistical design of experiments (DOE) approach using central composite design (CCD)/response surface methodology (RSM) was employed in this part of the study to further evaluate and establish potential relationships between LA production (concentration and yield) and the two influencing factors (SRT and SC). To note, a SC greater than 50 g L^{-1} was examined in this experiment however, incomplete mixing was observed using a magnetic stirrer and therefore higher concentrations were not considered. Thereafter, a DOE approach based on a 2^3 full factorial CCD/RSM was conducted with three solid retention time (SRT, same with hydraulic retention time) levels of 2, 3, and 4 days and three feed solids content (SC) levels of 30, 40, and 50 g L^{-1} (total solid basis, same for other solid contents mentioned below). The mixed solution was manually drawn off and then immediately fed with the same volume of gelatinized PPW slurry once a day according to the SRT and SC (for example, SRT of 2 d and SC of 30 g L^{-1} reactor, 400 mL mixture was removed and same volume of gelatinized PPW with SC of 30 g L^{-1} was added daily), no sedimentation phase was conducted. Sampling and analysis was conducted after at least 3 SRT cycles to ensure steady state conditions of the bioreactors were obtained. A total of thirteen conditions composed of 4 cubic points and 4 axial points and 5 replicates at the center point were conducted. The experimental data were processed using Design Expert 8.0 software (Stat-Ease, Inc., MN, USA).

The larger scale fermentation study used a working volume of 3 L HDPE reactor equipped with mechanical stirring (300 rpm) and temperature control (35 °C). PPW sample without dilution at a SC of 80 g L^{-1} from the Caldwell plant was used. The fermenter was fed either un-gelatinized or gelatinized PPW according to the operating parameter settings for a total of 40 d. Four conditions were assessed in sequence: (i) SRT1.5, fed daily with un-gelatinized PPW at a SRT of 1.5 d for 8 d; (ii) SRT2, fed daily with un-gelatinized PPW at a SRT of 2 d for 10 d; (iii) SRT1.5G, fed daily with gelatinized PPW at a SRT of 1.5 d for 14 d; (iv) SRT1G, fed twice a day with gelatinized PPW at a SRT of 1 d for 8 d. The fermentation broth was sampled and analyzed according to methods described below. To ensure a steady state condition, only data obtained after 5 cycles of each stage (at least 3 observations) were processed and analyzed by SAS v9.3 software for the Tukey's HSD test at a probability level of $p = 0.05$.

2.3. Recovery of LA

The recovery of LA from the large scale fermentation broth was conducted according to Lin et al. (2010) with slight modification. The fermentation broth was centrifuged (7000 rpm for 1 h) and the supernatant was filtered through glass fiber paper (Whatman GFA) to separate the solid residue. The brown filtrate was mixed with 5% activated carbon (Aldrich) for 2 h to remove protein, color, and cell debris, and the mixture was again clarified by centrifugation and filtration as described above. The solution was then passed through an ion-exchange resin column (Amberlite IR $120H^+$, ∅ 2 cm × 40 cm) at 5 mL min^{-1} to protonate the acids. The eluent (pH 2) was vacuum distilled at 70–80 °C, filtered (0.45 μm nylon), to obtain a concentrated solution of LA.

2.4. Analytical techniques

Chemical analysis of solid materials was carried out as previously described (Liang and McDonald, 2014): Moisture content was determined using a HB 43-S Mettler Toledo moisture analyzer (Ohio, USA). Starch was estimated in PPW (2.5 g in 0.02 M citrate/Na_2HPO_4 solution, 97.5 mL, pH 5), after gelatinization (placed in boiling water for 30 min), by enzymatic treatment (2.6 U α-amylase and 4.9 U glucoamylase (Sigma, USA)) for 240 min at 50 °C, deactivated in boiling water for 10 min, filtered (0.45 μm), and analyzed by HPLC for glucose using mannitol as an internal standard. PPW (5.0 g) was extracted using a Soxhlet apparatus with CH_2Cl_2 (150 mL) for 16 h, the lipid extract was concentrated to dryness, and the yield was determined gravimetrically according to ASTM D1108-96. Carbohydrate analysis was performed by high

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