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Lactic acid production with undefined mixed culture fermentation of potato peel waste

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ABSTRACT

Potato peel waste (PPW) as zero value byproduct generated from food processing plant contains a large quantity of starch, non-starch polysaccharide, lignin, protein, and lipid. PPW as one promising carbon source can be managed and utilized to value added bioproducts through a simple fermentation process using undefined mixed cultures inoculated from wastewater treatment plant sludge. A series of non-pH controlled batch fermentations under different conditions such as pretreatment process, enzymatic hydrolysis, temperature, and solids loading were studied. Lactic acid (LA) was the major product, followed by acetic acid (AA) and ethanol under fermentation conditions without the presence of added hydrolytic enzymes. The maximum yields of LA, AA, and ethanol were respectively, 0.22 g g^{-1}, 0.06 g g^{-1}, and 0.05 g g^{-1}. The highest LA concentration of 14.7 g L^{-1} was obtained from a bioreactor with initial solids loading of 60 g L^{-1} at 35 °C.

1. Introduction

Potatoes are a major crop for the US Pacific Northwest (PNW; Idaho, Washington and Oregon); 1.26×10^{10} kg were harvested with the total value of 1.9 billion U.S. dollar in 2011. Approximately 60% of harvested potatoes were manufactured for French fries, canned foods, starch and flour products in food processing plants while the remaining 40% are unprocessed whole potatoes (NPC, 2012). The processing of potatoes generates a significant waste stream. The typical steam peeling losses are approximately 8% of the potato weight, which amounts to about 1.01×10^9 kg of potato peel waste (PPW) in the PNW in 2011. PPW generated from the processing plants is conventionally considered a zero value waste (Arapoglou et al., 2010). Conventional waste management strategies for PPW are focused on direct physical elimination of organic pollutants, such as cropland composting application and feed for ruminant animals (Nelson, 2010). However, potential environmental issues associated with PPW land application and water drainage and contamination are a growing concern. Therefore, alternative uses of food processing waste streams for value added bioproducts promises a solution for waste management by waste reduction, reuse and recovery (Cossu, 2009).

The main chemical constituents of PPW are starch, non-starch polysaccharide (pectin, cellulose and hemicelluloses), lignin, protein, lipid, and ash (Camire et al., 1997). This chemical composition provides a good source of carbon for conversion into bioproducts. In this regard, and with an increasing worldwide energy demand, volatile fossil fuel prices, and environmental sustainability awareness, carbohydrate waste streams such as PPW (Jeon et al., 2008; Mars et al., 2010) are promising alternative carbon resources to make biofuels and chemicals via biochemical conversion of sugars using a carboxylate platform in a biorefinery (Chang et al., 2010; Holtzapple and Granda, 2009). With such a platform, sugars can be converted to various organic compounds by acid or enzymatic hydrolysis, glycolysis, acidogenesis, and acetogenesis (Agler et al., 2011); proceeding to complete biodegradation generates methane (CH_4). While CH_4 as the final product of anaerobic methanogenesis is a renewable form of natural gas, the economic feasibility of anaerobic digestion (AD) makes the biogas less favorable than other intermediate chemicals such as lactic acid (LA) valued at \$1.5–1.9 kg^{-1} (Pastor et al., 2004), ethanol, and volatile fatty acids (VFAs) (Voegele, 2011). However, combining AD with a portfolio of bioproducts from a waste stream could improve the economic feasibility of such ventures in the model of a biorefinery (Clarke and Alibardi, 2010).

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LA as a useful organic acid has been widely used in food, pharmaceutical, cosmetic and industrial applications, especially for making bioplastic, polylactic acid (PLA). The conventional industrial LA production starts with glucose derived from starch or lignocellulosic biomass by separate hydrolysis and fermentation or simultaneous saccharification and fermentation in the presence of enzymes and pure culture (Ou et al., 2011; Yamada et al., 2009). However, a single microbial strain only functions in a narrow range of substrates and operating conditions (Lin et al., 2011; Zhan et al., 2003). Although using known two or more mixed cultures can minimize this limitation and potentially widen the scope of usable carbohydrate materials to some extent (Cui et al., 2011; John et al., 2007), other restrictions such as sterilized conditions, consistent microbe concentration, pH, nutrients, and temperature are also critical for fermentation productivity (Cock and Stouvenel, 2006; Zhang et al., 2007). Using undefined mixed cultures can handle the complex conditions with the highest microbial diversity, great adaption, and self-evolution abilities. Mixed microbial consortia have been applied to complex substrates in the environmental sector to break down solids and purify wastewater under aerobic and anaerobic conditions for more than a century (Agler et al., 2011; Wanger et al., 2002). More recently, the production of VFAs by anaerobic fermentation with undefined mixed cultures from waste and wastewater has been studied (Bengtsson et al., 2008). However, to the authors' best knowledge there is no paper published that produced LA from PPW using an undefined mixed microbial culture.

In this study we explored the possibility of PPW fermentation with undefined mixed culture, and analyzed the influences of different bioreactor operational factors (PPW gelatinization, addition of hydrolytic enzymes, temperature, and PPW solid content) on the production of LA. This simple and novel approach can potentially maximize the value of PPW and further broaden the utilization of carbohydrate waste.

2. Materials and methods

2.1. Potato peel waste

PPW samples were obtained from a regional potato processing plant (JR Simplot Company, Nampa, ID) and frozen (−20 °C) before use. PPW was freeze dried for compositional analysis. 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 (Delgado et al., 2009). 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 performance liquid chromatography (HPLC) on the 2-stage sulfuric acid hydrolysates of lipid free PPW (0.200 g) (ASTM E 1758-01). More specifically: primary hydrolysis step using sulfuric acid (2 mL, 72%) for 60 min at 30 °C; secondary hydrolysis step using sulfuric acid (4%) for 30 min at 120 °C. The solution was made up to 200 mL in a volumetric flask, of which a 5 mL aliquot taken and mannitol added as an internal standard (1 mL, 0.20 mg mL^{-1}), neutralized with $PbCO_3$ (0.16 g), deionized through a mix-bed resin column (Amberlite IR-120 H^+ resin (0.5 mL) and Amberlite IRA-35 OH^- resin (0.5 mL)) and filtered (0.45 μm). The lignin and suberin components in potato cell walls are mixed together and the measurement of this complex was determined on lipid free PPW by acid insoluble (Klason lignin, precipitate from the 2-stage hydrolysis mentioned above) and acid soluble lignin (280 nm, Beckman 640 UV–Vis spectrometer) fractions according to ASTM D 1106-96 and Schoening and Johansson (1965) methods, respectively. *N* content was determined on a CE 440 elemental analyzer (Massachusetts, USA). Protein was estimated by multiplying total by 6.25. Ash was determined by dry oxidation at 600 °C. All analyses were performed in duplicate. The chemical compositional results are listed in Table 1.

2.2. Fermenter set-up

Air-locked glass bioreactors with a working volume of 800 mL were used for batch fermentation experiments. The temperature was controlled by a heated water-jacket, and at the start of each test all bioreactors were inoculated with 2% (16 mL) activated sludge from the aeration tank of the Moscow, Idaho municipal wastewater treatment plant and fed with PPW.

The PPW was homogenized in a Warning blender prior to fermentation. Gelatinization was performed to swell the starch granules in the PPW to improve the accessibility of enzymes/bacteria (Delgado et al., 2009). Four bioreactor operating factors were considered in this study (Fig. 1): (i) PPW pre-gelatinization (boiled for 30 min at 100 °C), (ii) addition of hydrolytic enzymes (6 μg^{-1} mixed *α-amylase* (Sigma, USA), *glucoamylase* (Sigma, USA) and 30 μg^{-1} *cellulase* (Fisher Scientific)), (iii) reactor temperature (20, 35 and 50 °C), and (iv) solids loading (20, 40, and 60 g L^{-1}). Operating factors (i) and (ii) were evaluated in the first round of experiments, followed by (iii) and (iv) sequentially. Bioreactors were operated for 12 d until no significant changes in organic acid concentration were observed. The fermentation experiments were performed in triplicate for experiments/operating factors (i) and (ii) and in duplicate for experiments/operating factors (iii) and (iv). Samples were analyzed in duplicate. The bioreactor labeling for experiments (i) and (ii) are as follows: (A) un-gelatinized PPW, (B) gelatinized PPW, (C) un-gelatinized PPW and hydrolytic enzymes, and (D) gelatinized PPW and hydrolytic enzymes.

2.3. Analytical methods

LA, acetic acid (AA), ethanol and other C_3–C_6 volatile fatty acids were quantified through ion exclusion HPLC analysis, using a Rezex ROA organic acid column (7.8 mm × 30 cm, Phenomenex, Torrance, CA) and a Waters HPLC pump and autosampler (Waters Corp., Milford, MA) equipped with differential refractive index detector (ERC-5710, ERMA, Japan), on elution with 0.005 N aqueous sulfuric acid (0.5 mL min^{-1}) at 65 °C. Sugars were quantified by HPLC using two Rezex RPM columns in series (7.8 mm × 30 cm, Phenomenex, Torrance, CA) and a Waters HPLC pump and autosampler (Waters Corp., Milford, MA) equipped with differential refractive index detector (Model 2414 Waters Corp., Milford, MA), on elution with water (0.5 mL min^{-1}) at 85 °C. HPLC data were acquired and analyzed using N2000 chromatography software (Surwit Science & Technology, China). DO and pH were measured with Orion-3-Star

Table 1
Chemical composition of potato peel waste (PPW).

Parameters	Dry weight (%)
Carbohydrate	63.2 ± 4.2
Starch	34.3 ± 2.7
Protein (N_{tot} 6.25)	17.1 ± 0.3
Lipids	1.2 ± 0.0
Lignin and suberin complex	
Acid soluble	6.2 ± 0.2
Acid insoluble	4.1 ± 0.0
Ash	9.6 ± 0.1