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Production and characterization of bio-oil and biochar from the pyrolysis of residual bacterial biomass from a polyhydroxyalkanoate production process

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ABSTRACT

Polyhydroxyalkanoate (PHA) production generates a significant amount of residual bacterial biomass (RBB) after PHA extraction. The RBB as a zero-value waste contains proteins, carbohydrates, phenolics, and ash, which can be managed and converted to bio-oil and biochar products by pyrolysis. Thermogravimetric analysis (TGA) was used to investigate the thermodegradation kinetics of RBB and pyrolysis–GCMS studies were employed to determine potential chemical products. Pyrolysis was conducted on a laboratory-scale auger reactor at 500 °C. The bio-oil and biochar yield were 28 and 46%, respectively. The pyrolysis bio-oil was characterized by the combination of GCMS, high-pressure liquid chromatography (HPLC), and electrospray ionization mass spectrometry (ESI–MS). The bio-oil was dominated by nitrogen-containing, hydrocarbons, and aromatic compounds. The biochar was studied for its specific surface area and pore size, chemical functionality by Fourier transform infrared (FTIR) and Raman spectroscopies, and butane absorption activity. Biochar was comprised of a large part of polycondensed phenolic and majorly disordered amorphous carbon.

1. Introduction

Driven by the awareness of environmental pollution by petroleum based plastics, the bioplastic market is expected to reach to 6.2 million metric tons globally by 2017 [1]. Within the diverse bioplastic family, polyhydroxyalkanoate (PHA) is a special group of polyesters produced by a wide variety of microorganisms as an internal (i.e., within bacterial cells) carbon and energy reservoir. Due to its good biocompatibility, biodegradability, and renewability, PHA has been widely used in biomedical, packaging, and agricultural areas [2,3].

Commercial PHAs are produced using pure or recombinant bacterial cultures at high cost (UD$ 4.4–6.1 kg^{-1}) [4], making it less competitive with conventional petroleum based plastics as bulk materials at the point of price. Recently, inexpensive carbon/nitrogen sources such as agricultural residues or industrial waste and woody biomass streams have become competitive carbon sources to reduce the PHA production cost by at least 50% [5–8]. The use of mixed microbial cultures (MMCs) has been proposed as an ideal alternative to current commercial PHA production (recently reviewed by Laycock et al. [9]). However, the PHA production process from waste streams using MMCs generates relatively lower PHA content as compared with that from pure substrates and pure microbial cultures [3]. In our recent study, PHA (specifically polyhydroxybutyrate-*co*-hydroxyvalerate, PHBV) was biosynthesized from fermented dairy manure by MMCs [7]. The PHBV content reached up to 40% of the dry cell weight with HV content ranging from 15 to 40 mol%. The remaining material, the residual bacterial biomass (RBB), is conventionally discarded as waste materials (>60% of the biomass dry weight) potentially result in a valuable byproduct.

The RBB is primarily comprised of lysed bacterial cells; specifically, proteins, nucleic acids, carbohydrates, and lipids [10]. Consequently, disposal of this RBB resulting from PHA production is an issue. Given their diverse composition, opportunities exist to upgrade this material into useful bioproducts. Pyrolysis of biomass,

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mainly lignocellulosics (e.g., wood and herbaceous species), has gained considerable attention as an option for producing a crude bio-oil that can be further upgraded into drop-in fuels [11,12]. However, the pyrolysis of lignocellulosics carry some limitations: (i) derived pyrolysis bio-oil contains an abundance of oxygenated compounds and (ii) the bio-oil is too acidic [13]. Aside from lignocellulosic materials, other suitable feedstocks for pyrolysis include N-containing wastes, such as algae [14], manure [15,16], potato peel [17], and activated sludge [18–23]. Of these, activated sludge is arguably the most similar to RBB, due to both being comprised primarily of bacteria. For example, Sánchez et al. studied sewage sludge, focusing on the effect of pyrolysis temperature (350, 450, 550, and 950 °C) on the composition of the bio-oils [22]. They obtained the liquid products (bio-oil and water) and biochar products with the yield of about 30% and 50%, respectively. Different classes of compounds were identified in the oils by GCMS analysis, such as hydrocarbons (alkane and alkene), monoaromatic hydrocarbons (e.g., benzene, toluene, styrene, phenol, etc.), N-containing aromatic compounds (e.g., pyridine, alkyl pyridines, pyrrole, indole, and quinolones derivatives), aliphatic and aromatic nitriles, and carboxylic acids [22]. With these constituents, activated sludge has the potential to be used as a fuel oil after upgrading or as a source of commercially useful chemicals. Determining if the similar RBB holds the same potential, knowledge of the chemical composition and product distribution following pyrolysis is needed.

PHA was biosynthesized from a pilot-scale PHA bioreactor fed by fermented dairy manure liquor with MMCs from a waste water treatment plant. N-rich RBB waste was generated after PHA bioplastic was isolated. The objective of this study was to thermochemically convert (via pyrolysis) the RBB into value added bioproducts and minimize the biological waste and impact to the environment. The resulting pyrolysis products were collected and characterized. The pyrolysis bio-oil products were chemically investigated by a combination of GCMS, ESI–MS, and HPLC. The biochar was characterized by proximate, ultimate, butane activity, surface area, and FTIR and Raman spectroscopies.

In the present study, it is the first time utilizing RBB waste for value added bioproducts. Bio-oil production from RBB via pyrolysis provides an alternative to the growing interest in pyrolysis liquids produced from agricultural residues. The yield and compositions of bioproducts from pyrolysis were compared with other N-containing waste and biomass.

2. Material and methods

2.1. Residual bacterial biomass (RBB) generated from PHA production process

Biomass used in this study was collected from a pilot-scale PHA producing bioreactor fed with fermented dairy manure [7]. Briefly, dairy manure was collected from the University of Idaho North Farm Dairy and fed to a continuously operated 800 L anaerobic fermenter operated under a 24 h cycle time with solid and hydraulic retention times of 4 days. Following solid-liquid separation by screening and settling, the volatile fatty acid rich liquid was pumped into the second and third stage 700 L bioreactors for PHA production. After PHA was accumulated in the bioreactor, biomass was recovered and suspended in 6.25% sodium hypochlorite to lyse the cells and cease metabolic activity during the recovery process [24]. PHA-rich biomass was centrifuged and frozen at −20 °C. Samples were lyophilized prior to extraction and characterization.

Lyophilized biomass (500 g) was washed with acetone (4 L) for 24 h to remove lipids, followed by Soxhlet extraction system with $CHCl_3$ (2 L; 60 °C) for 24 h to extract the PHBV bioplastic. The yield (from duplicate experiments) of crude PHBV, lipids, and RBB was in the range of 15–26%, 5–10%, and 60–80%, respectively. The HV content of the PHBV copolymer was ranging from 15 to 40 mol% as determined by ^{1}H NMR [7].

The RBB was vacuum-dried prior to use or analysis. A flow diagram showing the processes used and experiments performed is shown in Fig. 1.

2.2. RBB characterization

2.2.1. Calorific value, proximate and ultimate analyses

Calorific value of RBB sample was determined using a Parr oxygen bomb calorimeter (Model 1261 according to the ASTM D5865-04). Pre-dried biomass sample (1.0 g) was pressed into a pellet (6 mm Ø) on a Carver Laboratory hydraulic press to a pressure of 15 MPa. The proximate analysis (ash, volatile matters, and fixed carbon) on vacuum-dried RBB was performed in accordance with ASTM standard E870-82. More specifically, the content of volatile matter (VM) was determined following 7 min of combustion in a muffle furnace at 950 °C. Ash content was carried out at 580 °C, and moisture content (MC) was measured using a moisture analyzer (HB43-S Mettler Toledo). Fixed carbon (FC) content was a calculated value by subtracting MC, VM and ash from 100. The ultimate (elemental) analysis for C, H and N were determined using a CE-440 elemental analyzer (Exeter Analytical), and O was calculated by the difference of C, H, N and ash results from 100%.

2.2.2. Chemical composition

The lipids from the RBB were extracted with CH_2Cl_2 and quantified according to the method of Osman et al. [25]. Carbohydrate content of lipid-free RBB after CH_2Cl_2 extraction was analyzed as previously described [26,27]. In short, polysaccharides were first hydrolyzed into monosaccharides with 2 M trifluoroacetic acid (TFA) at 105 °C and then reduced by $NaBD_4$ and followed by acetylation with acetic anhydride. The alditol acetate derivatives were analyzed by $GCMS_{EI}$ (FOCUS-ISQ, ThermoScientific). Separation was achieved using a BPX-70 capillary column (30 m × 0.25 mm Ø, SGE Analytical Science) with a temperature gradient from 190 °C (1 min) to 250 °C (20 min) at a rate of 2 °C min^{-1}. The compounds were identified with authentic derivatized sugars and mass spectral analysis. The crude protein content was estimated from the measured N content (a multiple of 6.25 was assumed). The lignin content was determined on lipid-free RBB as acid insoluble (Klason) and acid soluble lignin as described by ASTM E1758-01 with the modification proposed by Liang and McDonald [28].

Amino acid composition (free and bound) was performed according to the method described by McDonald et al. [29]. Briefly, protein in the RBB (20 mg) was hydrolyzed in hydrochloric acid into amino acids, and then free/hydrolyzed amino acids were sequentially derivatized using acetylchloride/isobutanol (1:4 v/v) mixture followed by heptafluorobutylanhydride in CH_2Cl_2. The heptafluorobutyl-isobutyl esters products were dissolved in $CHCl_3$ (1 mg mL^{-1}) and analyzed by $GCMS_{EI}$ (PolarisQ iontrap, Thermo Electron Corp.). Separation was achieved on a ZB1 capillary column (30 m × 0.25 mm Ø, Phenomenex) using a temperature program from 40 °C to 250 °C at 5 °C min^{-1}. The eluted compounds were identified with authentic derivatized amino acids and mass spectral matching.

2.2.3. Thermogravimetric analysis (TGA)

TGA was performed on a TGA-7 (PerkinElmer) instrument. RBB samples (3–5 mg, in duplicates) were heated from 50 to 900 °C at rates of 5, 10, 20, 30, 40, and 50 K min^{-1} under nitrogen (30 mL min^{-1}). TGA and differential thermogravimetry (DTG) data were analyzed using Pyris v8 software. Activation energy (E) and pre-exponential factor (A) were calculated according to ASTM