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.

Keywords: Residual bacterial biomass; polyhydroxyalkanoate; compositional analysis; bioproducts

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, 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 d. 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 ^1H 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 CH₂Cl₂ and quantified according to the method of Osman et al. [25]. Carbohydrate content of lipid-free RBB after CH₂Cl₂ extraction was analyzed as previously described [26, 27]. In short, polysaccharides were first hydrolyzed into monosaccharides with 2M trifluoroacetic acid (TFA) at 105 °C and then reduced by NaBD₄ 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 CH₂Cl₂. The heptafluorobutyl-isobutyl esters products were dissolved in CHCl₃ (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 (Perkin-Elmer) 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 E1641-07. The decomposition kinetics was assumed to be first-order to determine *E* and *A* as follows:

$$E = -(R/b) \times \Delta(\log \beta) / \Delta(1/T)$$

$$A = -(\beta'/E_r) \times R \times \ln(1-\alpha) \times 10^a$$

where, *R* is the gas constant, *b* is the approximate derivative from ASTM E1641 numerical integration constants, β is the heating rate at K min⁻¹, β' is the heating rate nearest the midpoint of the experimental heating rates at K min⁻¹, *T* is the temperature (K) of the experimental heating rate at constant conversion, *E_r* is the refined value of Arrhenius *E*, α is the conversion value of decomposition, and *a* is the approximation integral taken from ASTM E1641 of numerical integration constants.

2.2.4. Pyrolysis GCMS analysis

Analytical pyrolysis GCMS (Py-GCMS) was performed on RBB (< 0.1 mg) and pyrolyzed at 500 °C in He with a Pyrojector II unit (SGE Analytical Science), coupled to a FOCUS-ISQ

GCMS_{EI} (ThermoScientific). The compounds were separated on the RTx-5MS capillary column (30 m × 0.25 mm Ø, Restek) with temperature programmed to be 40 to 250 °C at 5 °C min⁻¹. The eluted compounds were identified by their mass spectra, authentic standards, and with the NIST 2008 library matching. The relative abundance of each compound was calculated relative to the CO₂ peak [28].

2.3. Bio-oil and biochar production from RBB pyrolysis

Pyrolysis was conducted at 0.2 kg h⁻¹ feed rate (K-Tron weight loss twin-screw feeder) in a custom built auger reactor (Φ 5 cm × 90 cm) at 500 °C with N₂ purge (6 L min⁻¹), and the temperature of transfer tube was set at 450 °C (shown schematically in Fig. 2) [17]. The auger speed was adjusted to obtain a residence time of 8 s. Vapors were condensed through a two stage ice-water-cooled tube and shell condensing system and bio-oil was recovered and yield recorded. Biochar was collected at the end of reactor and yield recorded.

The pH value of the pyrolysis bio-oil was measured with a portable DO/pH meter (Orion-3-Star). Simplifying the characterization, the bio-oil (5 g) was vigorously mixed with water (10 g) for 15 min and centrifuged for 30 min with the centrifugal force of 3900 g. The bio-oil was 100% dispersible in water, which is noted to be WD fraction. The WD bio-oil fraction was further extracted by adding diethyl ether (40 mL) to obtain ether-soluble (ES) and aqueous (AQ) fractions (Fig. 1).

2.4. Bio-oil characterization

GCMS (FOCUS-ISQ, Thermoscientific) was used to analyze the ether soluble (ES) fractions (1 mg/mL) with anthracene added as an internal standard (50 µg mL⁻¹), in triplicate, where the separation was achieved using an RTx-5MS capillary column (30 × 0.25 mm Ø, Restek). The

temperature program was set to be from 40 to 320 °C at 5 °C min⁻¹. Compounds were identified as described above for pyrolysis GCMS.

The AQ fraction was analyzed by HPLC, in triplicate, using a Rezex ROA organic acid column (7.8 × 30 cm, Phenomenex) equipped with a differential refractive index detector (ERC-5710, ERMA), on elution with 0.005 N aqueous sulfuric acid (0.5 mL min⁻¹) at 65 °C. Data were acquired and analyzed using the N2000 chromatography software (Science Technology Inc., China).

The WD fraction was also analyzed directly by ESI-MS following dissolution in 1% acetic acid in methanol. The sample was introduced to a Finnigan LCQ-Deca instrument (Thermoquest) at a flow rate of 10 µL min⁻¹. Both negative and positive ion ESI-MS scans in the range m/z 50-2000 were performed for the same sample. The ion source and capillary voltages were 4.48 kV and 47 V, respectively, and the temperature was 275 °C. Both the number average molar mass (M_n) and the weight average molar mass (M_w) were computed using all the ions detected in the ESI-MS spectra of the ion with higher intensity. M_n and M_w were computed as $M_n = \sum M_i N_i / \sum N_i$ and $M_w = \sum M_i^2 N_i / \sum M_i N_i$, respectively, where the M_i is the m/z and N_i is the intensity of the i^{th} ion.

The boiling point distribution of bio-oil was determined by TGA from 110 to 900 °C at 10 K min⁻¹ under N₂ flow of 30 mL min⁻¹.

2.5. Biochar characterization

Biochar was vacuum dried prior to analysis. The butane activity (BA) of biochar was determined according the method of ASTM D5742-95 where dry biochar (16.7 ± 0.05 mL, of known weight) was placed into the sample tube flushed with butane (250 ± 5 mL min⁻¹) until a constant weight gain was obtained. The sample tube was placed in a water bath at 25 ± 0.2 °C. The proximate

analysis and calorific values of biochar samples were determined according to the aforementioned methods.

The specific surface area of the bacterial biomass char (0.10 g, in duplicate) was determined by N₂ and CO₂ (g) adsorption using a TriStar II plus automatic physisorption analyzer (Micromeritics Instrument Corporation). Before analysis the sample was vacuum degassed for 10 h at 200 °C. N₂ isotherms were collected at 77K and a partial pressure range of 0.0001-0.99, within this range 50 absorption and 35 desorption points were specified to fully resolve the isotherm. CO₂ isotherms were collected at 273K and a partial pressure range of 0.00001-0.03 with 75 points specified for the absorption isotherm. BET analysis was used to determine the apparent surface area (SA) from the N₂ isotherm using data from points collected at partial pressures between .001 and 0.1. SA, micropore volume (V_{mi}), and average pore width (W_{avg}) were determined by the Dubinin-Radushkevich (DR) equation fit to data points between $2 < \log(P_0 P^{-1})^2 < 6$ for both N₂ and CO₂ isotherms. W_{avg} was calculated from the characteristic binding energy (E_o) given by the DR equation [30].

$$W_{avg} \text{ (nm)} = 10.8 / (E_o - 11.4 \text{ kJ mol}^{-1})$$

Total pore volume (V_T) was determined from the maximum adsorption quantity at a partial pressure of approximately 0.99 for the N₂ isotherms. Total mesopore volume (V_{me}) was determined by subtracting the micropore volume determined by N₂ from V_T.

The RBB and biochar samples were characterized by FTIR spectroscopy using a Thermo Scientific iS5 spectrometer with an attenuated total reflection (ATR) iD5 probe (ZnSe). Samples were analyzed in triplicate, absorbance spectra averaged and baseline corrected using Omnic v9.0 software (ThermoScientific).

Raman spectra (9 replicates) of RBB biochar were acquired on Alpha 300 R confocal Raman microscope (Witec) spectrometer with 532 nm excitation, 0.5 s integration time and 10 scans. The spectra were averaged and baseline corrected using Omnic v9 (ThermoScientific) software. Band fitting of the spectrum was performed using a Gaussian function with Igor Pro v6.3 (Wavemetrics) software.

3. Results and discussion

3.1. RBB characteristics

3.1.1. Chemical composition, proximate, ultimate, and calorific value analyses

Table 1 shows the results obtained from the proximate, ultimate and chemical composition analyses, and the calorific values of the RBB. The RBB has a high amount of ash (21%), which was probably due to accumulated minerals (from the fermented dairy manure) that provided nutrients to the mixed microbial culture growth necessary for PHA biosynthesis. Additionally, the FC and VM of this RBB were close to the proximate analysis of sewage sludge by Shen and Zhang [23].

The ultimate analysis shows that C (36%) and H (5%) contents of RBB were slightly lower than hybrid poplar [17] and herbaceous biomass (e.g. corn stover and switchgrass) [12]. The calorific value of RBB was 16.1 MJ kg^{-1} , which is comparable with the values for some hardwoods ($13.5\text{--}19.4 \text{ MJ kg}^{-1}$) [31], but higher than those for seaweed algae samples ($10.6\text{--}13.5 \text{ MJ kg}^{-1}$) [14], as well as a mixture of mixed-culture algal biomass and swine manure [32]. This observed increase in calorific value was due to the significant N (attributed to protein) content of RBB which exceeded 5% ($\text{C/N (mole)} = 7.52$), and is comparable with other N-rich biomass, such as

activated sludge from waste water treatment and sewage sludge [21, 33]. The C/H and C/O ratios of RBB were 0.58 and 1.52, respectively.

The RBB contained approximately 21 wt% carbohydrate, 27 wt% acid insoluble residues (e.g., lignin/protein), and 10 wt% lipids (fatty acids and PHBV). It is worth noting that protein content of this RBB was > 30 wt%; similarly high protein content has been observed in activated sludge from waste water treatment processor [10, 20, 33]. Taken together, these findings suggest that the RBB could be a potential waste stream to be converted into more valuable bio-products through pyrolysis.

3.1.2. Protein and amino acid analysis

With protein constituting a major portion of the RBB, further distribution between free and bound amino acids is necessary to understand the origin of the pyrolysis products. The RBB contained 13 free amino acids, and upon hydrolysis, 16 amino acids were detected. The most abundant free amino acid was glutamic acid, followed by alanine and aspartic acid. The three major amino acids after hydrolysis, were glutamic acid, aspartic acid, and lysine. All amino acids have the same peptide backbone; similar degradation (e.g., decarboxylation and deamination) could be experienced for either free or bound form.

3.1.3. TGA/DTG analysis of waste biomass feedstock

TGA was conducted to investigate the thermal degradation behavior (e.g., pyrolysis temperature and decomposition kinetics) of RBB (Fig. 3). The thermal degradation of RBB occurred in three distinctive stages between 50 and 600 °C: (i) <200 °C, (ii) 200-375 °C, and (iii) > 375 °C. The first two stages could be attributed to the moisture/volatile matter evaporation and the decomposition of carbohydrates (150-350 °C) and cellulosic polysaccharides (275-350 °C). The

third stage was possibly due to lignin degradation (250-500 °C). Lipids residue would occur through the whole temperature range (200-600 °C) [28, 34, 35]. Proteins from bacteria decompose in a temperature range of 300 to 400 °C [18]. About 21% char/ash was observed, which is in accordance with ash content determination.

The activation energy, E , before 75% conversion of samples at 5% conversion intervals were according to ASTM methods. The average E and A values were 199 kJ mol⁻¹ and 1.07×10^{24} , respectively. These parameters were similar to other lignocellulosic biomass [28, 36] and protein-rich sewage sludge [18].

3.1.4. Py-GCMS analysis of residual bacterial biomass

Analytical Py-GCMS at 500 °C was used to identify potential pyrolysis products (Table 3). A total of 32 pyrolysis compounds were identified with the most abundant products being CO₂ followed by acetic acid (9.8%), docosenamide (10.9%), and acetaldehyde (9.1%). Approximately 20% carbohydrate derived compounds (including acetic acid) were identified. Acetaldehyde could be generated from the thermal decomposition of protein or free amino acid, especially alanine [37], which was the second most abundant amino acid identified in the RBB (Table 2). A small but noteworthy amount of diketopiperazine (2.1%) was identified, which is caused by the dimerization of two glycine units [38, 39]. Several fatty acids compounds such as undecenoic acid (5.0%) and hexadecenoic acid (4.4%) were produced, which were derived from lipids in the RBB. Aromatic hydrocarbons, phenols, guaiacol and syringol were also detected. For example, guaiacol and syringol (and their derivatives) comprised 12.8 and 7.8% of the pyrolysis products, respectively. This finding supports the presence of lignin or lignin fragments in the RBB [40] and reinforces the chemical compositional analysis. Lignin is likely carried over as fine particles from the fermented dairy manure liquor feed to the PHA bioreactor. Furthermore, some of these

aromatic compounds (e.g. phenol) may originate from aromatic amino acids, such as phenylalanine and tyrosine [41]. Additionally, N-containing compounds (e.g., pyrrole, benzonitrile, indole, and docosenamide, etc.) derived from protein were produced, constituting approximately 36% of the pyrolysis products. This agrees with the ultimate (C, H, and N) and chemical compositional analyses of the RBB (Table 1). Regarding indoles and aromatic nitriles (e.g., benzonitrile), these products could also be possibly derived from protein decomposition. Pyrrole, indole and their derivatives have been observed in pyrolytic oil from sewage sludge [22, 42]. The indole derivatives were possibly formed from pyrolysis of tryptophane, while pyrrole derivatives were from serine and asparagines [43, 44].

3,5-Diethylpyridin-4-amine was also present at a level of 2.6% of all the compounds detected. The pyrolysis product octahydrodipyrrolo[1,2-*a*:1', 2'-*d*]pyrazine-5,10-dione (OHDPPD) was most likely a proline condensation product [42, 45]. Acetamide (4.4%) was also produced and could arise from microbial cell membranes and amino sugars [42]. A small amount of butenoic (1.6%) and pentenoic acids (1.7%) were identified, and likely arose from the thermal degradation of residual PHBV in the RBB.

3.2. Pyrolysis yield and products fractionation

TGA analysis of RBB showed almost complete decomposition by 500 °C, therefore, pyrolysis was conducted at this temperature. The bio-oil and biochar yields and properties (e.g., pH and water solubility) were recorded. A bio-oil yield of 28% was obtained, which was similar to that from sewage sludge (30%) [22]. A biochar yield of 46% was obtained, which lied in the range of 36-56% that was observed from pyrolyzed activated sludge (thickened excess activated sludge, dewatered digested sludge, and dried excess activated sludge) [21]. These high biochar yields are ascribed to the high ash content of the biomass. The nature of the biomass, the heat transfer rate,

and efficiency of the pyrolysis reactor can influence bio-oil and biochar yields [17]. The efficiency of the transfer tube can be potentially improved to condense and recover more bio-oil. Moreover, the RBB pyrolysis product yields and characteristics can be optimized by varying the pyrolysis parameters (residence time, temperature, etc). For example, pyrolysis temperature could be increased to produce biochar with relatively higher surface areas.

The bio-oil was shown to have a high pH of 8.2 as compared to woody lignocellulosic biomass pyrolysis bio-oils, which are acidic (pH ~ 2.5) [11]. This high pH was most likely a result of a high amine content of the bio-oil which was derived from protein. Compared with pyrolysis oil from low-protein lignocellulosic biomass, the lower acidity of bio-oil from RBB could make it favorable for direct use as boiler fuel. The bio-oil was highly dispersible in water (WD fraction) and this is probably due to the polar pyrolysis products derived from proteins and carbohydrates [13]. Due to its good water dispersibility, nitrogen-rich bio-oil could be fed back for PHA biosynthesis which typically occurs under aqueous medium.

The WD fraction was partitioned into ether (ES, 15% w/w) and aqueous (AQ, 85% w/w) fractions (Fig. 1) that were characterized by GCMS and HPLC, respectively, to simplify the identification of the generated compounds (Table 4).

The WD fraction was comprised of carbohydrate derived products such as acetic acid (41.3%), levoglucosan (3.1%), and formic (1.9%) and propanoic (1.9%) acids. About 27.3% of the products were N-based compounds, such as imidazole and pyrrole derivatives, and were similar to the Py-GCMS analysis. This further confirmed thermal decomposition or recombination of amino acids (free or hydrolysates from proteins) during pyrolysis. Moreover, some aromatic hydrocarbons were formed, including phenol, methylphenols, guaiacol, syringol and toluene

derivatives were also formed. According to Fabbri et al. [41], phenols are characteristic thermal fragmentation products of free aromatic amino acids such as phenylalanine and tyrosine.

Guaiacol and syringol based compounds are derived from lignin. In addition to compounds formed from simple cleavage of amino acids, the formation of 5-methyl-2,4-imidazolidinedione is possibly from protein pyrolysis by lactam ring formation. This compound was also observed in keratin pyrolysis products [46].

The average molar mass (M_w and M_n) of the pyrolysis bio-oil was obtained by the ESI-MS analysis. The positive and negative ion ESI-MS spectra are shown in Fig. 4. The positive ion MS spectrum was dominated by $< m/z$ 400 ions than those in the negative mode ($< m/z$ 300). The M_w and M_n determined in positive mode were 496 and 592 g mol⁻¹, respectively, with a polydispersity (PD = M_w/M_n) close to 1.2. The M_w , M_n , and PD values determined from the negative ion spectrum were respectively, 394 g mol⁻¹, 258 g mol⁻¹, and 1.5. These results clearly show that the products were mono- to oligo-meric compounds. The molar mass was higher than other biomass types, i.e., hybrid poplar and potato peel waste [17]. More N-containing compounds are observed in the positive mode, whereas more oxygen-rich compounds were seen in the negative mode. In the positive mode (Fig. 4a) protonated species $[M+H]^+$ were observed and tentatively assigned at m/z 97 (C₅H₉N₂), 111 (N₆H₁₁N₂), 125 (C₇H₁₃N₂), 139 (C₈H₁₅N₂), 153 (C₉H₁₇N₂ or C₉H₁₂O₂), 167 (C₁₀H₁₉N₂), 180 (C₁₀H₁₄O₂N), 197 (C₁₁H₁₉O₂N), 209 (C₁₂H₁₉O₂N), 223 (C₁₃H₂₁O₂N), 231 (C₁₄H₁₉ON₂), and 253 (C₁₆H₁₇ON₂). These compounds were also found in pyrolysis products of N-containing species of switchgrass [47] and sewage sludge [22]. They were possibly derived from protein or free amino acid of the RBB. For example, the compound C₁₄H₁₉ON₂ is likely a dipeptide of aspartic acid ($[M+H]^+ = 133$, Table 2). The peak at m/z 82 was assigned to methylpyrrole and compliments the findings from the Py-GCMS analysis. Major

peaks observed in the negative ion spectrum ($[M-H]^-$) with tentative assignment include m/z at 109 ($C_6H_5O_2$, benzenediol), 123 ($C_7H_7O_2$, guaiacol), 137 ($C_8H_{10}O_2$), 149 ($C_9H_9O_2$, vinyl guaiacol), 191 ($C_7H_{11}O_6$), and 207 ($C_7H_{11}O_7$). The presence of these compounds supports the presence of phenolics and lignin in the RBB, which could be carried over from the fermented dairy manure liquor fed for the PHBV biosynthesis.

The boiling point distribution of the bio-oil was evaluated using TGA. This method was considered a miniature “distillation” unit according to Chen et al. [32]. The boiling temperature range was calibrated with an alkane wax standard ($C_{10} - C_{44}$). Table 5 shows the major weight loss ($\sim 89\%$) of pyrolysis bio-oil occurred below $220^\circ C$. This indicated the bio-oil was mainly comprised of small compounds ($< C_{12}$). The coke oil with boiling point ranging from 25 to $200^\circ C$ can be used as bottle gas (chemicals) and gasoline [32]. At this stage, the RBB could become an attractive potential for bio-oil feedstock yet, nitrogen remains in the pyrolysis products, which should be removed in the subsequent chemical upgrading process. Therefore, this oil can be potentially upgraded or refined for transportation fuels and chemicals production.

3.3. Biochar characterization

FTIR spectroscopic analysis was used to investigate the chemical structure of biochar samples, while the raw biomass sample was analyzed for comparison (Fig. 5). The band assignments are summarized in Table 6. The broad band in the region of $3600-3200\text{ cm}^{-1}$ is attributed to the O–H (e.g., water, alcohol, and phenol) and N–H (amines) stretching vibrations [20]. For example, the N–H stretching (amide II) vibration at $3380-3304\text{ cm}^{-1}$ was observed in the RBB (Fig. 5a), whereas it was not seen in the biochar sample. This indicated that the N-containing protein was mostly converted into pyrolysis bio-oil products. Aliphatic bands C–H (CH_3 and CH_2) stretching vibrations between 2950 and 2850 cm^{-1} were present in both RBB and biochar samples. The

weak band at 1724 cm^{-1} in RBB sample could be from the C=O stretching of PHBV bioplastic or lipid residues (fatty acids derivatives) (Fig. 5a) [48]. The broad band at 1641 cm^{-1} (Fig. 5a) was from Amide I (C=O stretching), while the one at 1538 cm^{-1} was ascribed to the C–N stretching and N–H in-plane bending absorption due to Amide II of secondary amide in protein, respectively, which was not present in the spectra of the biochar (Fig 5b). The bands in the RBB between 1300 and 1500 cm^{-1} (i.e. 1451 and 1416 cm^{-1}) could be assigned to the deformation of CH_2 and CH_3 groups (Fig. 5a). The small band at 1232 cm^{-1} was attributed to C–O stretching and O–H bending vibrations of carboxylic acids [20].

The broad band centered at 3338 cm^{-1} in biochar sample was from O–H group stretching (Fig. 5b). The broad absorption band in biochar from 1700 to 1300 cm^{-1} (centered at 1586 cm^{-1} and a shoulder at 1510 cm^{-1}) was assigned to C=C skeletal vibration of the aromatic ring of lignin. Hence, the biochar sample could be comprised of a large part of polycondensed phenolic structures. The intense band centered at 1052 cm^{-1} for both RBB and biochar sample was assigned to C–O and C–O–C stretching of carbohydrates. As stated earlier, the biomass is comprised of a large amount of bacterial constituents (nucleic acids, proteins, carbohydrates and lipids).

The proximate analysis, BA, calorific value, and surface area of biochar samples are given in Table 1. The biochar had an ash, VM, and FC content of 60%, 18%, and 22%, respectively. The ratio of VM and FC of the biochar sample was much higher than that of hybrid poplar and potato peel waste (PPW). This could be contributed to the relatively lower extent of condensed aromatic structures (number of aromatic C's per aromatic cluster) [17]. The calorific value for biochar was 20 MJ/kg , which is similar to the biochar samples obtained from pyrolysis of PPW. BA is an indirect measure of surface area and adsorption of alkane for activated carbon. The BA value of

pyrolysis biochar was 2.1%. This value was lower than commercial activated carbon (~ 25%) [49], possibly due to lower total surface area. Higher pyrolysis temperature would likely increase BA as well as carbonization or ozonolysis [17, 50].

Gas adsorption isotherms, Fig. 6, indicate that the predominant pore structures in the RBB biochar are microporous. Analysis of the CO₂ adsorption isotherm by the DR equation identifies a SA of $102 \pm 2 \text{ m}^2 \text{ g}^{-1}$. The SA determined by N₂ absorption was considerably lower, but shows strong agreement when calculated by either the BET or DR equation, $7.7 \pm 0.3 \text{ m}^2 \text{ g}^{-1}$ or $8.1 \pm 0.6 \text{ m}^2 \text{ g}^{-1}$ respectively. The SA determined by N₂ is consistent with the results obtained for sewage sludge pyrolysis biochar ($10.8 \text{ m}^2 \text{ g}^{-1}$) produced at 500 °C [51]. The low SA value corroborates the low BA value obtained for this biochar.

The V_{mi} determined by N₂ was low, only $0.0029 \pm 0.002 \text{ cm}^3 \text{ g}^{-1}$. In Fig. 6, the hysteresis seen in the N₂ isotherm indicates that much of the pore volume detected is located within mesopores. This is confirmed by a V_T of $0.046 \pm .001 \text{ cm}^3 \text{ g}^{-1}$. When compared with V_{mi} (N₂) a V_{me} of $0.043 \pm .003 \text{ cm}^3 \text{ g}^{-1}$ was obtained. More complete analysis of the microporous region by CO₂ results in a V_{mi} (CO₂) of $0.041 \pm 0.001 \text{ cm}^3 \text{ g}^{-1}$. Analysis of the E_o confirms that the micropores determined by CO₂ were largely inaccessible to N₂. Using the DR equation a W_{avg} of 0.6 nm was determined for pores probed by CO₂ absorption. In comparison, the E_o for N₂, $11.0 \pm 0.8 \text{ kJ mol}^{-1}$, was too low to be assessed by the DR equation, a result of substantially larger average pore size.

Ultimate analysis showed 27.7% of C, 1.2% of H, and 4.9% of N in the biochar sample. Due to the high ash (60%) content the O content was estimated at 5.9%. The biochar can be used for soil

amendment to enhance soil aggregation, water holding capacity, and organic carbon content. Compared to the parent biomass feedstock, lower C, H, and N was found in biochar. The C/H ratio was increased by 230% in biochar as compared to parent RBB, likely as a result of increasing content of aromatic compounds in the biochar sample. The C/O ratio of the biochar sample was 410% higher than the parent RBB. A similar result was found in one previous study [13], pyrolytic deoxygenation of the high protein feedstock, such as defatted pennycress presscake which produced a pyrolysis products with a relatively higher C/O ratio. The C/N ratio was lower in biochar sample than that of RBB, which further confirmed the denitrogenation via deamination of protein and amino acids. Considering the N balance, the non-condensable gas fraction also contained some of the N from the starting biomass, possibly in the form of NH_3 .

Raman spectroscopy was performed on the biochar to obtain information on the structure of this carbonaceous material. The spectrum showed two broad bands at Raman shift of 1355 cm^{-1} and 1585 cm^{-1} , which are respectively assigned to the D-band (amorphous or disordered C) and G-band (graphitic crystallites) of carbon (Fig. 7) [52]. These spectral bands are consistent with Raman spectra of biochar derived from pyrolyzed sewage sludge [51]. An estimation of disordered carbon in the sample was calculated from the ratio of D/G band intensity (I_D/I_G) to be 1.3. The I_D/I_G value obtained was in the range of values for biochar (0.8-1.5) from various biomasses pyrolyzed around 500°C [52, 53], however was lower than sewage sludge biochar (5.7) produced at 500°C [51]. These results show that the RBB biochar is mainly disordered amorphous carbon.

4. Conclusions

In this study, residual bacterial biomass (RBB) from an MMC-based PHA production process was characterized for the chemical composition and thermal decomposition. The RBB was comprised of carbohydrate, lignin, and N-rich compounds (protein/nucleic acid). Similar calorific values and thermal decomposition kinetic energy further showed the RBB could be suitable for use as a renewable fuel. Pyrolysis was successfully used to convert the RBB into bio-oil and biochar products. The bio-oil yield (28%) was relatively low compared to that of lignocellulosic biomass. Biochar yield (46%) was high due to high ash and protein contents of RBB. Py-GCMS showed pyrolysis products were comprised of aromatics, hydrocarbons, and N-containing compounds. The primary compounds identified in the bio-oil can be grouped into different classes: hydrocarbons, such as mono-aromatic hydrocarbons and phenolic compounds, which include toluene and phenolic compounds; aliphatic ketone, including pentanone and cyclopentanedione; nitrogen and oxygen containing aromatic compounds, such as pyrrole, indole, and pyridinyl products; aliphatic compounds and amines; and carboxylic acids. This preliminary study shows the RBB can be utilized to produce co-products to improve the economic viability of PHA production. Meantime, the results help understand the utilization of dairy manure as a feedstock for PHA production, for example, phenolics and lignin seemed originated from the fermented liquor. Future research will focus on optimizing pyrolysis operational parameters (temperature and residence time) to improve the bio-oil or biochar yields and quality. Nitrogen-rich pyrolysis oil could be considered as a potential feedstock for PHA production in the future.

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Table 1. Characterization of RBB feedstock and biochar.

Analyses	% dry basis [*]	
	Biomass	Biochar
Proximate analysis		
Ash	21.4 ± 0.5	60.3 ± 0.0
VM	65.5 ± 0.3	18.1 ± 0.7
FC	13.1 ± 0.3	21.6 ± 0.8
Ultimate analysis		
C	36.1 ± 0.1	27.7 ± 0.4
H	5.3 ± 0.0	1.2 ± 0.0
N	5.6 ± 0.0	4.9 ± 0.0
O [§]	31.6 ± 0.1	5.9 ± 0.3
C/H atomic ratio	0.58	1.92
C/N atomic ratio	7.52	6.60
C/O atomic ratio	1.52	6.26
Calorific value (MJ kg ⁻¹)	16.1 ± 0.5	20.0 ± 0.6
Chemical compositional analysis		
Lipid	10.1 ± 0.1	
Carbohydrate	20.9 ± 0.2	
Acid insoluble residue	27.1 ± 0.1	
Crude protein (N% × 6.25)	35.0 ± 0.0	
BA (%)		2.1 ± 0.0
Surface area (m ² g ⁻¹)**		
N ₂ (BET)		7.7 ± 0.3
CO ₂ (DR)		102 ± 2

^{*} Data are means of three replicates ± standard deviations.

^{**} Data are means of two replicates ± standard error.

[§] O = 100 – (C + H + N + ash)

Table 2. Amino acid composition of protein hydrolysis and free amino acid in RBB (% relative abundance) determined by GCMS.

Amino acid	Retention time (min)	M ⁺ (<i>m/z</i>)	Formula	Amino acid composition after hydrolysis (%)	Free amino acid (%)
Alanine	6.2	89	C ₃ H ₇ NO ₂	4.2	13.5
Glycine	6.5	75	C ₂ H ₅ NO ₂	2.6	3.9
Valine	8.7	117	C ₅ H ₁₁ NO ₂	3.3	5.5
Threonine	9.1	119	C ₄ H ₉ NO ₃	4.8	3.5
Serine	9.4	105	C ₃ H ₇ NO ₃	5.1	4.5
Leucine	10.3	131	C ₆ H ₁₃ NO ₂	6.7	7.8
Iso-leucine	10.6	131	C ₆ H ₁₃ NO ₂	2.6	3.5
Proline	12.6	115	C ₅ H ₉ NO ₂	7.1	7.0
Hydroxyproline	14.9	131	C ₅ H ₉ NO ₃	0.8	trace
Methionine	15.9	149	C ₅ H ₁₁ NO ₂ S	0.3	trace
Aspartic acid	17.4	133	C ₄ H ₇ NO ₄	13.3	10.3
Phenylalanine	18.4	165	C ₉ H ₁₁ NO ₂	5.4	3.0
Glutamic acid	20.3	147	C ₅ H ₉ NO ₄	24.5	26.4
Lysine	21.6	146	C ₆ H ₁₄ N ₂ O ₂	11.7	4.9
Tyrosine	22.4	181	C ₉ H ₁₁ NO ₃	7.3	6.2
Arginine	24.1	174	C ₆ H ₁₄ N ₄ O ₂	0.3	trace

Table 3. Compounds released by Py-GCMS of extractive free RBB.

No.	RT (min) ^a	Compound	M ⁺ (<i>m/z</i>)	Formula	Relative abundance (%) ^b
1	1.23	Acetaldehyde	44	C ₂ H ₄ O	9.13 ± 0.00
2	1.37	Acetamide	59	C ₂ H ₅ NO	4.40 ± 0.01
3	1.78	Acetic acid	60	C ₂ H ₄ O ₂	9.80 ± 0.00
4	2.06	Pyrrole	67	C ₄ H ₅ N	0.35 ± 0.01
5	2.15	2-methylcyclopentanol	100	C ₆ H ₁₂ O	0.51 ± 0.02
6	3.25	Toluene	92	C ₇ H ₈	0.46 ± 0.05
7	3.95	3-methylpyrrole	81	C ₅ H ₇ N	3.30 ± 0.03
8	4.42	1,4-dimethyl-1H-pyrazole	96	C ₅ H ₈ N ₂	2.44 ± 0.01
9	5.14	Butenoic acid	86	C ₄ H ₆ O ₂	1.05 ± 0.10
10	5.64	Benzonitrile	103	C ₇ H ₅ N	3.37 ± 0.10
11	6.92	4-methyl-1,3-dioxolane	88	C ₄ H ₈ O ₂	2.35 ± 0.05
12	7.16	Pentenoic acid	100	C ₅ H ₈ O ₂	1.65 ± 0.05
13	8.21	Phenol	94	C ₆ H ₆ O	1.74 ± 0.00
14	9.21	Diketopiperazine	114	C ₄ H ₆ N ₂ O ₂	2.05 ± 0.00
15	9.78	2,6-dimethyl-1-piperidinamine	128	C ₇ H ₁₆ N ₂	1.63 ± 0.01
16	10.28	2-methylphenol	108	C ₇ H ₈ O	0.86 ± 0.00
17	10.93	3-methylphenol	108	C ₇ H ₈ O	1.08 ± 0.05
18	11.10	Guaiacol	124	C ₇ H ₈ O ₂	1.58 ± 0.10
19	13.25	1,4:3,6-dianhydro-hexopyranose	144	C ₆ H ₈ O ₄	3.26 ± 0.13
20	13.87	Propionic acid, pentyl ester	144	C ₈ H ₁₆ O ₂	2.59 ± 0.10
21	14.01	Methyl guaiacol	138	C ₈ H ₁₀ O ₂	3.12 ± 0.00
22	15.01	3,5-diethylpyridin-4-amine	150	C ₉ H ₁₄ N ₂	2.59 ± 0.05
23	16.34	4-ethyl-guaiacol	152	C ₉ H ₁₂ O ₂	2.40 ± 0.07
24	16.87	Indole	117	C ₈ H ₇ N	2.18 ± 0.07
25	17.30	4-vinyl guaiacol	150	C ₉ H ₁₀ O ₂	2.26 ± 0.07
26	18.33	Syringol	154	C ₈ H ₁₀ O ₃	4.12 ± 0.07
27	20.77	Methoxymethyl-guaiacol	168	C ₉ H ₁₂ O ₃	3.32 ± 0.03
28	22.07	Undecenoic acid	184	C ₁₁ H ₂₀ O ₂	5.04 ± 0.03
29	23.55	Propioguaiacone	180	C ₁₀ H ₁₂ O ₃	2.82 ± 0.07
30	26.59	OHDPPD ^c	194	C ₁₀ H ₁₄ N ₂ O ₂	3.63 ± 0.02
31	29.23	Hexadecenoic acid	254	C ₁₆ H ₃₀ O ₂	4.44 ± 0.07
32	43.94	Docosenamide	337	C ₂₂ H ₄₃ NO	10.86 ± 0.01

^a RT: Retention time; ^b relative abundance (%) in the water dispersible fraction; ^c OHDPPD: octahydrodipyrrolo[1,2-*a*:1', 2'-*d*]pyrazine-5,10-dione.

Table 4. GCMS and HPLC analyses results of ES and AQ fractions of pyrolyzed RBB, respectively.

No.	RT (min) ^a	Compound	M ⁺ (m/z)	Formula	Concentration (% WD) ^b
GCMS					
1	7.60	3-methyl-2-pentanone	100	C ₆ H ₁₂ O	3.15±0.06
2	8.81	Phenol	94	C ₆ H ₆ O	9.22±0.56
3	10.13	3-methyl-1,2-cyclopentanedione	112	C ₆ H ₈ O ₂	0.91±0.00
4	10.45	2,3-dimethyl-2-cyclopentanedione	110	C ₇ H ₁₀ O	0.83±0.06
5	10.92	2-Methylphenol	108	C ₇ H ₈ O	0.68±0.07
6	11.55	3-Methylphenol	108	C ₇ H ₈ O	2.22±0.04
7	11.85	Guaiacol	124	C ₇ H ₈ O ₂	1.39±0.15
8	12.06	5-methyl-2,4-imidazolidinedione	114	C ₄ H ₄ N ₂ O ₂	5.08±0.00
9	12.62	N-(2-Propenyl)-N-(2-Propynyl)-N-ethylamine	123	C ₈ H ₁₃ N	2.93±0.06
10	13.47	1,2,5-Trimethylpyrrole	109	C ₇ H ₁₁ N	5.74±0.52
11	14.24	3-ethyl-2,4-dimethyl-pyrrole	123	C ₈ H ₁₃ N	2.42±0.08
12	14.79	Methyl-guaiacol	138	C ₈ H ₁₀ O ₂	1.30±0.17
13	15.35	2-(2-propynyloxy)cyclohexanol	154	C ₉ H ₁₄ O ₂	0.73±0.01
14	15.62	2-methyl-1-vinylimidazole	108	C ₆ H ₈ N ₂	1.57±0.09
15	16.01	Histidine	155	C ₆ H ₉ N ₃ O ₂	0.37±0.08
16	16.20	2-phenyl-4-pentenal	160	C ₁₁ H ₁₂ O	0.21±0.00
17	16.74	Histamine-2-carboxylic acid	155	C ₆ H ₉ N ₃ O ₂	0.76±0.21
18	17.02	5H-Pyrrolo-(3,2-d)pyrimidin-4-amine	134	C ₆ H ₆ N ₄	1.50±0.04
19	17.42	Indole	117	C ₈ H ₇ N	3.91±0.10
20	17.87	N,2,4,6-tetramethyl-bezenamine	149	C ₁₀ H ₁₅ N	3.06±0.09
21	19.27	Syringol	154	C ₈ H ₁₃ O ₃	0.88±0.06
22	19.68	Eugenol	164	C ₁₀ H ₁₂ O ₂	0.47±0.01
23	23.00	Butylated hydroxytoluene	220	C ₁₅ H ₂₄ O	2.55±0.21
HPLC					
1	15.91	Levoglucosan	162	C ₆ H ₁₀ O ₅	3.12±0.53
2	17.60	Formic acid	46	CH ₂ O ₂	1.90±0.36
3	21.03	Acetic acid	60	C ₂ H ₄ O ₂	41.25±0.27
4	24.29	Propanoic acid	74	C ₃ H ₆ O ₂	1.86±0.10

^a RT: retention time. ^b Concentration: % in the water dispersible (WD) fraction. The values were calculated from duplicates, while standard deviation was shown after ±.

Table 5. Boiling point distribution of pyrolysis bio-crude.

Temperature range (°C)	Product distribution (%)	Compound
Pyrolysis bio-crude		
50-220	89.0	<C ₁₂
220-440	10.0	C ₁₃ -C ₃₀
440-500	1.0	>C ₃₀
Wax standard		
50-210	60.5	<C ₁₂
210-270	32.1	C ₁₃ -C ₁₆
270-450	7.3	C ₁₇ -C ₃₀
450-550	0.1	>C ₃₀

Table 6. FTIR spectral band assignments for residual bacterial biomass (RBB) and pyrolysis biochar samples.

Band assignment	Band frequency (cm ⁻¹)	
	RBB	Biochar
Bonded -O-H, -N-H stretching	3380, 3304	
-O-H stretching		3338
-C-H stretching	2928	2928 (weak)
-C=O stretching	1724 (weak)	
Amide I band	1641	
Aromatic C-C/C=C stretching		1586
Amide II band	1538	
Deformation of -CH, -CH ₃ , and -CH ₂	1451, 1416	
C-O stretching and O-H bending	1232	
Silicate; C-O stretching	1052	1050

Fig. 1. Flow diagram of experiment steps performed, sample generation and analyses performed.

Fig. 2. A schematic diagram of the laboratory auger pyrolysis reactor apparatus.

Fig. 3. TG and DTG thermograms of residual bacterial biomass (RBB) at different heating rates (5 - 50 K min⁻¹).

Fig. 4. (a) Positive and (b) negative ion ESI-MS of water dispersible (WD) bio-oil from pyrolysis of residual bacterial biomass (RBB).

Fig. 5. FTIR spectra of (a) residual bacterial biomass (RBB) and (b) biochar.

Fig. 6. Absorption isotherms for residual bacterial biomass (RBB) biochar. Solid line represents CO₂ absorption while dashed line represents N₂ absorption/desorption.

Fig. 7. Raman spectrum of residual bacterial biomass (RBB) biochar.