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Disassembly and reassembly of polyhydroxyalkanoates: Recycling through abiotic depolymerization and biotic repolymerization

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HIGHLIGHTS

- Base depolymerizes polyhydroxyalkanoates (PHAs) to hydroxyacids and alkenoates.
- Thermal treatment depolymerizes PHAs to alkenoates.
- A microbial enrichment repolymerizes PHAs from hydroxyacids and alkenoates.
- Nitrogen-limited cells produce high quality PHA homopolymer and copolymer.
- Polyphosphate hydrolysis accompanies repolymerization.

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ABSTRACT

An abiotic–biotic strategy for recycling of polyhydroxyalkanoates (PHAs) is evaluated. Base-catalyzed PHA depolymerization yields hydroxyacids, such as 3-hydroxybutyrate (3HB), and alkenoates, such as crotonate; catalytic thermal depolymerization yields alkenoates. Cyclic pulse addition of 3HB to triplicate bioreactors selected for an enrichment of *Comamonas*, *Brachymonas* and *Acinetobacter*. After each pulse, poly(3-hydroxybutyrate) (P3HB) transiently appeared: accumulation of P3HB correlated with hydrolysis of polyphosphate; consumption of P3HB correlated with polyphosphate synthesis. Cells removed from the cyclic regime and incubated with 3HB under nitrogen-limited conditions produced P3HB (molecular weight > 1,000,000 Da) at 50% of the cell dry weight (<8 h). P3HB also resulted from incubation with acetate, crotonate, or a mixture of hydrolytic depolymerization products. Poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (PHBV) resulted from incubation with valerate or 2-pentenoate. A recycling strategy where abiotic depolymerization of waste PHAs yields feedstock for customized PHA re-synthesis appears feasible, without the need for energy-intensive feedstock purification.

1. Introduction

Polyhydroxyalkanoates (PHAs) are a diverse class of biopolymers that can potentially replace petroleum-based plastic products (Houmiel et al., 1999). Many species of bacteria synthesize PHA granules when they are supplied with carbon and electron equivalents but lack another nutrient needed for cell replication, such as nitrogen or phosphorus (Anderson and Dawes, 1990; Lee, 1996). Microorganisms use the granules as reservoirs of carbon and reducing equivalents for later use when conditions become favorable for cell replication (Doi, 1990; Pieja et al., 2011). While PHAs are currently more expensive than their fossil carbon-derived counterparts (Arun et al., 2006), they are of great interest due to their diversity of function, biocompatibility, and lack of persistence in the environment (Johnson et al., 2009; Steinbuchel, 2001). As shown in Fig. 1, PHAs are renewable both aerobically and anaerobically. Under aerobic conditions, they degrade to CO_2 (Akmal et al., 2003), and the CO_2 can be recycled by photosynthesis back into PHAs. Under anaerobic conditions, they degrade to methane-rich biogas that can be captured and used to create virgin poly (3-hydroxybutyrate) (P3HB) through the activity of type II methanotrophic (methane-utilizing) bacteria (Pieja et al., 2011). Molecular

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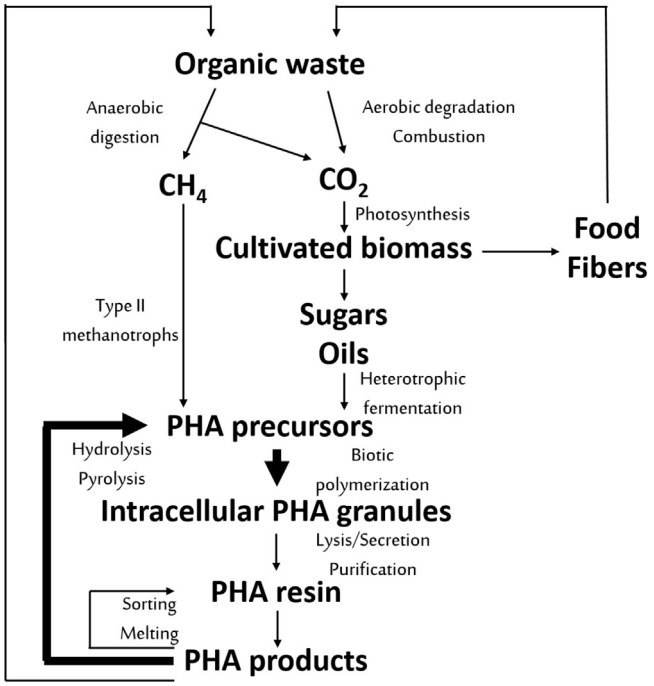

Fig. 1. Recycling paths for PHAs. The thick lines highlight chemical routes for PHA recycling based upon (1) abiotic depolymerization of PHAs into precursor molecules, including chemical precursors, such as hydroxyalkanoate monomers and alkenoic acids, and biochemical precursors, such as acyl-CoA and acyl-ACP (Chen, 2010), and (2) biotic polymerization of precursors into PHA granules.

recycling of PHAs through such lengthy ecological pathways leads to energy inefficiencies and longer time scales for polymer recycling. Shorter recycling pathways are needed. In principle, PHAs can be recycled through conventional sorting and re-melting (Fig. 1), but downcycling results, as desirable properties are lost with successive reuse and shortening of the polymer chains (Chan Sin et al., 2010). Strategies are needed for regeneration of PHAs without downcycling.

In this study, we propose and test a "short-circuit" PHA recycling strategy that combines abiotic depolymerization (via base hydrolysis or pyrolysis) with biochemical repolymerization, enabling rapid regeneration of PHAs without downcycling (Fig. 2). Specifically, we demonstrate that microbial enrichments can repolymerize abiotic depolymerization products to high quality PHAs (high molecular weight, low polydispersity), as illustrated in Fig. 2. Abiotic PHA depolymerization has been previously investigated using both chemical and thermal methods (Ariffin et al., 2010; Yu et al., 2005), but these studies had limited scope in terms of materials tested and the methods used. No study to date has coupled abiotic depolymerization to biotic repolymerization or evaluated the feasibility of repolymerization without energy-intensive purification steps.

2. Methods

2.1. Base-catalyzed hydrolysis of poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (PHBV)

Hydrolysis of PHBV was carried out by methods similar to that reported for P3HB (Yu et al., 2005). Twelve mg of microbially-generated PHBV with 5 mol% 3HV content (Sigma–Aldrich, St Louis, MO, USA) was added to 10 mL of 0.1 M sodium hydroxide solution and charged into sealed cylindrical glass vials (pH 13). The vials were placed in a water bath, shaken continuously at 200 rpm, and incubated at 60 °C. Vials were periodically removed from the water bath and analyzed for soluble degradation products.

Two-milliliter samples were extracted and centrifuged for 15 min at 14,000 rpm. After centrifuging, 1.25 mL of supernatant was added to a 2 mL centrifuge tube containing 25 μL of 1.44 M HEPES Buffer and 200 μL of 800 mg/L sodium benzoate. The solution was frozen at −80 °C, lyophilized until dry, then amended with 250 μl of methanol. Twenty microliters of the dissolved sample was transferred to a glass GC vial and amended with 120 μL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). The solution was microwaved in a conventional microwave oven (Emerson Model # MW9332BFC) at a power level of 6 for 3 min. Samples of this solution were analyzed by GC/MS using an Agilent 6890 N gas chromatograph (GC) coupled to an Agilent 5973 mass selective detector (MSD). The GC was equipped with a HP-5MS column (Agilent Model Number 19091s-433 30.0 m long × 250 μm i.d. × 0.25 μm film thickness). The oven temperature program was as follows: 50 °C for 3 min, ramp increase to 60 °C over 9 min, 60 °C for 3 min, ramp increase to 260 °C over 9 min, ramp increase to 300 °C over 6 min, and held at 300 °C for 6 min. Helium (1.0 mL min^{-1}) was the carrier with inlet pressure maintained at 56.7 kPa (8.22 psi). The Mass Selective Detector (MSD) was operated in scanning ion mode at mass to charge ratios (m/z) from 33 to 250. The ions used for quantification of 3HB, crotonate, 3HV, 2-pentenoate, 3-pentenoate, and benzoate were m/z = 147, 191; 143, 69; 147, 131; 83, 143, 157; 157, 117, 41, 83; and 77, 105, 135, 179, respectively. The ion peak areas for a particular product were summed and divided by the sum of the integrated benzoate peaks. For each sample, the result was compared to standard curves.

2.2. Base-catalyzed pyrolysis of P3HB

The pyrolysis of P3HB was carried by the method of Ariffin and coworkers (Ariffin et al., 2010). Unpurified P3HB (natural origin, Sigma–Aldrich, St Louis, MO, USA) was ground with magnesium hydroxide (Fisher Scientific, Pittsburgh, PA) (9 wt% relative to the P3HB) using a mortar and pestle. This mixture (370–400 mg) was placed on a metal scoopula, and the scoopula inserted into a 2.54 cm diameter quartz tube. The tube was inserted into a Thermo Scientific Lindberg Mini-Mite Tube Furnace, and subjected to a

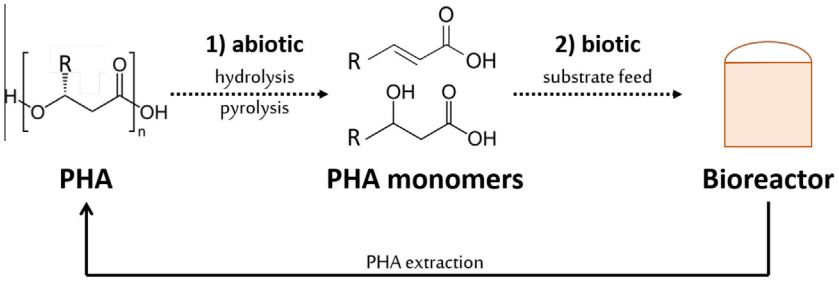

Fig. 2. Overall scheme of direct recovery of PHA from waste PHA (two dotted lines represent the key steps proposed in this paper).