Bioresource Technology 148 (2013) 61–69

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Biosynthesis of poly(hydroxybutyrate-hydroxyvalerate) from the acclimated activated sludge and microbial characterization in this process

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HIGHLIGHTS

- Biosynthesis of various mix of P(HB/HV) from the acclimated activated sludge.
- Significant liner correlation between concentration of propionate and HV fraction.
- Desired HV fraction requires proper selection of carbon sources and acclimation.
- Specific species in the sludge were dominant for PHB and PHV accumulation.
- Mechanisms for P(HB/HV) accumulation were proposed.

ARTICLE INFO

Article history:
Received 12 June 2013
Received in revised form 14 August 2013
Accepted 17 August 2013
Available online 28 August 2013

Keywords:
Activated sludge
P(HB/HV)
HV
Microbial community
Substrate composition

ABSTRACT

This study investigated the effects of substrate composition (acetate and propionate) on synthesis of various mix of poly(hydroxybutyrate-hydroxyvalerate) (P(HB/HV)) from activated sludge, which was acclimated using a single carbon (acetate) and mixed carbons (acetate and propionate). Results of batch P(HB/HV) production tests indicated that the yield and synthesis rate of P(HB/HV) decreased as the proportion of propionate in the substrate increased. However, mixed-carbon-acclimated sludge with acetate and propionate exhibited better P(HB/HV) production performance than with acetate-acclimated sludge in terms of substrate utilization, yield of P(HB/HV) and HV fraction in P(HB/HV). The desired hydroxyvalerate (HV) fraction (0–74%) of the P(HB/HV) could be obtained based on the substrate composition and sludge acclimation. Furthermore, PCR–DGGE analysis indicated that specific species dominated the activated sludge used for P(HB/HV) production. *Acidobacteria* and *Burkholderiales* were the dominant bacterial populations and played an important role in HV synthesis.

1. Introduction

Polyhydroxyalkanoates (PHAs), which are microbially synthesized polymers, are currently attracting much interest from researchers because their physical properties are similar to those of conventional thermoplastics such as polyethylene (PE) and polypropylene (PP) (Chang et al., 2011; 2012 and Morgan-Sagastume et al., 2010). The homopolymer poly(3-hydroxybutyrate) (PHB) was the first of the PHAs to be investigated (Bosco and Chiampo, 2010; Johnson et al., 2009; Johnson et al., 2010a). However, PHB is a crystalline and stiff material with a high melting point of approximately 179 °C, which is close to its thermal degradation temperature. This thermal property of PHB may increase its brittleness during industrial processing and therefore limit its industrial applications (Bengtsson et al., 2010; Johnson et al., 2010b). The copolymer of HB and 3-hydroxyvalerate (HV), namely P(HB/HV), has better physical properties, such as impact resistance, toughness, flexibility and others that are involved in manufacturing processes.

Activated sludge is a well-known mixed culture that is able to accumulate P(HB/HV) using microorganisms (Pan et al., 2011).

Abbreviations: COD, chemical oxygen demand; DO, dissolved oxygen; VSS, volatile suspended solid; MLVSS, mixed liquor volatile suspended solid; *MPCC*, maximum P(HB/HV) concentration; *MPCT*, maximum P(HB/HV) content; PCR, polymerase chain reaction; DGGE, denaturing gradient-gel-electrophoresis; PHAs, polyhydroxyalkanoates; PHB, polyhydroxybutyrate; PHV, polyhydroxyvalerate; P(HB/HV), poly(hydroxybutyrate-hydroxyvalerate); HB, hydroxybutyrate; HV, hydroxyvalerate; SBR, sequencing biological reactor; HRT, hydraulic retention time; SRT, solid retention time; X, biomass; $Y_{P(HB/HV)}$, P(HB/HV) yield; $q_{P(HB/HV)}$, specific P(HB/HV) synthesis rate; q_s, specific substrate consumption; TCA cycle, tricarboxylic acid cycle.

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http://dx.doi.org/10.1016/j.biortech.2013.08.102

The production cost of P(HB/HV) can be significantly reduced by replacing pure substrates with activated sludge because the process is easier to manipulate, does not require sterile conditions, and uses renewable substrates (e.g., sugars or fatty acids) as the carbon source (Bosco and Chiampo, 2010). More important, the composition of P(HB/HV) produced could be manipulated by the types of the supplemented carbon sources. Feeding activated sludge with different substrate composition produces different proportions of the HV monomer in P(HB/HV) (Hu et al., 2005 and Villano et al., 2010). With different proportions of the HV monomer, the performance of P(HB/HV) may vary greatly (Castilho et al., 2009).

Therefore, it is important to manipulate the carbon source composition for P(HB/HV) synthesized from activated sludge through reaction process conditions. Until now, most studies of P(HB/HV) production from activated sludge have been limited to a single cultivating carbon substrate feeding the P(HB/HV)-accumulating sludge. Chang et al. found that propionate-acclimated sludge exhibited better PHA production from propionate than from acetate in terms of kinetics and stoichiometry. However, acetate-acclimated sludge demonstrated superior PHA production capability from acetate than from propionate (Chang et al., 2012). Therefore, if activated sludge were to be acclimated using mixed carbons, the mixed-carbon-acclimated sludge would most likely perform better in PHA production, including with regard to rapid kinetics. However, there is still little information about P(HB/HV) production using mixed-carbon-acclimated sludge.

Carbon source composition plays an important role in synthetic P(HB/HV). Moreover, carbon influences the microbial composition of the sludge, and these microbes may produce and accumulate PHAs as carbon and energy storage materials. Liu et al. found PHB-accumulating microorganisms in excess activated sludge acclimated to a single carbon source for PHB accumulation (Liu et al., 2011). Thus, the characterization of this microbial composition is linked to developing promising strategies for improved process performance for PHA production (Ciesielski et al., 2008; Chen et al., 2010). Although several researchers have found that even-numbered (i.e., acetate and butyrate) or odd-numbered (i.e., propionate and valerate) carbon sources may produce the homopolymer PHB or the copolymer P(HB/HV), there is a lack of information about the microorganisms responsible for P(HB/HV) production in such an environment, which are a crucial factor for P(HB/HV) synthesis using activated sludge.

In the present study, activated sludge was acclimated under aerobic conditions using a single carbon (acetate) and mixed carbons (acetate and propionate). Then, the acclimated sludge was harvested to conduct aerobic batch tests with various ratios of acetate and propionate to investigate the effects of substrate composition on P(HB/HV) synthesis. Additionally, the substrate utilization and microbial communities under different substrate conditions were characterized. The results of this study improve the understanding of P(HB/HV) accumulation and production in activated sludge and are valuable for biosynthesizing polymers in industrial applications.

2. Methods

2.1. Acclimation of activated sludge

The activated sludge used in this work was obtained from the secondary sedimentation tank of a municipal wastewater treatment plant in Xiamen, China. Laboratory-scale sequencing biological reactors (SBRs) with a working volume of 5 L were used in this study to acclimate the excess sludge. The SBRs were situated in a room with a temperature range of 28–31 °C under aerobic conditions. The SBRs operated on a 24 h cycle consisting of a 23 h aerobic phase, a 40 min settling phase and a 10 min withdrawing phase. At the end of the aerobic phase, a defined volume of biomass was removed to keep the content of the mixed liquor suspended solids (MLSS) at approximately 2 g/L. The supernatant was removed, and 3 L of culture medium was replenished after the settling phase to maintain the hydraulic retention time (HRT) at 40 h. During the aerobic phase, dissolved oxygen (DO) was supplied through a ceramic membrane disperser using an air compressor and controlled at approximately 80% of the saturation value.

The culture medium for the SBRs consisted of the acclimating carbon substrate and nutrients. A single-carbon substrate of acetate (adjusted to pH 7.5, COD concentration 852 mg/L) and a mixed-carbon substrate of acetate and propionate (3:1 mol ratio, adjusted to pH 7.5, COD concentration 852 mg/L) were fed as carbon sources. The culture medium was composed as follows (mg/L): KCl, 234; NH_4Cl, 194; $KH_2PO_4{\cdot}3H_2O$, 150; $CaCl_2$, 54; $FeCl_3{\cdot}3H_2O$, 9.8; $MgSO_4{\cdot}7H_2O$, 306; peptone, 334; and yeast extract, 124 (Liu et al., 2011).

In each cycle, liquid- and solid-phase samples were taken regularly from the SBRs at the end of the aerobic phase to analyze the COD, pH, MLSS and MLVSS. The COD removal efficiency of both of the SBRs was maintained at approximately 95%. The pH in the SBR process was uncontrolled and ranged from 8.7 to 9.5. After operating for more than 2 months, the SBRs achieved stability, i.e., a sludge retention time (SRT) of 5 days.

2.2. Batch P(HB/HV) accumulation tests

Aerobic batch tests were performed to investigate the effects of substrate composition on P(HB/HV) synthesis. Sludge acclimated to a single-carbon or mixed-carbon substrate was transferred from the SBR into a glass reactor in each batch test. The mixed-carbon solution of acetate and propionate was then added to the reactor with an initial concentration of 4684 mg COD/L. The tested ratios of acetate to propionate in the COD concentration were 1:0, 4:1, 2:1, 1:1, 1:2, 1:4 and 0:1. The total mixed liquid volume in the batch reactor was 1 L. Afterwards, the mixed liquid in the reactor was subjected to intermittent aeration (30 min aeration and 30 min stirring in each cycle) (Liu et al., 2011). A DO content of approximately 80% in the mixed liquid was maintained by aeration through a ceramic membrane disperser with an air compressor. The stirring rate was maintained at 400 ± 5 rpm using a mechanical agitator. The initial pH value of the mixed liquid was adjusted by adding dilute sulfuric acid or dilute sodium hydroxide. The reactor was operated at a constant temperature of 30 °C.

2.3. P(HB/HV) content determination

The P(HB/HV) content in the sludge was determined using the procedures described by Albuquerque et al. A centrifuged sample from the batch test was lyophilized, digested, methylated, extracted, and then analyzed by gas chromatography (GC9560, China) using a flame ionization detector and a 30 m × 0.32 mm Chrompack SE-30 column (Albuquerque et al., 2010). Nitrogen was used as the carrier gas. The injection port and detector were maintained at 200 and 250 °C, respectively. The GC oven was programmed to begin at 100 °C (1 min) and then increase at a rate of 10 °C/min to 170 °C, at which point the condition was maintained for 2 min. The sample injection volume was 1.0 μL. PHB calibration was performed with a P(HB/HV) standard containing 5% wt. HV (Sigma–Aldrich Chem).