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Synthesis of poly(3-hydroxybutyrate/3-hydroxyvalerate) from propionate-fed activated sludge under various carbon sources

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ABSTRACT

This study investigated the potential of a propionate-fed PHAs accumulating sludge, which was submitted to aerobic dynamic feeding (ADF) condition, for producing poly (3-hydroxybutyrate/3-hydroxyvalerate), P(HB/HV). Results of batch P(HB/HV) production tests indicated that propionate-ADF sludge with propionate or valerate exhibited better PHAs production performance than with acetate in terms of kinetics and stoichiometry. However, acetate-ADF sludge obtained a superior PHAs production capability from acetate than from propionate. Choice of carbon source for PHAs production therefore relied significantly on the cultivating substrate of PHAs accumulating sludge. Furthermore, mixture of acetate and valerate in molar ratio of 50:50 achieved higher P(HB/HV) content than in molar ratio of 75:25, and obtained a P(HB/HV) copolymer with optimum HV fraction of 45 mol%. The above findings propose that elevating the applicability of P(HB/HV) production require simultaneously two conditions: cultivating a propionate-fed sludge and providing the sludge with mixture of odd- and even-number carbon sources.

1. Introduction

Polydroxyalkanoates (PHAs) are biodegradable plastics that are stored within numerous microorganisms as energy and carbon storage materials. The accumulation of PHAs as intracellular granules usually occurs under conditions of nutrients limitation in the presence of excess carbon source (Anderson and Dawes, 1990; Lee, 1996). Mixed culture (e.g. activated sludge) have been confirmed by a number of past studies (Takabatake et al., 2000; Dionisi et al., 2004) to be capable of producing PHAs. The process of mixed culture has more economic advantages than that of pure culture, because the process is simpler to control, requires no sterile conditions, and uses the renewable substrate (sugars and fatty acid) as the carbon source (Satoh et al., 1998; Reis et al., 2003). Recently, PHAs accumulation was observed in activated sludge submitted to consecutive period of external substrate accessibility (feast phase) and unavailability (famine phase) under fully aerobic conditions, i.e. aerobic dynamic feeding (ADF) process (Lemos et al., 2004; Dionisi et al., 2005; Johnson et al., 2010). Operation of the mixed culture under these conditions would enhance the sludge with high PHAs production capacity (Salehizadeh and Van Loosdrecht, 2004; Dias et al., 2006).

The homopolymer of poly(3-hydroxybutyrate) (PHB) is the first of PHAs to be investigated and has attracted considerable commercial interests, due to its thermoplastic property, biodegradability, hydrophobicity, and low oxygen permeability (Steinbuchel and Fuchtenbusch, 1998; Mothes et al., 2004). However, PHB is a crystalline and stiff material with high melting point of about 179 °C, which is close to its thermal degradation temperature (Reis et al., 2003). The above thermal property of PHB may increase the brittleness during industrial processing and therefore limit its industrial applications (Dai et al., 2007; Khanna and Srivastava, 2007). The thermal properties of PHB may be modified, as was proposed by previous studies (Ojumu et al., 2004; Sudeh et al., 2000), by incorporating 3-hydroxyvalerate (HV) monomer into HB polymer chains; this modification thus leads to the copolymer of poly(3-hydroxybutyrate/3-hydroxyvalerate) (P(HB/HV)). Serafim et al. (2008) indicated that increasing HV content from 0 to 50% could decrease the melting point of the copolymer P(HB/HV). However, a gradual increase in melting point would occur if the HV fraction increased from 50 to 100%. The minimum melting point for P(HB/HV) is observed at 40–50 mol% of HV, and this low melting point facilitates the industrial processing of the polymer (Wang et al., 2001).

The composition of PHAs produced could be manipulated by the types of the supplemented carbon sources. Feeding activated sludge with substrate of even-number carbon sources (i.e. acetate and butyrate) produces the homopolymer of PHB; while a copolymer of P(HB/HV) with HV as dominant unit is formed when

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Nomenclature

ADF	aerobic dynamic feeding
COD	chemical oxygen demand (mg/L)
CODac	acetate concentration on a COD basis (mg/L)
CODprop	propionate concentration on a COD basis (mg/L)
CODval	valerate concentration on a COD basis (mg/L)
DO	dissolved oxygen concentration (mg/L)
Ac	acetate concentration (mg/L or C mmol/L)
Prop	propionate concentration (mg/L or C mmol/L)
Val	valerate concentration (mg/L or C mmol/L)
HB	3-hydroxybutyrate (mg HB/g MLVSS)
HV	3-hydroxyvalerate (mg HV/g MLVSS)
MLSS	mixed liquor suspended solid (mg/L)
MLVSS	mixed liquor volatile suspended solid (mg/L)
PHAs	polyhydroxyalkanoates (mg PHAs/g MLVSS)
P(HB/HV)	poly(3-hydroxybutyrate/3-hydroxyvalerate) (mg P(HB/HV)/g MLVSS)
$-q_s^{max}$	maximum specific substrate uptake rate (C mmol substrate/C mmol X.h)
$-q_{Ac}^{max}$	maximum specific acetate uptake rate (C mmol acetate/C mmol X.h)
$-q_{val}^{max}$	maximum specific valerate uptake rate (C mmol valerate /C mmol X.h)
$q_{P(HB/HV)}^{max}$	maximum specific P(HB/HV) production rate (C mmol P(HB/HV)/C mmol X.h)
q_{HV}^{max}	maximum specific HV production rate (C mmol HV/C mmol X.h)
SBR	sequencing batch reactor
SCOD	soluble chemical oxygen demand (mg/L)
SMPs	soluble microbial products (mg/L as COD)
SRT	sludge retention time (day)
TCA cycle	tricarboxylic acid cycle
X	active biomass concentration (C mmol)
$Y_{P(HB/HV)/S}^{max}$	maximum yield of P(HB/HV) from total substrate (C mmol P(HB/HV)/C mmol substrate)
$Y_{HV/Val}^{6h}$	yield of HV from valerate in the initial 6 h of batch test (C mmol HV/C mmol Val)
$Y_{HV/Val}^{max}$	maximum yield of HV from valerate (C mmol HV/C mmol Val)
$Y_{X/S}^{max}$	maximum yield of active biomass (C mmol X/C mmol substrate)

odd-number carbon sources (i.e. propionate and valerate) is fed (Dias et al., 2006). To incorporate HV units in PHAs, propionate was usually used by previous studies of mixed culture as the carbon source (Takabatake et al., 2000; Hu et al., 2005). The effect of valerate supplement for P(HB/HV) production, nevertheless, was mostly used in pure culture process (Ishihara et al., 1996; Mothes et al., 2004; Khanna and Srivastava, 2007) and was seldom reported in studies of mixed culture. Moreover, past researches of PHAs production concerning mixed culture were mostly investigated by the PHAs accumulating sludge fed with acetate as the cultivating carbon substrate (Beun et al., 2002; Martins et al., 2003; Serafim et al., 2004; Johnson et al., 2010). There is still little information about the PHAs production by using the propionate-fed PHAs accumulating sludge.

In this study, a sequencing batch reactor (SBR) submitted to ADF condition was constructed by using propionate as the cultivating carbon substrate (COD_{prop} = 400 mg/L). Then, the propionate-fed sludge was harvested to conduct aerobic batch tests under various carbon sources (acetate, propionate, valerate, and mixture of acetate and valerate), so as to compare the composition and the performance of P(HB/HV) synthesis. Besides, a separate ADF SBR with acetate (COD_{ac} = 400 mg/L) as cultivating carbon substrate was also constructed in this study for comparing the effects of odd- and even-number cultivating carbon substrates on the P(HB/HV) producing behavior.

2. Methods

2.1. Operation of propionate- and acetate-fed ADF SBRs

Two laboratory-scale SBRs (each with a working volume of 10 L) subjected to ADF condition were constructed in this study, so as to enrich PHAs accumulating cultures for batch P(HB/HV) production tests. These two SBRs were separately fed with propionate and acetate as carbon sources. The propionate- and acetate-fed ADF SBRs were seeded from other steady-state propionate- and acetate-fed anaerobic–oxic (A/O) SBRs, respectively. After being seeded, these two ADF SBRs were both operated for more than 150 days. The SBRs were situated in a constant-temperature room of 20 °C. The SBRs, operated in two cycles per day, consisted of 600 min of aerobic phase (feast and famine) and 120 min of settling phase. At the end of aerobic phase, 660 ml of mixed liquid was removed so as to maintain the sludge retention time (SRT) at 15 days. After settling phase, 4.34 L of supernatant was withdrawn and 5 L of fresh culture medium was replenished.

The culture medium for each SBR consisted of the cultivating carbon substrate and nutrients. Propionate and acetate were respectively the cultivating carbon substrates for propionate- and acetate-fed SBRs; the nutrients were the same for both SBRs. The nutrients solution was composed of 68 mg NH_4Cl, 20 mg peptone, 11.07 mg KH_2PO_4, 8.42 mg K_2HPO_4, 48 mg $MgSO_4{\cdot}7H_2O$, 21.6 mg $CaCl_2$, 20 mg thiourea (to prevent nitrification), and 0.28 mL of trace elements solution (per liter of distilled water). The trace elements solution consisted of 6 g $FeCl_3$, 0.6 g H_3BO_3, 0.12 g $CuSO_4$, 0.72 g KI, 0.48 g $MnCl_2$, 0.24 g Na_2MoO_4, 0.48 g $ZnSO_4$, 0.6 g $CoCl_2$, and 40 g EDTA (per liter of distilled water). These medium solutions were mixed together and adjusted to pH 7.0 ± 0.2 by using 1 M HCl and 1 M NaOH. The synthetic influents into these SBRs therefore contained 400 mg COD/L of carbon substrate, 20 mg/L of ammonia–nitrogen (NH_4^+-N), and 4 mg/L of phosphate (PO_4^{3-}-P), respectively.

2.2. Batch P(HB/HV) production tests

Aerobic batch tests under various carbon sources were performed to investigate the potential of P(HB/HV) production by ADF sludge from the propionate-fed SBR. For each batch test, the ADF sludge was taken from the propionate-fed SBR at end of famine phase (named propionate-ADF sludge). Then, the propionate-ADF sludge was put into the batch reactor equipped with magnetic stirrer, pH probe, aeration rock, etc. The carbon source solution was then added into the reactor so as to attain an initial concentration of 124 °C mmol/L. The total mixed liquid volume in the batch reactor was 500 mL. Two different types of carbon sources were adopted for batch P(HB/HV) production tests: single carbon source (acetate, propionate and valerate) and mixed carbon sources (acetate to valerate in the mole ratios of 75:25 and 50:50). Besides, the batch tests by using acetate-ADF sludge were also conducted when acetate or propionate was used as the carbon source. During the steady-state operation of the two SBRs, the concentrations of MLSS