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# Coupling of polyhydroxyalkanoate production with volatile fatty acid from food wastes and excess sludge

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ABSTRACT

In this paper, the synthesis of polyhydroxyalkanoates (PHAs) by activated sludge with aerobic dynamic feeding process was conducted in a sequencing batch reactor by using food wastes and excess sludge fermentation liquid as the carbon source. The volatile fatty acids (VFAs) in the fermentation liquid were divided into even-numbered (acetic and butyric acid) and odd-numbered (propionic and valeric acid). The experiments conducted by central-composite design (CCD) showed that the pH could significantly affect the ratio of even-numbered to odd-numbered VFAs. Statistical analysis indicated a positive correlation ($R^2 = 0.97$, $P < 0.05$) between the consumption of even-numbered VFAs and the synthesized of PHB, while the consumption of odd-numbered VFAs were correlated with the synthesized PHV. By controlling the ratio of even-numbered to odd-numbered VFAs, the contents of PHV in the PHAs could be controlled within the range of 22–30%. When fermentative VFAs were used as the substrate for the synthesis of PHAs, the microbial synthesis of PHA and biomass was higher than that mixture of analytically pure acids was used. These results are of vital significance for the comprehensive utilization of solid wastes.



*Keywords:* Polyhydroxyalkanoates; Activated sludge; Volatile fatty acids; Food waste

## 1. Introduction

Polyhydroxyalkanoates (PHAs) as an alternative to plastics have attracted much interest because they can be completely biodegradable and biocompatible and are produced from renewable sources. PHAs are a type of polyesters that can be synthesized and accumulated by various microorganisms as intracellular energy-and carbon-storage materials when there is an essential growth-limiting component such as nitrogen, phosphate or oxygen (Salehizadeh and Van Loosdrecht, 2004). So far, industrial production of PHAs is mainly based on pure cultures (in wild and recombinant strains) and well-defined substrates. The application of PHAs is prevented mainly by their high production cost compared with the oil-derived plastics (Choi and Lee, 1997), and the production cost is 8–10 times more than that of conventional plastics. Thus, with the aim of commercializing PHA, a substantial effort has been devoted to reducing the production cost through the development of bacterial strains, more efficient fermentation/recovery processes and cheap substrates (such as organic waste). It has been reported that the production expense of PHAs can be reduced by more than 50% if mixed cultures and cheaper substrates (sewage sludge and food wastes) were used (Reis et al., 2003). And many efforts have been made toward the study of efficient PHA production techniques and use of renewable carbon resources (Jiang et al., 2009; Cai et al., 2009).

Properties of PHAs vary considerably, which depend on their composition, i.e. PHV/PHB ratios. PHAs can have physical properties that range from brittle and thermally unstable to soft and tough. For example, the HB homopolymer has thermoplastic processability, 100% resistance to water and 100% biodegradability, but it is stiffer and more brittle polymer of high crystallinity (Pornpa et al., 2007) compared with example polystyrene, while poly[(R)-3-hydroxybutyrate-co-(R)-3-hydroxy valerate] (P[3HB-co-3HV]) is characterized by increased elongation to break and low melting temperature and more flexible and tougher than P(3HB) (Castilho et al., 2009). Currently, numerous studies on PHA synthesis using activated sludge focused on the optimization of the operational or nutritional conditions, such as carbon source (Dhawal

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et al., 2010; Bengtsson et al., 2008), temperature (Johnson et al., 2010b), carbon and nitrogen limitation (Johnson et al., 2010a), pH (Marianna et al., 2010; Charuvan and Chalermraj, 2007), sludge-retention time (Chua et al., 2003) and phosphorus and nitrogen limitation (Wen et al., 2010), aimed at increasing the yield of PHAs. However, there seem little studies concerning the effect of the ratio of even-numbered to odd-numbered VFAs in the fermentation on the PHA synthesis process. It has been proved that the homopolymer of poly(3-hydroxybutyrate) [P(3HB)] was produced from *n*-alkanoates with even carbon numbers, whereas the copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate units [P(3HB-co-3HV)] was produced from *n*-alkanoates with odd carbon numbers (Akiyama et al., 1992), but no definite quantitative relation was explored in their literatures.

The present work was an attempt to research the effect of different operational conditions on VFAs content and the ratio of even-numbered to odd-numbered VFAs and its relationship with PHB and PHV accumulation by active sludge. In order to select a culture with high PHA storage capacity, activated sludge was submitted to aerobic dynamic feeding (ADF) in a sequencing batch reactor (SBR) and the fermentative VFAs were used as the substrate for PHA synthesis. In order to compare the difference in the synthesis of PHAs caused by the change of substrates, analytically pure VFAs have also been used as the substrates for PHA synthesis.

## 2. Materials and methods

### 2.1. Sludge source and wastes characteristics

Dewatered excess sludge from municipal wastewater treatment plant and food wastes were used to generate VFAs-containing fermentative liquid as the raw material to produce PHAs. The seed sludge used for acidogenic fermentation and PHAs synthesis was collected from an anaerobic digester of Sibao Municipal Wastewater Treatment Plant in Hangzhou. The concentrations of total solids (TS) and volatile suspended solids (VSS) in the sludge were 18.32 ± 0.1 g/L and 10.70 ± 0.1 g/L, respectively. The food wastes was collected from supermarket, which consisted of 11 types of food (%, w/w) and included cabbage (17.0%), banana peels (8.5%), orange peels (10.0%), sponge gourds (5.0%), pears (7.0%), apples (8.5%), celery (5.0%), noodles (8.0%), boiled rice (9.0%), potatoes (17.0%), and carrots (5.0%). The food wastes were crushed with an electric blender and diluted with tap water to obtain TS levels of 13.4 ± 2.0 (%, w/w) and VSS levels of 94.7 ± 2.0 (%, TS). The dewatered excess sludge was obtained from the Sibao Municipal Wastewater Treatment Plant with 23.4 ± 1.6% TS and 45.0 ± 2.0% VSS.

### 2.2. Experiment design on the ratio of even-numbered to odd-numbered VFAs

This experiment to determine the potential impact factors and their interactive effects on the ratio of even-numbered to odd-numbered VFAs were designed with the help of Design Expert Version 7.1.2® (Stat-Ease Inc., Minneapolis, NC) software package. A central-composite design (CCD) for four independent variables with each at five levels was employed to fit a second-order polynomial model which indicated that 30 experiments with 16 cubic points and eight axial points and six replicates at the center point were required for this procedure. The variables in this study were food waste composition, hydraulic retention time (HRT), organic loading rate (OLR) and pH as shown in Table 1. Table 2 presented the CCD design at the given range of the above parameters in terms of coded and actual terms.

### 2.3. Reactor and operation

#### 2.3.1. Acclimation operation and batch tests for acidogenic fermentation

The anaerobic sludge was aerated for 7 days, using the dissolved organic matter in the anaerobic sludge as carbon source to improve the hydrolysis acidification bacteria activity. A 3 L acclimation tank (added 1 L aerated sludge) was used in acclimation stage and operated in semi-continuous mode (once-a-day draw-off and feeding). The acclimation tank was kept at 35 °C with a rotation speed of 200 r/min. The mixture of food wastes and dewatered excess sludge was added to ensure the organic loading was 4 g VSS/L day. The acclimation tank was flushed with $N_2$ gas, and the pH was not controlled in this stage. Reactor was considered to be in a steady state when the concentrations of soluble chemical oxygen demand (SCOD) and VFAs in the draw-off were stable. Steady state was usually achieved within 4 h of HRT, but experiments were conducted for 5–6 h HRT to ensure steady-state conditions (Lim et al., 2008a,b).

Batch tests ware conducted in 500 ml serum bottles (with 200 ml acclaimed sludge), and sealed tightly with a rubber plug linked to a silicone gas release tube. The pH in each reactor was adjusted by adding 3 M NaOH or 3 M HCl after feeding everyday. Each reactor was operated under different conditions, as shown in Table 2. The hydraulic retention time (HRT) was equal to the solid retention time (SRT) in batch tests because solid was withdrawn with the mixed liquor everyday.

#### 2.3.2. Acclimation operation and batch tests for PHA synthesis

The seed sludge from aeration tank was aerated for 4 days to exhaust the original existed substrate. Two identical SBR reactors were operated in this acclimation process, with a working volume of 5 L. The aerated activated sludge was added to the reactors and fermentative VFAs were added as carbon source. The feed also contained the following minerals: $CaCl_2{\cdot}2H_2O$ (50 mg/L), $MgSO_4{\cdot}7H_2O$ (100 mg/L), $FeCl_3{\cdot}6H_2O$ (2 mg/L), $Na_2EDTA$ (3 mg/L), $ZnSO_4{\cdot}7H_2O$ (0.1 mg/L), $MnCl_2{\cdot}4H_2O$ (0.03 mg/L), $H_3BO_3$ (0.3 mg/L), $CoCl_2{\cdot}6H_2O$ (0.2 mg/L), $NiCl_2{\cdot}6H_2O$ (0.02 mg/L), $CuCl_2{\cdot}2H_2O$ (0.01 mg/L), and $NaMoO_4{\cdot}2H_2O$ (0.03 mg/L). Each reactor operation cycle includes three stages: 10 min for the feeding stage; the biological reaction stage (1.5 h of “feast” phase, 5 h of “famine” phase); and the last stage was 2 min for final withdrawal. Sludge mixed with liquor was withdrawn without settling phase. In this way, SRT was equal to HRT which was kept at 10 days. Oxygen was supplied by an air compressor to ensure that the oxygen concentration was greater than 4 mg/L in the biological reaction stage. In this acclimation process, the pH value was not controlled, but the reactor temperature was maintained at 25 °C with an air conditioner. SBR cycles were characterized by measurements of biomass concentration, substrates and PHA at the end of the cycle. With the SBR operating performance under pseudosteady-state conditions for 2 months, the excess biomass was utilized in the batch tests.

Two SBR reactors with a working volume of 1 L were inoculated with the acclimated sludge and the MLSS of the sludge