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Optimal production of polyhydroxyalkanoates (PHA) in activated sludge fed by volatile fatty acids (VFAs) generated from alkaline excess sludge fermentation

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ABSTRACT

To reduce the production cost of polyhydroxyalkanoates (PHA) and disposal amount of excess sludge simultaneously, the feasibility of using fermentative volatile fatty acids (VFAs) as carbon sources to synthesize PHA by activated sludge was examined. At pH 11.0, 60 °C and fermentative time of 7 d, the VFAs yield was 258.65 mgTOC/gVSS. To restrain cell growth during PHA production, the released phosphorus and residual ammonium in the fermentative VFAs was recovered by the formation of struvite precipitation. Acetic acid was the predominant composition of the fermentative VFAs. PHA accumulation in excess sludge occurred feeding by fermentative VFAs with aerobic dynamic feeding process. The maximum PHA content accounted for 56.5% of the dry cell. It can be concluded from this study that the VFAs generated from excess sludge fermentation were a suitable carbon source for PHA production by activated sludge.

1. Introduction

As a fully biodegradable and biocompatible plastic, polyhydroxyalkanoate (PHA) is an interesting alternative to petrochemical derivate plastic due to their similar characteristics (Anderson and Dawes, 1990). PHA are the polyesters that accumulate as intracellular carbon, energy and reducing-power storage material in over 300 various microorganisms (Steinbüchel, 1992). PHA can be biosynthesized from renewable resources, allowing for a sustainable and closed-cycle process for the production and use of such polymers (Braunegg et al., 1998).

Currently, PHA synthesis at industrial scale is based on microbial isolates and well defined substrates (Patnaik, 2005). However, the cost of PHA produced thus is still too high for PHA to compete with the conventional plastic commodities. Economic evaluation showed that the production expense of PHA can be reduced over half if renewable waste materials and activated sludge were used (Serafim et al., 2004). Besides, investigation of the process optimization techniques to increase the PHA production efficiency can help to widen the application of PHA.

Above all, almost 30% of total PHA production cost is attributed to the carbon source (Salehizadeh and Van Loosdrecht, 2004). Additionally, a great amount of excess sludge is generated daily worldwide. Handling, treatment and ultimate disposal of the excess sludge accounts for 40–60% of the total operational cost of an activated sludge treatment plant (Liu, 2003). One strategy for excess sludge management is moving towards reutilization of sludge as useful resources, such as fermenting the excess sludge to generate carbon source for PHA production by pure culture (Lee and Yu, 1997) and for phosphorus removal by activated sludge (Tong and Chen, 2007). Volatile fatty acids (VFAs) is one of the main intermediates in the anaerobic sludge fermentative liquid (Yuan et al., 2006; Tong and Chen, 2007) and the most suitable substrate for PHA storage. Under alkaline conditions, the yield of VFAs was enhanced significantly from excess sludge anaerobic fermentation (Yuan et al., 2006; Chen et al., 2007).

PHA synthesis by activated sludge is possible to reduce PHA production cost, since its sterilization, equipment and control requirements are lower and the microbial communities in activated sludge can adapt well to the complex substrates present in the agroindustrial wastes (Salehizadeh and Van Loosdrecht, 2004). Basic and applied research on this field has been implemented in the past decade (Lemos et al., 2003; Dias et al., 2005, 2006; Dionisi et al., 2006; Serafim et al., 2006, 2007), focusing on areas such as process configuration, reactor operational strategies, process modeling and control, metabolic pathway analysis, microbial characterization and polymer characterization.

Improving the intracellular PHA content is important for decreasing the extraction and recovery cost of PHA downstream processing. The PHA production process known as "feast and famine" or as "aerobic dynamic feeding (ADF)" has a high potential to enhance the intracellular PHA content and specific storage rate of PHA by activated sludge (Serafim et al., 2004; Dias et al., 2005;

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Dionisi et al., 2006). Since under transient conditions, e.g. insufficiency of an essential growth limiting component or temporary presence of excess carbon source, some bacterial in activated sludge can adapt physiologically to the exposure of high substrate concentration rapidly, uptake substrate quickly and store PHA in a more balanced way (Daigger and Grady, 1982; van Loosdrecht et al., 1997). Periodic substrate feeding aerobically creates an alternation of excess and lack of extra carbon source that will favor the microorganisms most able to store the substrate quickly during the feast phase and then reutilize it for growth during the famine phase (Majone et al., 1999).

To reduce the PHA production cost and the disposal amount of excess sludge simultaneously, based on the above considerations, this research investigates the feasibility of PHA production by activated sludge by using VFAs generated from excess sludge fermentation. Up to now, no work had been done on the PHA production by using excess sludge fermentative liquid as substrate and activated sludge as inoculation. A new process involving two stages is proposed here. The first stage is carbon source generation by means of anaerobic thermophilic alkaline excess sludge fermentation. Ammonia and phosphorus in the fermentative VFAs are removed under thermophilic and alkaline conditions and by adding magnesium to form struvite deposition. The second one is PHA accumulation by inoculating activated sludge submitted to aerobic transient carbon supply. In the first stage, to provide carbon source for PHA production, the excess sludge having treated municipal wastewater, was fermented under anaerobic, thermophilic and alkaline conditions. Acidogenic fermentation transforms most of biodegradable component in the municipal sludge at high rate into a mixture of VFAs, carbohydrates and proteins. In the second stage, the municipal sludge taken from the secondary sedimentation tank was used immediately and directly to degrade the VFAs-containing fermentative liquid and accumulate PHA, which means the sludge used as inoculation was not domesticated or enriched previously, so as to economize the volume of PHA production reactor. This stage was operated by aerobic periodic feeding in a lab-scale batch reactor.

2. Methods

2.1. Sludge source and the characteristics

Excess sludge treating municipal wastewater was used both to generate VFAs-containing fermentative liquid as the raw material to produce PHA and to accumulate PHA as the inoculum.

The excess sludge used as the raw material to generate VFAs was sampled from the secondary sedimentation tank of a municipal wastewater treatment plant at Shatin in Hong Kong, which was operated with a traditional activated sludge process. The sludge samples were concentrated by settling at 4 °C for 24 h before they were fermented to generate VFAs. The average characteristics of the sludge after settlement are as follows: pH 6.8 ± 0.2, TSS (total suspended solids) 13 115 ± 125 mg/L, VSS (volatile suspended solids) 9 901 ± 65 mg/L, DOC (dissolved organic carbon) 53 ± 12 mg/L, TOC (total organic carbon) 5 470 ± 76 mg/L, VFAs (as TOC) 9.5 ± 1.8 mg/L, and the analysis was replicated three times for every sludge sample. In the excess sludge samples, proteins and carbohydrates, which account for about 55 ± 10% of TOC together, are the two predominant organic compounds in excess sludge; the lipid and oil in the excess sludge accounts for around 1% of TOC.

The activated sludge used as the inoculum to produce PHA came from a 15 L lab-scale sequencing biological reactor (SBR) treating real municipal wastewater, which had the influent TOC of 19–55 mg/L, sludge retention time (SRT) of 10 d, and operated aerobically under room temperature (21 ± 1 °C). Before inoculation, the sludge was concentrated by centrifuge at 1000 rpm for 20 min and utilized immediately to accumulate PHA without previous acclimation.

2.2. VFAs generation by sludge fermentation under various pH and temperature

Batch experiments were conducted under anaerobic conditions to investigate the influence of temperature (21 ± 1 °C, 35 ± 1 °C and 60 ± 1 °C) and pH (pH 8.0, 9.0, 10.0, 11.0 and no pH control as blank) on the optimization yield of VFAs. The operation conditions were summarized in Table 1. In each airtight beaker with working volume of 500 mL, pre-settled excess sludge with 500 mL was added to generate VFAs. The beakers were sparged with nitrogen gas for 30 s to remove oxygen from the headspace, enveloped by aluminium foil, and mixed by magnetic stirrer at 100 rpm. Five beakers were surrounded outside with thermo-resistance wire to set the temperature at 35 ± 1 °C and 60 ± 1 °C, respectively. The temperature was monitored by thermometer inside the reactors to maintain the temperatures consistent. Another five beakers were placed at room temperature (21 ± 1 °C). Every 4 h throughout the experiments, the pH in each beaker was adjusted by adding 3 M NaOH or 3 M HCl to maintain at a certain pH value according to Table 1. A beaker without pH adjustment was used as a blank control.

To enhance the VFAs yield, sodium dodecylbenzene sulfonate (SDBS) was added at the dosage of 0.02 g/gVSS in each batch experiments. Since the VFAs yield after SDBS addition is over 7 times of that in the absence of SDBS (Jiang et al., 2007). SDBS is a widespread used surfactant, which can be easily found in excess activated sludge. The presence of SDBS could strengthen the solubilization of sludge particulate organic-carbon, hydrolysis of solubilized substrate and acidification of hydrolyzed products, and the decrease of methanogenic bacteria activity (Jiang et al., 2007).

A batch fermentor of 20 L was established to generate VFAs to provide carbon source for PHA production. This 20 L fermentor was operated under the optimal VFAs generation conditions obtained from the above batch beaker experiments. After fermentation, the mixture was centrifuged at 100*g* for 10 min, and the centrate was autoclaved and collected for ammonium recovery. Before immediately used as substrate to produce PHA, the fermentative liquid was adjusted to pH 7.0 ± 0.5 by adding 10 M HCl.

2.3. Ammonium and phosphorus recovery from sludge fermentative liquid by formation of struvite

The ammonium solubility (wt.%) even at 60 °C was still as high as 18.1% under neutral condition. Alkaline conditions would lead to

Table 1
Batch experiment conditions to generate VFAs from excess sludge fermentation

Batch	Temperature (°C)	pH	Initial excess sludge characteristics (mg/L)
A1	35 ± 1	8.0 ± 0.1	TSS:13260, VSS: 9845, DOC: 43, TOC:5395, VFAs (as TOC) 11.5
A2	35 ± 1	9.0 ± 0.1	
A3	35 ± 1	10.0 ± 0.1	
A4	35 ± 1	11.0 ± 0.1	
A5	35 ± 1	No control	
B1	60 ± 1	8.0 ± 0.1	TSS:13110, VSS: 9878, DOC: 54, TOC:5461, VFAs (as TOC) 9.3
B2	60 ± 1	9.0 ± 0.1	
B3	60 ± 1	10.0 ± 0.1	
B4	60 ± 1	11.0 ± 0.1	
B5	60 ± 1	No control	
C	21 ± 1	10.0 ± 0.1	TSS:12990, VSS: 9985, DOC: 63, TOC:5541, VFAs (as TOC) 7.9