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Polyhydroxyalkanoate recovery and effect of in situ extracellular polymeric substances removal from aerobic granules

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HIGHLIGHTS

- PHA recovery methods from aerobic granules were evaluated for the first time.
- Sodium hypochlorite method produced the highest PHA recovery yield.
- EPS removal found to be influential in PHA recovery from aerobic granules.
- Highest PHA recovery produced when EPS was completely removed.
- Degradation of PHA was minimal with sodium hypochlorite dispersion method.

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ABSTRACT

Polyhydroxyalkanoate (PHA) recovery from aerobic granules was investigated using four cell digestion agents, namely, sodium hypochlorite, sodium hydroxide, acetone and sodium chloride. Simultaneously, the removal of extracellular polymeric substances (EPS) and its effect on PHA yield were investigated. The highest PHA recovery yield was obtained using sodium hypochlorite, accounting for 89% cell dry weight (CDW). The highest PHA was recovered after the sodium hypochlorite completely removed the EPS from the aerobic granules. The average molecular weight (M_w) of the PHA recovered using sodium hypochlorite was 5.31×10^5 g/mol with only 1.8% molecular weight degradation. The energy and duration analysis for PHA recovery revealed that the sodium hypochlorite method required the least amount of energy and time at 0.0561 MJ/g PHA and 26 h, respectively. The PHA that was recovered was a P3(HB-co-HV) co-polymer.

1. Introduction

When an external carbon source is available, polyhydroxyalkanoate (PHA), a biodegradable polymer, is accumulated by microorganisms as an energy storage material (Salehizadeh and Van Loosdrecht, 2004). When an external carbon source is unavailable, stored PHA is oxidized to produce energy for the metabolic activities of the microorganism (Salehizadeh and Van Loosdrecht, 2004). Pure culture microorganisms and sugar substrate have traditionally been used to produce and accumulate PHA. However, because pure culture microorganisms and sugar substrates are expensive, mixed culture microorganisms and wastewaters (as substrate) have been used to accumulate PHA. Various parameters, including organic loading rates (Albuquerque et al., 2010), feast to famine ratios (Chen et al., 2013) and oxygen concentrations (Moralejo-Gárate et al., 2013) have been studied for their effect on PHA accumulation using mixed culture microorganisms and wastewater. Wastewater such as palm oil mill effluent (POME) which is rich in organic compounds produces volatile fatty acid (VFA) as an intermediate product during the anaerobic treatment. VFA produced from POME has been successfully used as substrate for PHA production using mixed culture (Md Din et al., 2012). The usage of VFA from POME for PHA production fits well into the concept of waste product valorization (5-stages Universal Recovery Processing) as proposed by Galanakis (2012).

A potential mixed culture PHA producer that has been largely ignored is the aerobic granule. Aerobic granules are spherical agglomerations of activated sludge with defined boundaries and are generally used in wastewater treatment (Liu and Tay, 2004). Due to the compactness of aerobic granules, culture reactors are small in size and only require a small footprint, and the use of a clarifier is not necessary. The simultaneous treatment of wastewater in a compact area and the production of PHA make the aerobic

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granules worthwhile to be studied. The accumulation of PHA in aerobic granules is possible due to the sharing of common formation factors. Both aerobic granules and PHA accumulation require a feast-famine phase in their formation stages. The feast-famine phase enhances the agglomeration process for aerobic granules, and enriches the PHA accumulation by microorganisms in a mixed culture (Johnson et al., 2009). To date, only a handful of researchers have reported on the formation of PHA in aerobic granules (Fang et al., 2009; Gobi and Vadivelu, 2014).

Despite the promising prospect of using aerobic granules to accumulate PHA, very little information is available on PHA recovery methods from the aerobic granules. Generally, the reported PHA recovery methods from aerobic granules have been directly adapted from methods used for pure culture or activated mixed culture sludge. In aerobic granules, one aspect that is often overlooked is the presence of extracellular polymeric substances (EPS). The EPS is actually a part of the cell and contains various amounts of proteins (PN) and polysaccharides (PS). The EPS is typically secreted by microorganisms under stress, such as in carbon deprived environment, high mechanical shear stress and high carbon-to-nitrogen ratio (Adav and Lee, 2011; McSwain et al., 2005). The secreted EPS bridges microorganisms and facilitates the aerobic granulation process (Adav and Lee, 2011). Additionally, the EPS envelops and protects cells from adverse conditions (Liu and Tay, 2004). As such, the EPS could pose a barrier in the extraction of PHA from the aerobic granules. Therefore, the application of pure culture or mixed culture based recovery methods on aerobic granules without considering the presence of the EPS would be ill-advised.

Discussions about the role of the EPS in PHA recovery from aerobic granules or any other mixed culture are lacking in the literatures. Earlier findings show that the protein content in the EPS exhibited 8-fold increases during the transition from floccular to granular sludge (Zhu et al., 2012). This increase strengthens the structure of the aerobic granules and against toxic attacks.

Conventionally, PHA recovery from microorganisms first requires cell digestion. Many solvents and oxidizing agents have been explored for cell digestion. The most common cell digesting agent is sodium hypochlorite (Hahn et al., 1994). Other solvents, such as acetone, and sodium dodecyl sulfate (SDS), have occasionally been used (Duque et al., 2014). In the subsequent PHA recovery step, chloroform has primarily been used to dissolve PHA (Heinrich et al., 2012). The combination of cell digestion and PHA recovery methods have produced yields over 60% with purities varying between 85% and 99% for common mixed cultures (Chen et al., 2013; Serafim et al., 2008). However, none of these PHA recovery methods have been considered for aerobic granules and much less for EPS-coated aerobic granules. Thus, there is a strong need to investigate the effects of EPS removal on PHA yield.

This work aimed to evaluate various PHA recovery methods on aerobic granules. The methods used herein were analyzed in terms of the amount of EPS removed, the PHA recovery yield, and the energy required for the overall recovery. In addition, the recovered PHA was analyzed for its molecular weight. To date, this study is the first to evaluate PHA recovery methods from aerobic granules.

2. Methods

2.1. Aerobic granules

Aerobic granules were obtained from a lab scale sequencing batch reactor (SBR) treating palm oil mill effluent (POME). The SBR was in steady state operation and operated on a 6-h cycle. The cultivation of aerobic granules in an SBR using POME was reported in our previous work (Gobi et al., 2011). The chemical oxygen demand (COD) removal efficiency remained above 90% throughout the SBR operation. The aerobic granules collected from the SBR at the end feast period were washed with distilled water to remove any impurities. The characteristics of the aerobic granules, such as settling velocity and EPS concentration, were analyzed. The collected aerobic granules were lyophilized before performing the PHA recovery method using four extraction methods. All extraction methods were done in triplicate.

2.2. Cell digestion methods

The experimental flow diagram of the study is shown in Fig. 1. It is hypothesized that the cell digestion step plays a more crucial role than the PHA dissolving step. Thus, more focus was given to the cell digestion step. These two steps were followed by the precipitation of the PHA using ice-cold methanol. The details of the cell digestion steps are discussed in the following subsections.

2.2.1. Sodium hypochlorite (NaOCl)

The sodium hypochlorite method for PHA recovery was adapted from Hahn et al. (1994). Briefly, for each gram of lyophilized aerobic granules, 12.5 mL of sodium hypochlorite (10% (v/v)) and 12.5 mL of chloroform were added. The sodium hypochlorite lyophilized aerobic granules loading was 8% (w/v). The mixture was vortexed and incubated in a water bath shaker at 37 °C for 90 min. Thereafter, the solution was centrifuged at 4400 rpm for 30 min. Upon centrifugation, three distinct layers formed. The top layer consisted of sodium hypochlorite was pipetted out. The remaining cell debris (2nd layer) and PHA-enriched chloroform (3rd layer) fractions were filtered using a simple filtration method. The filtrate (chloroform layer) was subsequently subjected to a PHA dissolving step (Fig. 1).

To study the effect of the sodium hypochlorite volume on cell digestion, three different volumes (8, 12.5 and 16 mL) were used. The PHA recovery yields obtained using the three different volumes were quantified and analyzed.

2.2.2. Acetone

The acetone method was adapted from Gamal et al. (2013). Briefly, for each gram of lyophilized aerobic granule 12.5 mL acetone (65 °C) was added to remove any impurities and non-PHA cellular particles. Thereafter, the acetone was discarded and the aerobic granules were subjected to a PHA dissolving step. To investigate the effect of acetone volume on cell digestion, the three different volumes of acetone were added to the aerobic granules (i.e., 8, 12.5 and 16 mL).

2.2.3. Sodium hydroxide (NaOH)

The sodium hydroxide method was adapted from Mohammadi et al. (2012). Briefly, 0.05 M NaOH was used to digest 1 g of lyophilized aerobic granules. The amount of aerobic granules used was adjusted to obtain a concentration of 20 g/L in the NaOH solution. The aerobic granules were digested in NaOH for 1 h. Thereafter, the solution was centrifuged at 15,000 for 15 min. The supernatant was discarded, and the pellet was further treated with 1% ethanol. The pellet with 1% ethanol was placed in an incubator shaker for 3 h at a speed of 200 rpm. Subsequently, the solution was centrifuged at 15,000 for 15 min. The supernatant was discarded, and the pellet was washed with deionized water. The solution containing the pellet and deionized water was centrifuged at 15,000 for 15 min. The pellet obtained after centrifugation was dried using a freeze dryer. The pellet was later subjected to a PHA dissolving step. The experiment was repeated using different concentrations of NaOH (0.05, 0.1 and 0.5 M) to study the effect of NaOH concentration on the PHA recovery.