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Short Communication

Dynamics of polyhydroxyalkanoate accumulation in aerobic granules during the growth–disintegration cycle

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HIGHLIGHTS

- Aerobic granules accumulated PHA at every stage of the growth–disintegration cycle.
- Small-sized granules accumulate more PHA than large granules.
- Substrate and oxygen diffusion affects the PHA yield and accumulation rate.
- The composition of PHA changes with the aeration rate.

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ABSTRACT

The polyhydroxyalkanoate (PHA) accumulation dynamics in aerobic granules that undergo the growth–disintegration cycle were investigated. Four sequencing batch reactors (SBR) were inoculated with aerobic granules at different stages of development (different sizes). Different sizes of aerobic granules showed varying PHA contents. Thus, further study was conducted to investigate the diffusion of substrate and oxygen on PHA accumulation using various organic loading rates (OLR) and aeration rates (AR). An increase in OLR from 0.91 to 3.64 kg COD/m^3 day increased the PHA content from 0.66 to 0.87 g PHA/g CDW. Meanwhile, an AR increase from 1 to 4 L/min only accelerated the maximum PHA accumulation without affecting the PHA content. However, the PHA composition only changes with AR, while the hydroxyvalerate (HV) content increased at a higher AR.

1. Introduction

Polyhydroxyalkanoate (PHA) is a biodegradable polymer that is formed in microorganisms as energy storage (Liu and Tay, 2004). To accumulate PHA in wastewater treating microorganisms, an aerobic dynamic feeding strategy is generally used. This feeding strategy is in fact a common factor for PHA accumulation and aerobic granule formation (Gobi and Vadivelu, 2014). However, in wastewater treatment using aerobic granules, the accumulation of PHA has rarely been reported. Aerobic granules are three-dimensional spheres that are made of layers of microorganisms (Liu and Tay, 2004). Due to its multi-layered structure, the diffusion of oxygen and substrate plays a major role in its structural integrity. As the aerobic granules grow in diameter, the diffusion of oxygen and substrate would be limited due to the internal resistance of aerobic granules (Li and Liu, 2005). Consequently, the area near to the core would be under starvation and anaerobic conditions. As a countermeasure, the cells at the core of the granules would consume the extracellular polysaccharide (EPS) layer for survival (McSwain et al., 2005). In aerobic granules, the disintegration of cells were attributed towards the consumption of EPS at the core during the starvation phase (Wang et al., 2007).

In PHA accumulating aerobic granules, it is expected that the structure of it would be able to withstand the starvation period longer than the conventional aerobic granules. This assumption is based on the function of PHA in the aerobic granules. The accumulated PHA will be oxidized first instead of EPS during the starvation phase. As such, the aerobic granules would be able to survive longer than conventional aerobic granules. Yet, the disintegration of PHA accumulating aerobic granules is inevitable due to the development of anaerobic zone at the core (McSwain et al., 2005). Disintegration may result in physiological shocks to the broken cells. The disintegrated cells are directly exposed to substrate without any protection layer (multilayer cells and EPS). Thus, the natural ability of the cells to respond to the substrate and oxygen might be altered. In a reactor containing aerobic granules, this growth–disintegration cycle of granules occurs continuously. As a

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result of the growth–disintegration cycle, various sizes of aerobic granules could be found in a reactor at any time. However, very little is known about the effect aerobic granules size on the PHA accumulation.

Thus, aim of this work was to study the correlations between the substrate concentrations, diffusion of oxygen and granular size on PHA accumulation by manipulating the organic loading rate (OLR) and aeration rate (AR). Till date, no study has focused on these aspects of aerobic granules for PHA accumulation.

2. Methods

2.1. Reactor setup

Four 2-L acrylic sequencing batch reactors (SBR) were used. In each reactor, air is supplied from the bottom of the reactor and controlled using a flow meter. The inner diameter of these SBR is 0.7 m each. At the beginning of each study, each reactor was inoculated with 2 g of aerobic granules and 1 L of palm oil mill effluent (POME). The volume exchange ratio was maintained at 25%. The reactors were operated in a 24-h cycle. Data were collected only after the reactor established a steady-state operation.

2.2. Wastewater and aerobic granules

POME was used as the sole feed for the reactor and was collected from Tali Ayer Palm Oil Mill, Bagan Serai, Perak. It was stored in a cold room at approximately 5 ± 1 °C. The aerobic granules that were used in this study were grown under an OLR of 2.73 kg COD/m^3 day and an AR of 3 L/min in the parent SBR (which is equivalent to 38 cm/s). Further reading on the operation of the parent SBR and development of aerobic granules can be found in Gobi and Vadivelu (2014).

2.3. Growth–disintegration cycle experiment

The aerobic granules obtained from the parent reactor were segregated into specified size classes using strainers (mesh sizes of the strainers used were 0.35, 0.5, 1 and 2 mm). Reactor 1 (R1) was inoculated with aerobic granules sizes of 0.35–0.5 mm, Reactor 2 (R2) with 0.5–1.0 mm, Reactor 3 (R3) with 1–2 mm, and Reactor 4 (R4) with aerobic granules that were larger than 2 mm. The granules size in each reactor was maintained in the specified range throughout the experimental period. Floccular biomass/granules less than 0.35 mm were removed from all the reactors on a constant basis. An equal amount of aerobic granules (in terms of weight) was inoculated in each reactor. Each of these four reactors was supplied with an identical OLR and AR. Liquid and solid samples were collected at specified time intervals and analyzed for PHA and COD. PHA analysis was done using the sodium hypochlorite-chloroform dispersion method as stated in Gobi and Vadivelu (2015).

2.4. Organic loading rate (OLR) and aeration rate (AR) effect

To analyze the effect of the substrate concentration on the PHA accumulation in aerobic granules, the organic loading rate (OLR) of the POME was adjusted by dilution to achieve 0.91, 1.82, 2.73 and 3.64 kg COD/m^3 day. Meanwhile, the effect of oxygen diffusion was investigated using four different ARs (1, 2, 3 and 4 L/min). PHA, COD and volatile fatty acid (VFA) analyses were performed for both OLR and AR studies. The VFA content at the specified time was determined using gas chromatography (GC) according to the method of Gobi and Vadivelu (2014). The granules were wasted periodically to maintain SRT of 2 days. The oxygen diffusion rate (K_La) was determined for each flow rate using Eq. (1) (Jamnongwong et al., 2010).

$$\frac{C_s - C_t}{C_s - C_o} = e^{-(K_La)t} \tag{1}$$

where K_La is the overall oxygen coefficient; C_t is the concentration of oxygen at time t, mg/L; C_s is the concentration of oxygen at equilibrium, mg/L; and C_o is the concentration of the initial oxygen level, mg/L. A linearized form of Eq. (1) is given as Eq. (2) (Jamnongwong et al., 2010).

$$\ln(C_s - C_t) = \ln(C_s - C_o) - \frac{K_La}{2.303}t \tag{2}$$

The gradient of the graph ln ($C_s - C_t$) against time (t) will indicate the value of K_La.

3. Results and discussion

3.1. Effect of aerobic granules size on PHA accumulation

During a cycle of growth–disintegration, different sizes of aerobic granules may appear in an SBR. Thus, the effect of this growth–disintegration cycle on PHA accumulation was studied using different sizes of aerobic granules. It is assumed that similar aerobic granules size would exhibit identical behavior on PHA accumulation. It was found that the maximum PHA accumulation decreased with increasing granule size (data shown in Supplementary section). The reactor with granules between 0.35 and 0.5 mm (R1) accumulated 0.68 g PHA/g CDW, and this value gradually decreased with increasing granule size. The lowest value was observed at R4 ($x > 2$ mm), at which only 0.60 g PHA/g CDW was accumulated.

It must be noted that in these four reactors, the OLR, AR and aerobic granule concentrations were kept constant throughout the study. Therefore, the possibility of imbalanced exposure of substrate and oxygen on aerobic granules was eliminated. Thus, it is postulated that diffusion could be the reason for different PHA accumulation as the size varies. Theoretically, small granules have a larger surface area and vice versa. The increase in the effective contact area between the organic compound and aerobic granules proportionally increases the diffusion process (Liu et al., 2005). The diffusion process occurring in smaller aerobic granules is faster and produces a higher PHA content. Meanwhile, in larger granules, the effective contact area of the substrate and granules is limited. Only the cells at the boundary are directly exposed to the substrate. As such, the layers near to the surface area are expected to easily accumulate PHA. Meanwhile, the accumulation of PHA by the cells beneath the first layer of cells is totally dependent on the diffusion process of the substrate. However, the limited surface area of large granules makes the substrate diffusion process slower. Moreover, as the outer layer already consumed a sizeable amount of substrate from the bulk solution, the diffusion process becomes difficult with reduced driving force to overcome the mass transfer resistance in aerobic granules. As a result, the amount of substrate that diffused into the cells decreases, and the cells beneath the first layer of cells are exposed with little/no amount of substrate. Per se, the accumulation of PHA is not possible in the cells that are not exposed to substrate. Consequently, with a limited number of cells taking part in the PHA accumulation process in large granules, the amount of PHA that accumulates in large granules decreases compared to that in small granules.

Based on these observations, there is a strong urge to demonstrate the role of the diffusion process in PHA accumulation in aerobic granules. Substrate and oxygen diffusion was investigated using various OLRs and ARs.