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Performance of a Tropical Enhanced Biological Phosphorus Removal Process at Different Carbon Loadings

Li Wen Chew, Adeline Seak May Chua*, Phiak Kim Poh, Gek Cheng Ngoh

Department of Chemical Engineering, Faculty of Engineering, University Malaya, Lembah Pantai, 50603 Kuala Lumpur, Malaysia.
adeline@um.edu.my

In recent years, efficient enhanced biological phosphorus removal (EBPR) processes operating at temperature higher than 28 °C have been reported in several studies. However, the operating strategy to maintain stable and high phosphorus removal efficiency at tropical temperature remains elusive. In EBPR process, glycogen accumulating organisms (GAOs) compete with polyphosphate accumulating organisms (PAOs) for the often limited carbon substrates. Carbon loading is apparently impactful on the EBPR performance. Although there have been many studies on the effect of carbon concentration on EBPR, the temperature range investigated was below 25 °C. It is of our interest to elucidate how carbon loading affects the EBPR performance at higher temperatures. In this study, EBPR is carried out at 30 °C in a laboratory-scale sequencing batch reactor (SBR) with a working volume of 1.6 L. The seed sludge was obtained from a local sewage treatment plant (STP). The reactor was operating under alternating anaerobic-aerobic condition and fed with acetate-rich synthetic wastewater. The acetate concentration is reduced consecutively from phase I (60 mg C/L) to phase II (40 mg C/L). The reactor exhibited EBPR characteristics one week after the start-up, showing an extremely short acclimatisation period. In phase I, we observed an increase in the anaerobic phosphorus release from 61 mg P/L to 125 mg P/L and the aerobic phosphorus uptake from 28 mg P/L to 121 mg P/L. The phosphorus content in the dry biomass also increased from 3 wt% to 11 wt%. In phase II, the reduction of acetate concentration led to the deterioration of EBPR performance whereby the phosphorus content reduced from 11 wt% to 4 wt%. The findings indicate that higher carbon loading may be a key to maintain efficient EBPR processes in the tropics.

1. Introduction

Phosphorus is one of the key components that cause eutrophication and subsequently deteriorate water quality. The increase of phosphorus content in water bodies is due to effluents from wastewater treatment plant (WWTP) and run off from nearby plantations. Urbanisation and rapid population growth has significantly increased the phosphorus load into the WWTP over the years, causing the effluent from WWTP one of the primary sources for phosphorus in the water bodies. The increasing severity of eutrophication worldwide has urged the need for phosphorus removal from wastewater. Enhanced biological phosphorus removal process (EBPR) was introduced into WWTP. Due to its relatively low cost and environmentally sustainability, EBPR has become a popular method for phosphorus removal (Mielczarek et al., 2013).

EBPR is a widely implemented process in WWTP with stringent discharge of phosphorus. In EBPR process, sludge is recirculated through anaerobic and aerobic conditions to promote the enrichment of a group of bacteria known as polyphosphate accumulating organisms, PAOs. During anaerobic phase, PAOs take up volatile fatty acids (VFAs) and store it as polyhydroxyalkanoates (PHA) by using energy from the hydrolysis of polyphosphate (poly-P). In the subsequent aerobic phase, PAOs use the stored PHA as energy source for biomass growth and P replenishment which result in P removal. There is another group of organisms known as glycogen accumulating organisms, GAOs which compete with PAOs for anaerobic VFA uptake without contributing to P removal (Oehmen et al., 2007). To optimise P removal efficiency in an EBPR, competition between PAOs and GAOs has to be minimised.

As PAOs and GAOs are competitors for carbon substrate, carbon loading could greatly influence their competition. Generally, EBPR performance is relatively stable using acetate as carbon source. Based on many

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previous studies done, PAOs are having a competitive advantage over GAOs at lower carbon concentrations of COD : P ratio less than 10 : 1 (Ong et al., 2013). According to Burow et al. (2008), it was proposed that active acetate transport contributes more to overall acetate uptake in Accumulibacter (PAOs) than Defluvicoccus (GAOs). Tu and Schuler (2013) also reported that PAOs produced 1 mol ATP/ acetate uptake in excess of internal metabolic requirement for acetate transformation to PHB, which can be used for active acetate transport, while GAOs had no excess ATP and so rely on slow diffusion process. Both studies have suggested that both PAOs and GAOs drive acetate uptake using cellular chemiosmotic gradient but PAOs are able to expand more energy to generate this gradient and can therefore utilise acetate permease activity which can scavenge low amount of acetate. Increased permease-mediated acetate uptake in PAOs provide them competitive advantage over GAOs at low acetate concentration. Most of these studies such as Yu et al. (2014), Tu and Schuler (2013) and Ahn et al. (2009) only limited to low temperature EBPR below 25 °C. This has urged the need to further examine the effect of carbon concentration on tropical EBPR process.

This paper aims to develop a strategy to cultivate highly enriched PAOs in a tropical based EBPR by focusing on the effect of carbon loading on the phosphorus removal performance as well as PAOs/GAOs competition. The approach is to operate a laboratory scale EBPR system at different acetate concentration with monitoring of system performance and abundance of intracellular poly-phosphorus granules, a characteristic of PAOs.

2. Materials and Methods

2.1 SBR operation

A laboratory scale sequencing batch reactor (SBR) with a working volume of 1.6 L is operated under anaerobic-aerobic (AO) condition at 30 °C. The reactor was seeded with the activated sludge collected from a local sewage treatment plant located in Petaling Jaya, Selangor, Malaysia. The reactor is operating in successive cycles of 4 h which consists of 2 min of anaerobic feeding, 1 h of anaerobic phase, 2 h of aerobic phase followed by 46 min of settling and 2 min of decanting. The pH is not controlled but fluctuated in the range of 7.3 to 8.4. The hydraulic retention time (HRT) and solid retention time (SRT) are 10 d and 10 h. The SBR is fed with acetate-rich synthetic wastewater of 60 mg C/L acetate in phase I for 30 days and 40 mg C/L acetate in phase II for 50 days. There is a transition period of 20 days between phase I and phase II. During transition period, the carbon loading is adjusted to the desired carbon loading and sludge is given time to adapt to the new environment. The synthetic wastewater composition was adopted from Ong et al. (2013). A final concentration of 15 mg P/L phosphorus is dosed together with 75 mg C/L and 50 mg C/L dissolved organic carbon which include acetate added together with yeast and peptone in phase I and phase II.

2.2 Chemical analyses

EBPR performance was monitored weekly for 100 days. Samples were collected at the beginning of anaerobic phase, the beginning of aerobic phase and the end of aerobic phase from selected cycle for the analysis of mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), dissolved organic carbon (DOC), acetate, ortho-phosphate (PO_4-P) and P content. The MLSS and MLVSS were analysed in accordance with standard methods (APHA, 1998). Mixed liquor samples were filtered with 0.45 µm membrane filter (Minisart NML, Sartorius, Germany) and subjected to a TOC analyser (TOC-V CSN, Shimadzu, Japan). The acetate and PO_4-P concentration were filtered through 0.2 µm membrane filter filter (Minisart NML, Sartorious, Germany) and determined using Ion Chromatography (861 Advanced Compact IC, Metrohm, Switzerland). P content was measured by using colorimeter adopted the molybdovanadate method with acid persulfate digestion (Method 10127) (HACH, DR/890, USA) (APHA, 1998).

2.3 Microscopic examination for polyphosphate accumulation

4'-6-Diamidino-2-phenylindole (DAPI) staining was conducted according to the procedure proposed by Kawaharasaki et al. (1999). DAPI working solution with a high concentration of 50 ppm was prepared in 25 mM Tris-HCl buffered saline at pH 7.0 and filtered through 0.2 µm pore size syringe filter (Minisart RC 15, Sartorious, Germany). A paraformaldehyde-fixed activated sludge was collected at the end of the aerobic phase and applied with the working solution. After 10 min of incubation, the slide was rinsed with ultra-pure water (arium® 611UF, Sartorious, Germany) and air dried in dark condition at room temperature. The stained samples were observed under fluorescence microscope (Leica DM2500, Leica, Germany).

3. 3. Result and Discussion

3.1 EBPR performance at different acetate concentration

In an EBPR process, the acetate uptake profile and the PO_4-P release and uptake profile during anaerobic and aerobic phase were served as an indication of the presence of PAOs. The concentration profiles of acetate and

P in selected cycles throughout the 100 days of reactor operation are shown in Figure 1. The first part denotes Phase I (high acetate concentration) where the acclimatisation of seed sludge to EBPR sludge happen on the 14th day, the second part denotes Transition Phase and the last part denotes Phase II (low acetate concentration

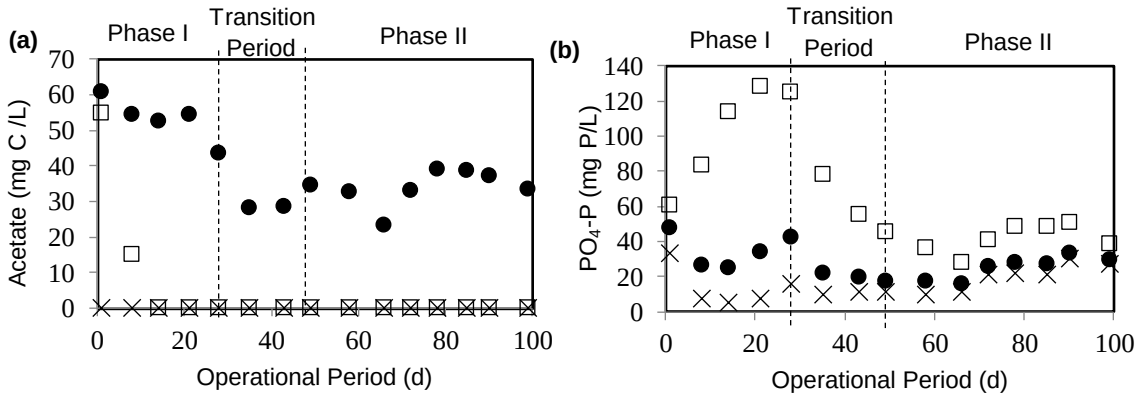

Figure 1: Variations of (a) Acetate and (b) PO4-P concentration in the weekly monitored SBR cycles from the 1st day to the 100th day: (×) Beginning of Anaerobic phase; (□) Beginning of Aerobic phase; (●) End of Aerobic phase.

Two weeks after the start-up, the reactor showed EBPR characteristics. The complete anaerobic acetate uptake that occurred with an increase of anaerobic poly-P release on 14th day gives an indication of PAOs enrichment. As agreed by most of the EBPR biochemical model (Comeau et al., 1986). PAOs have the ability to hydrolyse poly-P to enable anaerobic carbon uptake. Based on Figure 1, there was a drastic increase of anaerobic poly-P release into the bulk liquid from 14 mg P/L to 89 mg P/L from the 1st day to 14th day, The reduction of acetate was in line with the increment of PO_4-P concentration in the wastewater and this shows that poly-P acts as the source of energy for the anaerobic substrate uptake during anaerobic phase.

The acetate and PO_4-P concentration profile, P content in the sludge could act as another indicator for EBPR performance. Figure 2 shows the variations of P content on selected EBPR cycles from 1st day to 100th day. According to Figure 2, the P content in the dry biomass increased from 3 wt% to 10 wt% just within 2 week of system operation. It can be said that the sludge is enriched with PAOs just within 2 week of acclimatisation period. The extremely short acclimatisation period is very desirable for real EBPR operation.

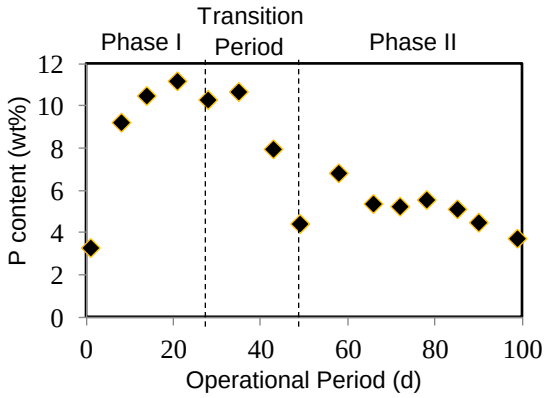

Figure 2: Variations of P content on selected SBR cycles from 1st day to 100th day.

Several evidences indicate that the phase I system is enriched with PAOs. Firstly, the anaerobic poly-P release/ acetate uptake (poly-P_{rel}/Ac) ratio was in the range of 0.56 mol/C-mol to 0.74 mol/C-mol, which was greater than the 0.5 mol/C-mol value suggested as a benchmark for PAOs dominance over GAOs (Schuler and Jenkins, 2003). There were also significant net removals of phosphorus (P) at the end of aerobic phase although effluent P still remained high in the range of 5 mg P/L to 16 mg P/L. Inconsistent P removal demonstrated by the EBPR system in phase I indicates that the EBPR process not yet reached its steady stage in P removal. This may be due to microbial-originated process perturbation (Mino et al., 1998). Second, the P content in the dry biomass was in the range of 9 wt% to 11 wt% which was much greater than the typical P content value of approximately

3 wt% in conventional activated sludge process, indicating high poly-P storage in the system. This value was also in the range of typical P content of an EBPR sludge, which usually ranges from 4 wt% to 15 wt% of the biomass dry weight (Panswad et al., 2003). The rapid increase in the P content which resembles PAOs shows that during phase I, PAOs were able to compete with GAOs for the acetate uptake. Complete acetate uptake with poly-P release during the anaerobic phase also suggested that PAOs were responsible for most or all acetate consumption.

During transition phase, carbon loading was adjusted from 60 mg C/L (phase I) to 40 mg C/L (phase II). From Figure 1 and 2 above, it can be seen that there was a significant decrease in the anaerobic poly-P release and aerobic poly-P uptake as well as the P content in the sludge. This shows that the decrease in carbon loading has a significant impact on the EBPR sludge. During phase II, the reduction in the acetate concentration has led to a decline in PAOs activity as indicated by the drop in the poly-P_{rel}/Ac value and P content in the biomass. First, the anaerobic poly-P release/ acetate uptake (poly-P_{rel}/Ac) ratio reduced significantly from 0.74 mol/ C-mol (phase I) to 0.03 mol/C-mol (phase II) on the 100^{th} day. The system also exhibited a poor P removal in which the poly-P release in the bulk liquid dropped from 94 mg P/L to 34 mg P/L and there was insignificant or almost zero net P removal. Complete acetate uptake during anaerobic phase with lesser amount of poly-P release indicating the reduction in the amount of PAOs and implying an increase of GAOs activity in the system. Second, the P content in dry biomass also reduced from 11 wt% (phase I) to 3.7 wt% (phase II). The reduction in poly-P_{rel}/Ac value and P content in the biomass are suggesting that GAOs or other heterotrophs may have dominated the culture during phase II.

According to the results shown, the performance of EBPR dropped significantly from phase I to phase II when carbon loading decreased. It was hypothesised that acetate permease activity in PAOs may behaved differently at tropical temperature higher than 28 °C, causing the competition between PAOs and GAOs to differ. At high acetate concentration, the system showed good EBPR performance and was enriched with PAOs. It has been suggested that the high acetate concentration may be sufficient to cater for the carbon requirement for both GAOs and PAOs. In another word, the limitation of the amount of GAOs in the system may allow PAOs to grow on acetate present in excess of GAOs requirement. It is hypothesised that GAOs and PAOs are able to coexist in EBPR processes in the tropics at high acetate concentration.High carbon loading may acts as a key strategy to cultivate PAOs in tropical based EBPR. Further research is required to elucidate PAOs and GAOs acetate uptake mechanisms in tropical EBPR process.

3.2 Detection of polyphosphate accumulation via DAPI staining

DAPI staining was applied to detect intracellular poly-phosphorus granules in the bacterial cells from 1st day, 29th day (phase I) and 72th day (phase II).

Based on Figure 3(b) and 3(d), there was a significant increase in the abundance of bright yellow fluorescence during phase I. However during phase II, there was an obvious decrease in the brightness of yellow fluorescence (Figure 3f). This result further demonstrates and confirms the enrichment of PAOs in the EBPR system during phase I and the significant decrease in the number of PAOs during phase II.

In phase I, we also noted the presence of tetrads and coccibacilli which resemble the morphologies of Defluvicoccus-GAOs and competibacter-GAOs (Seviour and Nielsen, 2010). This may suggest that GAOs and PAOs are able to coexist when acetate concentration is sufficient and that GAOs may not necessarily be the immediate cause of PAOs failure as stated in many previous studies. Since GAOs are able to coexist with PAOs in an EBPR system at high acetate concentration, other factors related to environmental changes such as low acetate concentration must have been responsible for the PAOs failures. During phase II, there was a significant increase in the number of coccobacillus resembles Competibacter in the sludge as shown in Figure 3(e). The dominance of Competibacter during phase II indicates that these GAOs was more tolerant to low acetate condition than Defluvicoccus and PAOs. When GAOs are present in significant number, they will compete with PAOs for limited carbon sources, which in turn limit the potential of PAOs for aerobic P uptake (Oehmen et al., 2007). Further investigation need to be done to confirm the identity of tetrads and coccibacilli present and the relation of the related microbial community.

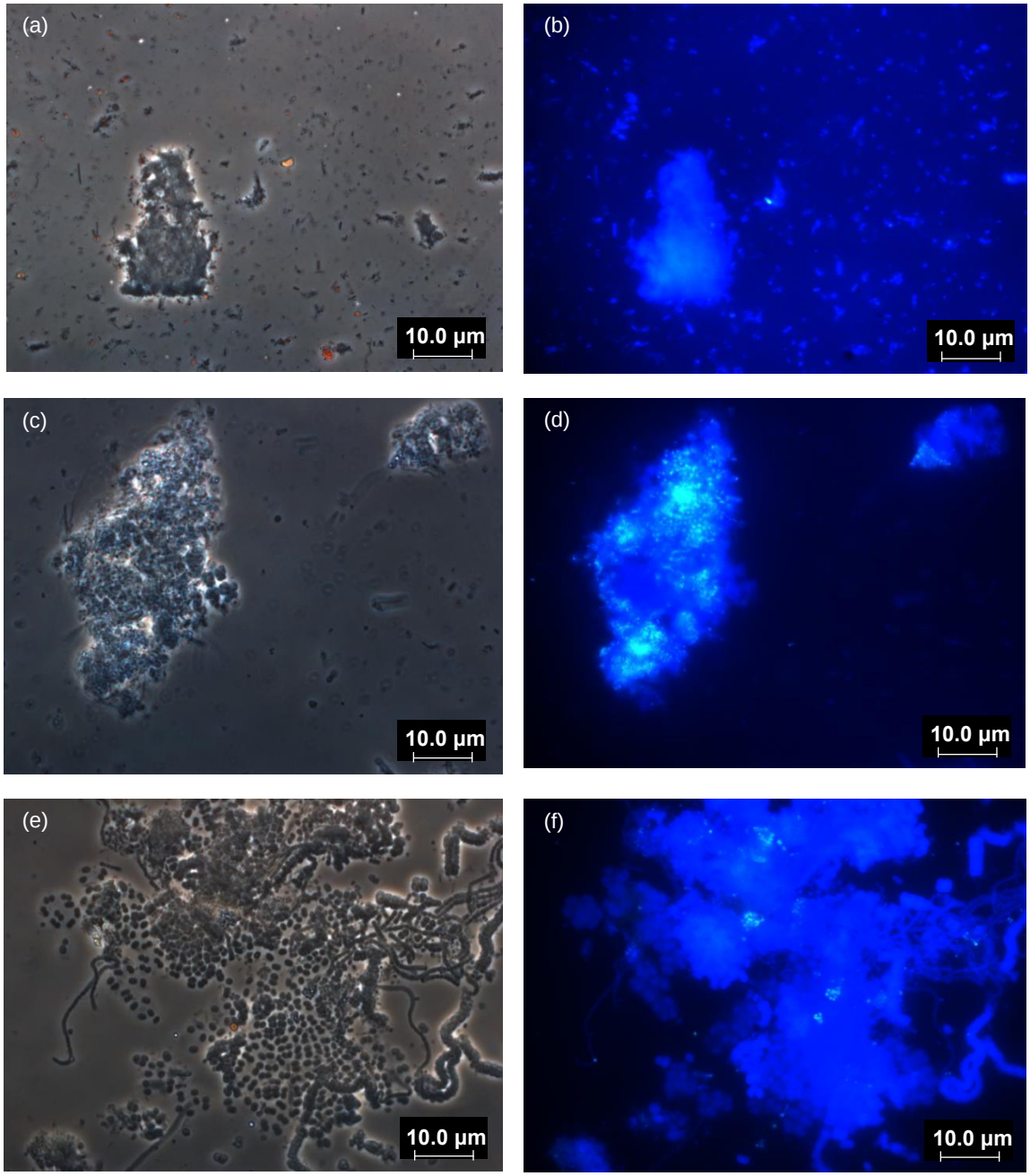

Figure 3: Microscopic observations of DAPI-stained EBPR sludge samples collected at the end of aerobic phase on 1st day, 29th day (phase I) and 72th day (phase II); Phase contrast image of sludge on a, c and e; Fluorescence image of sludge on b, d, and f; Bright yellowish fluorescence image indicates the presence of intracellular polyphosphate granules.

4. Conclusions

An EBPR reactor was successfully operated at 30 ºC within short acclimatisation period of 14 d at high acetate loading. This study revealed that acetate concentration below 40 mg C/L could significantly deteriorate tropical based EBPR performance and change the microbial community in the system. From an operational prospective, these results suggested that higher carbon loading may be a key to maintain efficient tropical-based EBPR processes. The poor EBPR performance seems to be due to the dominance of Competibacter at low acetate concentration. The presence of GAOs in successful EBPR system at high acetate concentration indicated that GAOs may coexist with PAOs at tropical based EBPR.

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