ESTABLISHMENT OF ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL PROCESS BY USING SEED SLUDGE FROM A CONVENTIONAL ACTIVATED SLUDGE PROCESS

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ABSTRACT

A lab scale sequencing batch reactor (SBR) was set up and operated to establish an Enhanced Biological Phosphorus Removal (EBPR) process. The SBR was seeded with biomass collected from a conventional activated sludge process. Development of EBPR characteristics in the SBR were monitored periodically throughout the 54-days of reactor operation. After 13 days of reactor operation, EBPR characteristics were observed. Effluent concentration of PO_4^{3-}-P were in the range of 5.4-9.5 mg/L from Day 13 to Day 54, except on Day 33 PO_4^{3-}-P was not detected. Although phosphorus removal performance was rather consistent in the period between Day 13 and Day 54 of reactor operation, the desired effluent concentrations of PO_4^{3-}-P have yet to achieve. It is believed that phosphorus removal efficiency can be further improved by optimizing the operational conditions of the EBPR system. The results of this study supported the enrichment of PAOs in the SBR within a relatively short sludge acclimatization period. Thus, it is feasible to establish EBPR process by using seed sludge from conventional activated sludge process.

Keywords: Enhanced Biological Phosphorus Removal; Polyphosphate Accumulating Organisms; Seed Sludge; Sequencing Batch Reactor

INTRODUCTION

Eutrophication, a worldwide aquatic environmental problem, which triggered by the enrichment of nutrients such as phosphorus and nitrogen in water bodies, has gained increasing attention. In comparison, phosphorus has significant impact in the occurrence of eutrophication (Chae et al., 2004; Thuyagarajan et al., 2007). Phosphorus can be released into environment via both natural and anthropogenic activities, the latter are the major sources (Miller, 2008). Jarvie et al. (2006) revealed that poorly treated municipal wastewater could be a significant point source of phosphorus to eutrophication. Thus, removal of phosphorus from municipal wastewater is one of the key strategies in preventing eutrophication (Liu *et al.*, 2007). Phosphorus can be removed at the wastewater treatment plants either through chemical precipitation with iron and aluminium salts, or through biological way termed enhanced biological phosphorus removal (EBPR). Costly chemical and large amount of sludge produced, hamper the implementation of chemical precipitation. In contrast, EBPR, an economical and sustainable method, has become an attractive alternative as no additional chemicals have to be added and less sludge volume is produced (Maurer and Gujer, 1994; Jardin et al., 1996; Metcalf and Eddy, 2003).

To protect the water environment against eutrophication, stringent water quality standards have been imposed on the wastewater treatment plants in many developed countries. For example, the discharge limit of phosphorus as low as 0.3 mg P/L has been adopted in Sweden since 1970s (Tykesson *et al.*, 2005). The current European regulation shows the guideline for total phosphorus discharge from the wastewater treatment plants should limit at 1-2 mg TP/L (Lesjean *et al.*, 2003). However, many developing countries including Malaysia have yet to introduce the discharge limit for phosphorus and nitrogen in their Environmental Regulations. In Malaysia, the present Environment Quality (Sewage and Industrial Effluents) Regulations, 1979, mainly concerns with the organic pollutants (BOD, COD) and heavy metals. Thus, the municipal wastewater treatment plants have been largely run on conventional activated sludge processes that remove mainly the organic matter. The aspect of nutrients removal has been long neglected. With the rapid population growth in big cities of Malaysia (Brinkhoff, 2007), the omission of nutrients removal from the municipal wastewater requires immediate attention from the authority. In Singapore, the neighbouring country of Malaysia, efforts have been taken to retrofit the existing wastewater treatment plant into EBPR system (Cao et al., 2009).

EBPR process is a modified conventional activated sludge process with the introduction of anaerobic stage. This process is capable of removing both organic matter and phosphorus from the municipal wastewater (Kawaharasaki *et al.*, 2002; Seviour *et al.*, 2003). The alternating anaerobic-aerobic condition in EBPR process enables the selection and proliferation of the microbial population named Polyphosphate-Accumulating Organisms (PAOs). The enrichment of PAOs in EBPR process contributes to the phosphorus removal. In EBPR process, influent wastewater is fed during the anaerobic condition. PAOs which are able to assimilate organic substrate under the anaerobic condition have an advantage over other bacteria in the substrate uptake competition (Satoh *et al*., 1996). Hydrolysis of intracellular polyphosphate in PAOs provide the energy for anaerobic substrate uptake (Mino et al., 1985). The assimilated carbon substrates are converted to polyhydroxyalkanoates (PHAs), an intracellular carbon and energy storage compound. As such, in the anaerobic phase, one can observe a reduction of organic concentration along with an increment of orthophosphate concentration in the wastewater. In the subsequent aerobic condition, PAOs utilize the stored PHA for cell growth, cell maintenance and also to generate energy for recovery of intracellular polyphosphate by polymerization of orthophosphate from the extracellular bulk liquid environment. Thus, orthophosphate in the wastewater is removed and stored in PAOs. Phosphorus-rich sludge can then be removed from the EBPR system by sludge wasting. The EBPR process has been successfully implemented in many developed countries and treated wastewater as low as 0.5 mg P/L has been achieved (Tykesson et al., 2005; López-Vázquez, et al, 2008).

The present study aims to investigate the feasibility of establishing an EBPR process by using seed sludge from a conventional activated sludge process. Besides, the municipal wastewaters from three local WTPs were also analyzed for the Chemical Oxygen Demand (COD), phosphate and ammonium contents.

MATERIALS AND METHODS

Sampling of municipal wastewater

Wastewater samples were collected from three municipal wastewater treatment plants (WTPs), namely A, B and C, located in Selangor, Malaysia. Influent wastewater was collected from each plant and filtered through 0.45μm membrane filter on site and preserved below 4^{o}C until analyses. The filtered samples were analyzed for Chemical Oxygen Demand (COD), phosphate (PO_4^{3-}) content and ammonium (NH_4^+) content within 24 hours after collection. Duplicate analyses were made for each sample.

Setup of sequencing batch reactor

A laboratory scale anaerobic/aerobic sequencing batch reactor (SBR) was setup for the operation of EBPR process. The seeding sludge used was collected from a conventional activated sludge process of a WTP located in Selangor. The SBR was operated in a working volume of 2 L, with temperature controlled at 28^{o}C. pH of the process was adjusted to 6.9 ± 0.1 by adding either 1.0 M HCl or 1.0 M NaOH. The SBR was operated in 6 cycles per day with 4 hours every cycle. Each SBR cycle consisted of five phases, 11 minutes of filling, 1 hour of anaerobic and 2 hours of aerobic conditions, followed by 40 minutes of settling and 9 minutes of decanting. Anaerobic period was achieved by nitrogen purging during the first 10 minutes of anaerobic period. Aerobic condition was maintained by delivering air from air compressor to mixed liquor. Solids retention time and hydraulic retention time was 10 days and 10 hours respectively. Operational parameters of the SBR are shown in Table 1 and operating sequence of SBR in one cycle is shown in Fig. 1.

Tab. 1: Operational parameters of SBR

Parameters	
Temperature	28^{o}C
pH	6.9 ± 0.1
DOC loading /cycle	50 mg/ L
PO_4^{3-}-P loading /cycle	15 mg /L
Solid retention time (SRT)	10 days
Hydraulic retention time (HRT)	10 hours

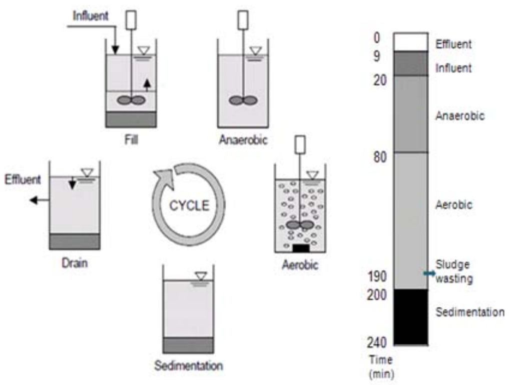


Fig. 1: Operating Sequence of SBR in One Cycle

The SBR was operating at 50 mg DOC/L feed with C:P ratio approximately 3. Feed was prepared from concentrated feed solutions and "P-water". The concentrated feed consisted of 1.79 g/L of sodium acetate (70% of carbon source) , 0.93 g/L of peptone (27% of carbon source), 0.15 g/L of yeast extract (3% of carbon source) , 0.32 g/L of NH_4Cl, 0.68 g/L of $MgSO_4.7H_2O$, 1.21 g/L of $MgCl_2.6H_2O$, 0.32 g/L of $CaCl_2.H_2O$, 0.02 g/L of allythiourea (ATU) to inhibit nitrification and trace elements solution (adapted from Liu et al., 2007). Concentrated "P-water" consisted 0.34 g/L of K_2HPO_4 and 0.39 g/L of KH_2PO_4. Concentrated feed and "P-water" was later diluted according to the designed DOC loading/ cycle and PO_4^{3-}-P loading /cycle before feeding into SBR. In order to follow the development of EBPR characteristic in the SBR, one SBR cycle was monitored weekly for the profile of DOC and $PO^{3-}{}_4$-P throughout the reactor operation period.

Analytical techniques

Mixed liquor samples from the SBR were filtered through 0.45µm cellulose acetate syringe filter for chemical oxygen demand (COD) and dissolved organic carbon (DOC) analysis; and 0.2 µm regenerated cellulose (RC) syringe filter for orthophosphate (PO_4^{3-}-P) analysis. COD was analyzed by using DR/ 890 HACH colorimeter (Method 8000). Dissolved organic carbon was assayed by TOC analyzer (TOC-V CSN, Shimadzu). PO_4^{3-}-P was measured by Ion Chromatograph (Methrom 861 Advanced Compact IC, column Metrosep A supp 5 150/4.0mm, anion eluent 3.2mM Na_2CO_3 and 1mM $NaHCO_3$, flowrate 0.7 mL/min). Ammonium (NH_4^+) was analyzed by using Ion Chromatograph equipped with column Metrosep C4 150 150/4.0mm with 65% Nitric acid and pyridine-2, 6-dicarboxylic acid as eluent, with elution rate of 0.9 mL/min.

RESULTS AND DISCUSSION

Characteristics of municipal wastewater

Fig. 2 shows the concentration of COD, PO_4^{3-} and NH_4^+ in the influent municipal wastewater of the three WTPs in Selangor. Selangor is one of the most densely populate states in Malaysia. The municipal wastewater channeled into the WTPs contained COD, PO_4^{3-} and NH_4^+ ranged from 107 mg/L to 206 mg/L, 4 mg/L to 5 mg/L, and 23 mg/L to 26 mg/L respectively.

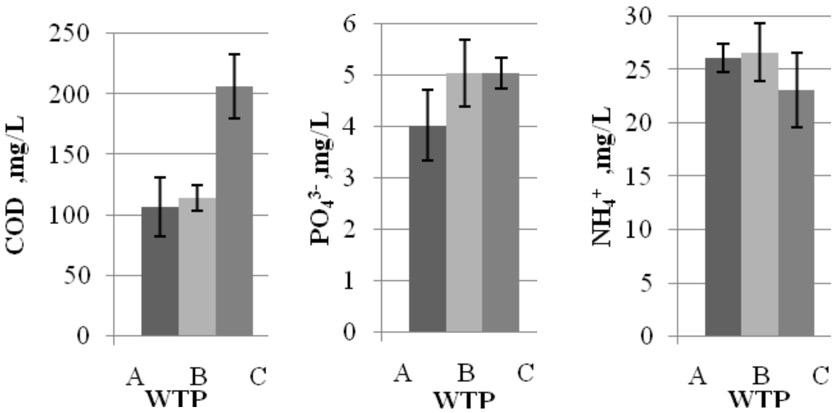
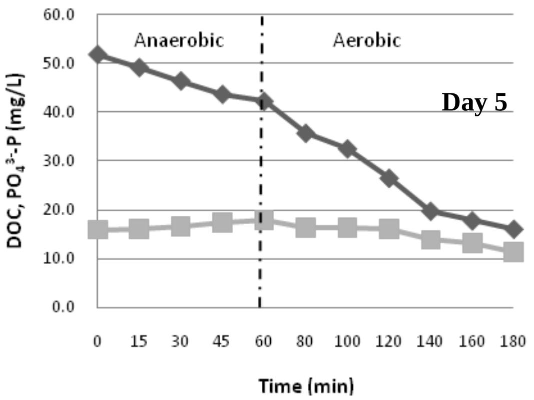


Fig. 2: COD, PO_4^{3-} and NH_4^+ concentration in the influent wastewater of WTP A, B and C in Selangor, Malaysia

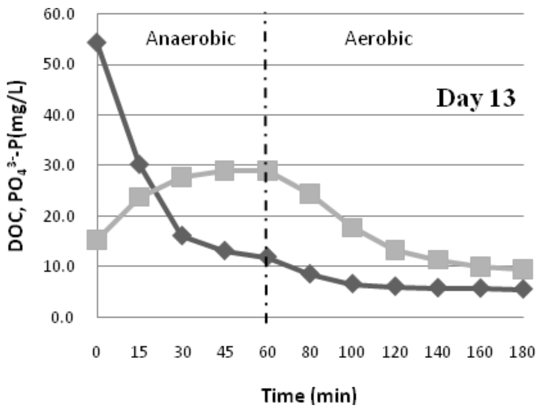
Although there is no report showing PO_4^{3-} concentration of local municipal wastewater, PO_4^{3-} concentration of municipal wastewater in Singapore reported by Cao et al (2009) is used as reference because both Malaysian and Singaporean having a relatively similar lifestyle. The PO_4^{3-} concentration measured was lower than the PO_4^{3-} values reported in Cao et. al. (2009) which ranged from 37.6 to 53.3 mg/L. The wastewater samples might contain condensed phosphate and organically bound phosphate that need to be oxidized to form orthophosphate. Thus, the obtained PO_4^{3-} concentration only represented a fraction of the soluble reactive phosphorus. Digestion methods such as microwave assisted digestion prior to ion chromatography analysis (Colina & Gardiner, 1999) were recommended for the measurement of PO_4^{3-} concentration in the wastewater samples.

Development of EBPR characteristics in SBR

Since PAOs exhibits distinct organic carbon and orthophosphate uptake and release trend in EBPR process, DOC and PO_4-P were monitored periodically throughout 54-days of reactor operation. Fig. 3 shows the concentration profile of DOC and PO_4^{3-}-P in the anaerobic and aerobic phase of one SBR cycle on Day 7, Day 13, Day 19, Day 26, Day 33, Day 40, Day 47, and Day 54. The dash line in each plot of Fig. 3 denotes the change from anaerobic to aerobic phases.



(i)



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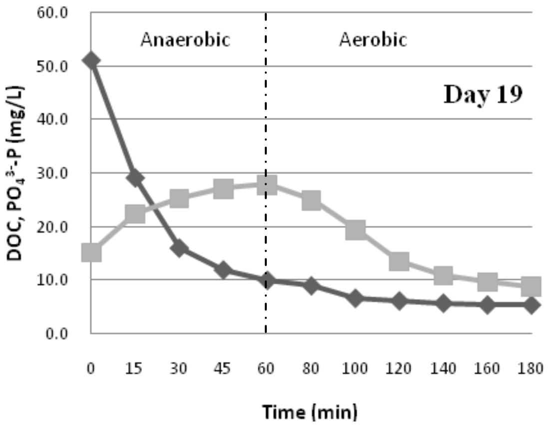

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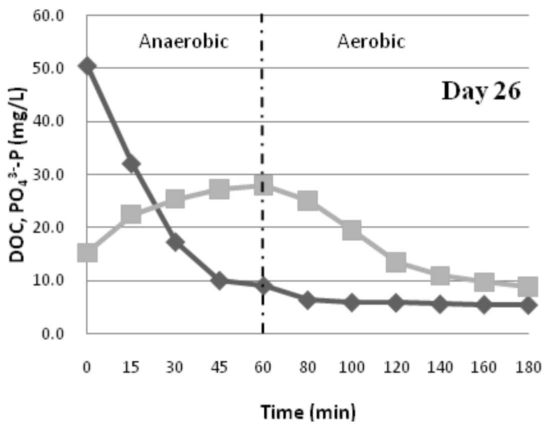

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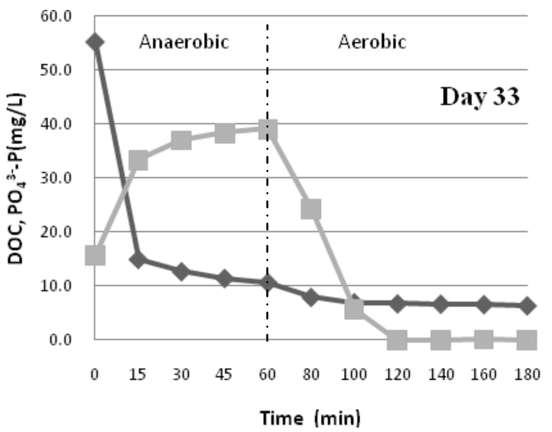

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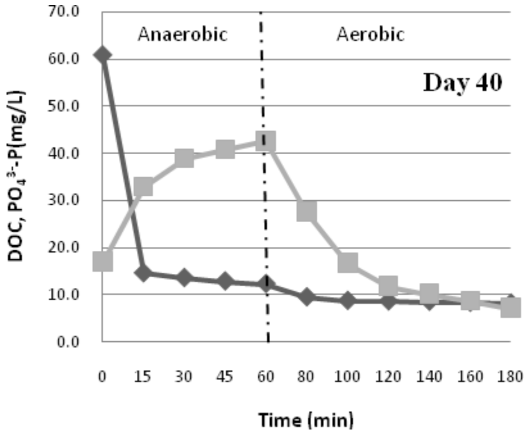

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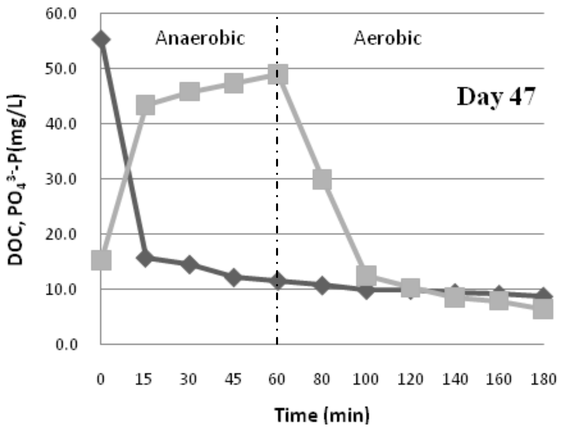

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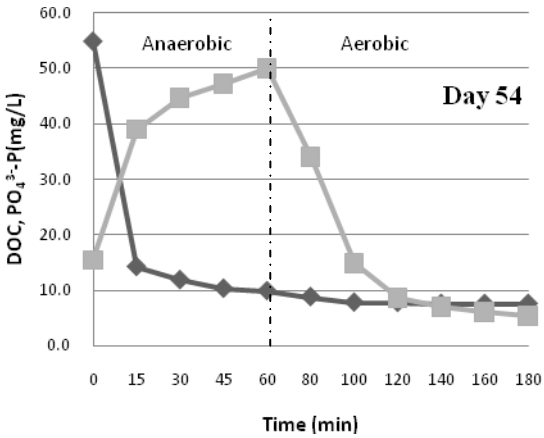


(viii)

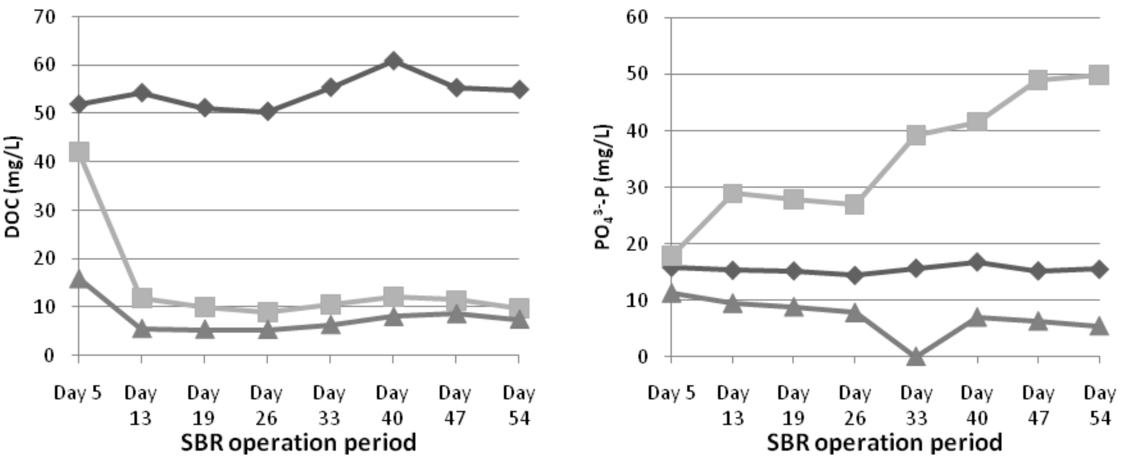
Fig. 3: Concentration profile of DOC and PO_4^{3-}-P in one SBR cycle on the (i) Day 5; (ii) Day 13; (iii) Day 19; (iv) Day 26; (v) Day 33; (vi) Day 40; (vii) Day 47; (viii) Day 54 of SBR operation period —◆—DOC —■—PO4-P

Fig 3(i) shows that there was no indication of EBPR after 5 days of SBR operation. Rapid assimilation of carbon substrate did not occur in the anaerobic phase. Carbon substrates were mainly taken up aerobically indicating the biomass still behaved as seed sludge from the conventional activated sludge process. DOC reduced from 51.9 mgC/L to 15.9 mgC/L giving a carbon removal of 69.1% on Day 5. As for the PO_4^{3-}-P profile, there was neither a significant increment in the anaerobic phase nor reduction in the aerobic phase. This suggested that PAOs had not been enriched in the SBR system. Slight reduction of PO_4^{3-}-P level in the bulk liquid environment of aerobic phase, from 17.8 mg/L to 11.3 mg/L, was due to bacterial growth and metabolism (Lesjean et al., 2003).

After Day 13 of SBR operation, EBPR characteristics had gradually emerged in the system. This can be seen from the rapid carbon reduction along with significant PO_4^{3-}-P increment in the bulk liquid environment of anaerobic stage; followed by a reduction in PO_4^{3-}-P level in the aerobic stage (Fig 3(ii) – (viii)).

As shown in Fig. 3(ii) to Fig. 3(viii), about 80% of the carbon substrate fed was taken up in the anaerobic stage of the SBR cycle monitored on Day 13, Day 19, Day 26, Day 33, Day 40, Day 47 and Day 54. The substrates taken up anaerobically mainly consist of acetate, the favorite carbon source for PAOs (Seviour et al., 2003). The average 20% of carbon substrates remained at the end of aerobic phase was contributed by yeast extract and peptone. Yeast extract and peptone were added as co-substrate in the synthetic wastewater (30% of carbon loading) because amino acids or proteins could enhance the growth of PAOs (Satoh et. al., 1994). A relatively short sludge acclimatization period obtained in this study supported the finding by Satoh et al. (1994).

In the anaerobic phase, the amount of PO_4^{3-}P released from biomass to the bulk liquid increased from 15 mg/L (Day13, Day 19, Day 26), to 25 mg/L (Day33, Day 40) and finally to 35 mg/L (Day 47, Day 54). The PO_4^{3-}-P uptake during the aerobic phase surpassed the release of PO_4^{3-}P during the anaerobic phase. This trend is supported by the models of EBPR system (Mino *et al.*, 1998). The effluent PO_4^{3-}-P concentration of the monitored cycles after the establishment of EBPR improved from 9.5 mg/L to 5.4 mg/L (Fig. 3(ii)-(iv) and Fig. 3(vi)-(viii)). On Day 33, complete P removal was achieved (Fig. 3(v)). The overall achieved effluent phosphorus concentration still higher than the discharged limit imposed on wastewater treatment plants in other countries, such as 0.3 mg P/L in Sweden (Tykesson *et al.*, 2005), 0.5 mg P/L in Germany and 0.15 mg TP/L in Netherlands (Lesjean *et al.*, 2003). A retrofitted full-scale activated sludge process in Singapore showed an average effluent PO_4^{3-}P concentration of 2.4 mgP/L (Cao et al., 2009). Thus, we believe that phosphorus removal efficiency of this developed EBPR process can be further improved by optimizing its operational conditions which are favorable to the growth of PAOs.



(i) (ii)

Fig 4: Concentration profile of (i) DOC, (ii) PO_4^{3-}-P in the monitored cycles throughout the 54-days of SBR operation

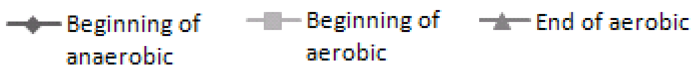


Fig. 4 (i) and (ii) summarized the SBR performance throughout the 54 days of reactor operation by highlighting the DOC and PO_4^{3-}-P concentration at the beginning of anaerobic phase, beginning of aerobic phase and end of aerobic phase of the monitored cycles on different days. It shows clearly that the seed sludge collected from the conventional activated sludge process had been acclimatized to the EBPR characteristics after Day 13 of reactor operation. This is also a sign of PAOs enrichment in the SBR within a relatively short sludge acclimatization period.

CONCLUSIONS

This study demonstrated the feasibility of establishing EBPR process by using seed sludge from a conventional activated sludge process in about 2 weeks. Effluent concentration of PO_4^{3-}-P were in the range of 5.4-9.5 mg/L from Day 13 to Day 54 of reactor operation, except on Day 33 PO_4^{3-}-P was not detected. Concentration of DOC in effluent were in the range of 8-10 mg/L which fulfills the standard implemented by local environment regulations. Result from this study is encouraging and it suggests the high potential of retrofitting the existing conventional activated sludge process to EBPR system. EBPR performance of this study can be further improved by optimizing the operational conditions. The identification of PAOs population can also be confirmed by microscopic study with fluorescent in situ hybridization (FISH).

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