Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 10 (21): 2643-2647, 2010
ISSN 1812-5654

Establishment of Enhanced Biological Phosphorus Removal in a Sequencing Batch Reactor by using Seed Sludge from a Conventional Activated Sludge Wastewater Treatment Process

Y.H. Ong, A.S.M. Chua and G.C. Ngoh
Department of Chemical Engineering, Faculty of Engineering, University Malaya,
50603 Kuala Lumpur, Malaysia

Abstract: A lab scale Sequencing Batch Reactor (SBR) was set up and operated to establish an Enhanced Biological Phosphorus Removal (EBPR) process. The reactor was fed with synthetic wastewater and activated sludge was recycled through alternating anaerobic and aerobic conditions. Seed sludge was collected from a conventional activated sludge wastewater treatment process. During the sludge acclimatization period, the performance of SBR was monitored periodically for the development of EBPR characteristic. Start-up period of about one month was required to acclimatize the seed sludge to establish EBPR. On day 8 of sludge acclimatization period, the system still showing characteristic of conventional activated sludge process where major part of carbon source were consumed during aerobic phase but not rapid carbon assimilation during anaerobic phase. The removal efficiency of organic matter and orthophosphate on day 8 were 80.4 and 14.9%, respectively. The trend of rapid carbon source assimilation and release of orthophosphate during anaerobic phase was getting significant started from day 19 to 28 of sludge acclimatization period. Efficiency of organic matter removal at the end of monitored cycles on day 19, day 24 and 28 were in the range of 70-80%. The efficiency of phosphorus (PO_4-P) removal was improving from day 8 to 28, 14.9% of removal on day 8, 20.9% on day 19, 21.6% on day 24 and 51.7% on day 28. Under microscopic analyses, the presences of gram-negative coccibacilli resemble Polyphosphate Accumulating Organismss (PAOs) detected when EBPR established. Both chemical and microscopic analyses 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.

Key words: Enhanced biological phosphorus removal, polyphosphate accumulating organisms, seed sludge, sequencing batch reactor

INTRODUCTION

Eutrophication, a worldwide aquatic environment problem triggered by enrichment of the nutrients such as phosphorus in water bodies, has gained increasing concern. Phosphorus can be released into environment via both natural and anthropogenic activities, the latter are the major sources. Removal of phosphorus from sewage and industrial is one of the key strategies in preventing eutrophication (Liu *et al*., 2007). Enhanced Biological Phosphorus Removal (EBPR) in activated sludge systems has thus become an attractive alternative to chemical precipitation of phosphorus (Maurer and Gujer, 1994) as no additional chemicals have to be added to the wastewater (Jardin and Popel, 1996).

Stringent water quality standards for phosphorus have been initiated in many developed countries, for example 0.3 mg L^{-1} P in Sweden since 1970s (Tykesson *et al*., 2005), 1-2 mg TP L^{-1} in European regulations (Lesjean *et al*., 2003), by the needs to protect surface water bodies against eutrophication. Besides, Singapore, the neighbouring country of Malaysia, has started investigation on retrofit existing wastewater treatment plant for phosphorus removal (Cao *et al*., 2009). In Malaysia, even though effluent standard for phosphorus is not regulated in the present environment quality (sewage and industrial effluents) regulations, 1979, it is imperative that immediate effort has to be directed to the effluent discharge limit of phosphorus.

Enhanced Biological Phosphorus Removal (EBPR) is modification of conventional activated sludge process with the introduction of anaerobic stage. EBPR, an anaerobic aerobic alternating process, removes organic substrates in the influent and releases phosphate from

Corresponding Author: Adeline Chua Seak May, Department of Chemical Engineering, Faculty of Engineering, University Malaya, 50603 Kuala Lumpur, Malaysia

sludge during the anaerobic phase (Kawaharasaki *et al*., 2002; Seviour *et al*., 2003). The EBPR highly relies on the selection and proliferation of microbial population termed Polyphosphate Accumulating Organisms (PAOs), which capable of storing orthophosphate from their growth environment (Liu *et al*., 2007; Seviour *et al*., 2003). Acclimatize sludge under anaerobic and aerobic alternating condition and feed it with organic substrates only in the anaerobic phase will enrich the presence of PAOs in the sludge. This is because the PAOs have an advantage in the competition toward organic substrates under anaerobic conditions (Satoh *et al*., 1996; Ichihashi *et al*., 2006). During anaerobic stage, PAOs rapidly assimilate carbon source and store as polyhydoxyalkanoates (PHA) by using energy generated from breaking down of intracellular polyphosphate (Seviour *et al*., 2003). Thus, phosphate is released into extracellular growth environment. In the subsequent aerobic conditions, PAOs utilize the stored PHA to generate energy for cell growth, cell maintenance and restoration of energy by polymerization of orthophosphate which uptake from extracellular growth. Phosphorus store in sludge can now be removed from EBPR system by regular wasting of excess sludge of high P content (Liu *et al*., 2007).

The aim of this study is to establish EBPR in a sequencing batch reactor by using seed sludge from a conventional activated sludge process.

MATERIALS AND METHODS

Setup of sequencing batch reactor: The EBPR process was operated in a laboratory scale anaerobic/aerobic Sequencing Batch Reactor (SBR). The seeding sludge for the SBR was collected from a conventional activated sludge wastewater treatment process in Selangor. With a working volume of 2.0 L, the SBR was operated with temperature controlled at 28°C. The pH used was adjusted to 6.9±0.1 by adding either 1.0 M HCl or 1.0 M NaOH. It was operated in 6 cycles per day with 4 h each cycle. The SBR cycle consisted of five phases, with 11 min filling, 1 h anaerobic and 2 h aerobic conditions, followed by 40 min settling and 9 min decanting. Anaerobic period was achieved by nitrogen purging during the first 10 min 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 h, respectively. Operational parameters of the SBR are shown in Table 1 and operating sequence of SBR is shown in Fig. 1.

The SBR was operating at 45 mg TOC L^{-1} 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^{-1} of sodium acetate (70% of carbon source), 0.93 g L^{-1} of peptone (27% of carbon source), 0.15 g L^{-1} of yeast extract (3% of carbon source), 0.32 g L^{-1} of NH_4Cl, 0.68 g L^{-1} of $MgSO_4.7H_2O$, 1.21 g L^{-1} of $MgCl_2.6H_2O$, 0.32 g L^{-1} of $CaCl_2.H_2O$, 0.02 g L^{-1} of allythiourea (ATU) to inhibit nitrification and trace elements solution (adapted from Liu *et al*., 2007). Concentrated P-water consisted 0.34 g L^{-1} of K_2HPO_4 and 0.39 g L^{-1} of KH_2PO_4. Concentrated feed and P-water was later diluted according to the designed TOC loading/cycle and PO_4-P loading/cycle before feeding into SBR.

Table 1: Operational parameters of SBR

Parameters	Values
Temperature	28°C
pH	6.9±0.1
TOC loading/cycle	45 mg L^{-1}
PO_4-P loading/cycle	15 mg L^{-1}
Solid Retention Time (SRT)	10 days
Hydraulic Retention Time (HRT)	10 h

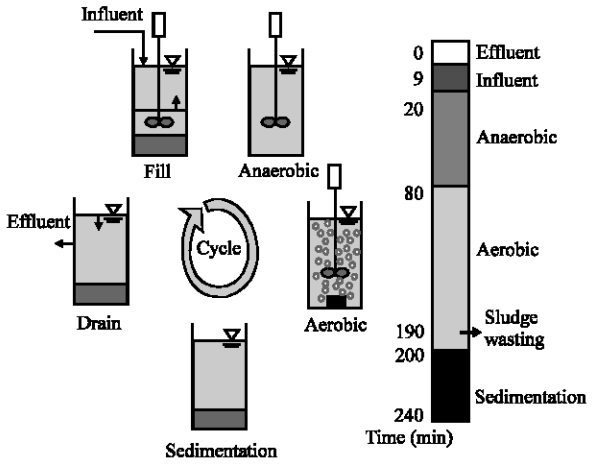

Fig. 1: Operating sequence of SBR in one cycle

Monitoring of EBPR characteristic in SBR

Analytical method: Mixed liquor samples from the SBR were filtered through 0.45 μm cellulose acetate syringe filter and 0.2 μm Regenerated Cellulose (RC) syringe filter for Total Organic Carbon (TOC) and orthophosphate (PO_4-P) analysis, respectively. Total organic carbon was assayed by TOC analyzer (TOC-V CSN, Shimadzu) Orthophosphate was analyzed by ion chromatography (861 Advanced Compact IC, Metrohm).

The Mixed Liquor Suspended Solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS) were analyzed as outlined in standard methods (APHA/AWWA/WEF, 1998).

Microscopic analysis: Gram's stain is the most commonly used stain in the preliminary identification of bacterial isolates. The method involves a differential double staining technique and procedures carried out in accordance to the manufacturer (MERCK) instructions. As dense flocs formed in the SBR, the samples were disaggregated and diluted with a homogenizer (Ziglio *et al*., 2002; Barat *et al*., 2006). The sludge smear was prepared and dried on glass slide and observed under phase contrast microscope (DMLS, Leica).

RESULTS AND DISCUSSION

Monitoring of EBPR characteristic: As PAOs exhibits distinct organic carbon and orthophosphate uptake and release trend in EBPR process, TOC and PO_4-P were monitored periodically throughout the sludge acclimatization period. A start-up period of about one month was required for sludge acclimatization and stable unit performance. Figure 2 shows the concentration profile of TOC and PO_4-P in one SBR cycle on day 8, 19, 24 and 28 of sludge acclimatization period.

From Fig. 2a, the trend of rapid assimilation of carbon source was not shown in anaerobic phase on day 8 of sludge acclimatization period. The system still showing characteristic of conventional activated sludge process as major part of carbon source were consumed during aerobic phase. There was slight increase in orthophosphate levels in the bulk liquid at the end of anaerobic phase, from 20.7 mg PO_4-P/L to 21.58 mg PO_4-P/L. Then in the subsequent aerobic phase, decreased in orthophosphate level in bulk liquid can be detected and resulted 17.59 mg PO_4-P/L at the end of the monitored cycle.

As shown in Fig. 2b-d, carbon source was utilized rapidly and phosphorus was also released during anaerobic phase. The TOC and PO_4-P concentration profile of EBPR process were gradually emerged as the trend of rapid carbon source assimilation in anaerobic stage were improving from day 19 to 28 of sludge assimilation period. Results show 48.6% (54.44 to 27.98 mg TOC L^{-1}) of carbon source was consumed at the end of anaerobic stage of the monitored cycle on day 19, 50.7% (42.74 to 21.07 mg TOC L^{-1}) on day 24 and 55.2% (39.92 to 17.88 mg TOC L^{-1}) on day 28. During the subsequent aerobic phase, results from all three monitored cycles on day19, 24 and 28 of sludge acclimatization period showed orthophosphate uptake from bulk liquid. The orthophosphate uptake during aerobic phase was surpassing the release of orthophosphate during anaerobic phase. This trend is supported by the models of EBPR system (Seviour *et al*., 2003). Removal of orthophosphate from the bulk liquid was from 14.21 to 11.24 mg PO_4-P/L on day 19, 16.85 to 13.22 mg PO_4-P/L on day 24 and 14.55 to 7.05 mg PO_4-P/L on day 28.

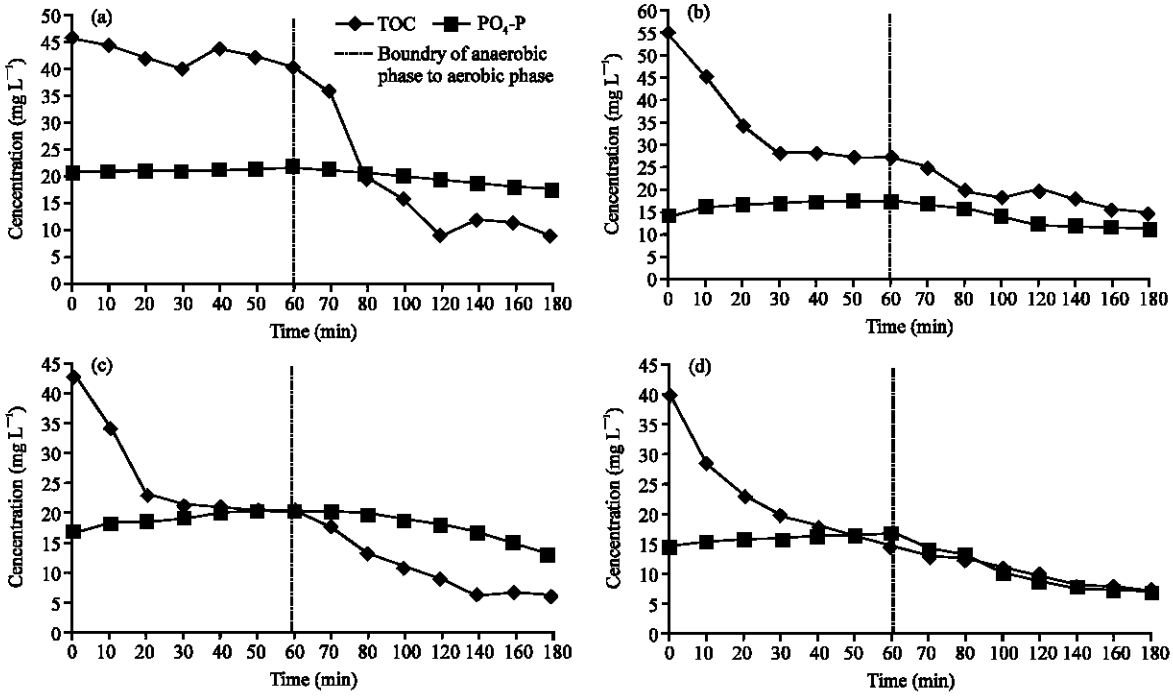

Fig. 2: Concentration profile of TOC and PO_4-P in one SBR cycle on the (a) day 8, (b) day 19, (c) day 24 and (d) day 28 of sludge acclimatization period

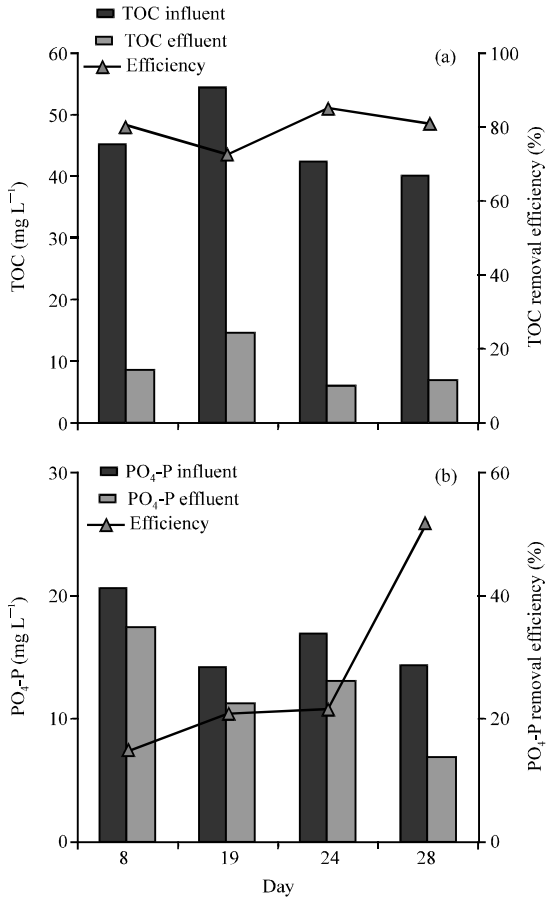

Fig. 3: Efficiency of (a) TOC removal and (b) PO_4-P Removal for one SBR cycle on day 8, 19, 24 and 28 of sludge acclimatization period

Efficiency of TOC removal and PO_4-P removal: The removal efficiency of organic matter and orthophosphate at the end of the monitored cycle on day 8, where EBPR still no established, were 80.4 and 14.9%, respectively, as shown in Fig. 3a and b. Efficiency of organic matter removal at the end of monitored cycles on day 19, 24 and 28 are in the range of 70-80%. As shown in Fig. 3, the efficiency of phosphorus (PO_4-P) removal was increasing from day 8 to 28, which resulted in 14.9% of removal on day 8, 20.9% on day 19, 21.6% on day 24 and 51.7% on day 28. This phosphate uptake and polyphosphate storage was believed to be performed by PAOs as only when conditions are completely anaerobic is phosphate released and phosphate in bulk liquid environment is taken up in aerobic condition (Seviour *et al*., 2003).

Microscopic analysis: The activated sludge was examined under microscope periodically during the sludge acclimatization period (Fig. 4). The Gram's stain result of

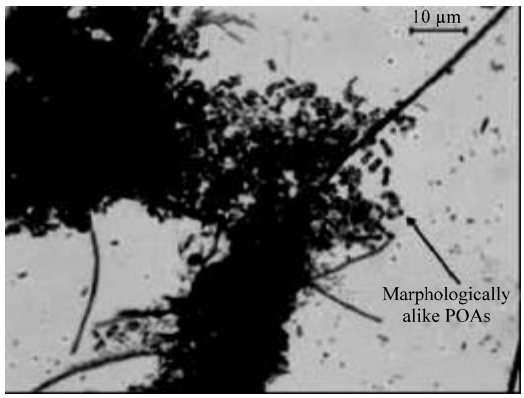

Fig. 4: Dense cluster of gram negative cocoid rods and Gram's stain result with morphologically alike PAOs

sample from one of the cycle on day 28 of sludge acclimatization period where EBPR was established. Counter stain by basic fuchsin give Gram-negative bacteria a pink or red colour. Under microscope, the Gram-stained activated sludge samples show large amounts of dense cluster of Gram-negative coccobacilli. Gram-negative coccobacilli resemble PAOs morphologically were detected.

Overall: Both chemical and microscopic analyses supported the enrichment of PAOs in 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. This finding is motivating and supported by the investigation result of retrofitting existing activated sludge process to EBPR in Singapore, neighbouring country of Malaysia, which shown by Cao *et al*. (2009). However, the performance of this lab scale EBPR system needs to be further improve to achieve higher efficiency of TOC and PO_4-P removal. Identification of coccobacilli resemble PAOs can be further confirmed with fluorescent *in situ* hybridization (FISH) as this method could give reliable result of the populations (Serafim *et al*., 2002; Barat *et al*., 2008).

CONCLUSION

The SBR operated for the enrichment of organisms which carrying out EBPR. Based on the experimental results after one month of sludge acclimatization, EBPR was successfully established. The potential and suitability of seed sludge from conventional activated sludge wastewater treatment process in EBPR establishment was shown. The trend of rapid assimilation

of carbon source from bulk liquid during anaerobic phase and uptake of phosphorus during aerobic phase gradually shown started from day 19 of sludge acclimatization period. Phosphorus removal efficiency achieved 51.7% and TOC removal efficiency was within the range of 70- 80% after a-month-long sludge acclimatization period. Under microscopic analysis, the presences of gram-negative cocci bacilli resemble PAOs detected when EBPR established. However, these initial indications of EBPR need to be confirmed from continuous operation of the SBR and further chemical analyses as well as microbial studies need to be carried out. Performances of the EBPR process can be further improved by investigate the optimum operational parameters.

ACKNOWLEDGMENT

This research project is financially supported by Peruntukan Penyelidikan Pascasiswazah (PPP) Grant (PS104/2008B), University Malaya.

REFERENCES

APHA/AWWA/WEF, 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edn., American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC., USA., ISBN: 0-87553-235-7.

Barat, R., T. Montoya, L. Borras, A. Seco and J. Ferrer, 2006. Calcium effect on enhanced biological phosphorus removal. Water Sci. Technol., 53: 29-37.

Barat, R., T. Montoya, L. Borras, J. Ferrer and A. Seco, 2008. Calcium phosphate precipitation in a SBR operated for EBPR: Interactions with the biological process. Water Sci. Technol., 58: 427-433.

Cao, Y., C.M. Ang, K.C. Chua, F.W. Woo and H. Chi *et al*., 2009. Enhancec biological phosphorus removal in the retrofitting from an anoxic selector to an anaerobic selector in a full scale activated sludge process in Singapore. Water Sci. Technol., 59: 857-865.

Ichihashi, O., H. Satoh and T. Mino, 2006. Sludge-sludge interaction in the enhanced biological phosphorus removal process. Water Sci. Technol., 53: 1-6.

Jardin, N and H.J. Popel, 1996. Influence of the enhanced biological phosphorus removal on the waste activated sludge production. Water Sci. Technol., 30: 333-343.

Kawaharasaki, M., A. Manome, T. Kanagawa and K. Nakamura, 2002. Flow cytometric sorting and RFLD analysis of phosphate accumulating bacteria in an enhanced biological phosphorus removal system. Water Sci. Technol., 46: 139-144.

Lesjean, B., R. Gnirss, C. Adam, M. Kraume and F. Luck, 2003. Enhanced biological phosphorus removal process implemented in membrane bioreactors to improve phosphorus recovery and recycling. Water Sci. Technol., 48: 87-94.

Liu, Y., Y.G. Chen and Q. Zhou, 2007. Effect of initial pH control on enhanced biological phosphorus removal from wastewater containing acetic and propionic acids. Chemosphere, 66: 123-129.

Maurer, M and W. Gujer, 1994. Prediction of the performance of enhanced biological phosphorus removal plants. Water Sci. Technol., 30: 333-343.

Satoh, H., W.D. Ramey, F.A. Koch, W.K. Oldham, T. Mino and T. Matsuo, 1996. Anaerobic substrate uptake by the enhanced biological phosphorus removal activated sludge treating real sewage. Water Sci. Technol., 34: 9-16.

Serafim, L.S., P.C. Lemos, C. Levantesi, V. Tandoi, H. Santos and M.A.M. Reis, 2002. Methods for detection and visualization of intracellular polymers stored by polyphosphate-accumulating microorganisms. J. Microbiol. Methods, 51: 1-18.

Seviour, R.J., T. Mino and M. Onuki, 2003. The microbiology of biological phosphorus removal in activated sludge system. FEMS Microbiol. Rev., 27: 99-127.

Tykesson, E., L.E. Jonsson and J.L.C. Jansen, 2005. Experience from 10 years of full scale operation with enhanced biological phosphorus removal at Oresundsverket. Water Sci. Technol., 52: 151-159.

Ziglio, G., G. andreottola, S. Barbesti, G. Boschetti, L. Bruni, P. Foladori and R. Villa, 2002. Assessment of activated sludge viability with flow cytometry. Water Res., 36: 460-468.