The characteristics of phosphorus removal in an anaerobic/aerobic sequential batch biofilter reactor

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Abstract Previous research has shown that alternated anaerobic/aerobic conditions are effective in removing phosphorus from wastewater using a biofilter system. However, few studies have been conducted on the features of polyphosphate (poly-P) accumulating organisms (PAOs) in biofilm on phosphorus removal. This study investigated the characteristics of the phosphorus removal mechanism in various hydraulic loads and anaerobic/aerobic time ratios using a sequential batch biofilter reactor. The storage and release of intracellular inclusions, especially polyhydroxyalkanoates (PHAs) and poly-P, would be an important factor for phosphorus removal. Under different operating conditions, total phosphorus removal was always determined by accumulation of PHAs and phosphorus release under the anaerobic phase. The PHA accumulation under the anaerobic phase was always in proportion to the biofilm phosphorus content under aerobic conditions. The result shows PAOs activity was closely related to PHA accumulation. However, the PHA accumulation under the anaerobic phase would be dependent on the hydrolysis of the complex carbon source into short chain fatty acids (SCFA). The result would be demonstrated by the simple carbon source effect. The effect of the An/Ox time ratio on TP removal was significant. Shorter anaerobic time would result in insufficient phosphorus release and greater time would result in inactive PAOs. The appropriate An/Ox time ratio was suggested as 1/2. Comparisons of the phosphorus removal characteristics between biofilm and suspended growth under the same growth conditions are discussed in detail.

Keywords Biofilm; sequential batch biofilter reactor; phosphorus release; phosphorus uptake; PHA accumulation

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Introduction

Phosphorus is an essential element to all organisms. However, the eutrophication of water resources is always consequent to the excess phosphate introduced by human activities. Conventional biological activated sludge system treatment accomplishes phosphorus removal by using enhanced biological phosphorus removal systems (EBPRs). Many researches have shown that biological phosphorus removal in EBPRs occurs via the exposure of microorganisms to alternating anaerobic and aerobic conditions. Under the anaerobic phase, poly-P accumulating organisms (PAOs) incorporate large amounts of short chain fatty acids (SCFA) and store them mainly as poly-b-hydroxybutyrate (PHB) while the phosphorus contained in the biomass is released as soluble phosphate. During the aerobic phase, PAOs degrade the intracellular organic matter while phosphate is taken up to replenish their internal poly-P-storage.

Compared with a suspended growth system, phosphorus removal in biofilm has some advantages, including: (1) avoiding the sludge bulking problem; (2) economizing on treatment plane space; (3) avoiding secondary phosphorus release in a clarifier; (4) a lower production of waste sludge and (5) a higher phosphorus content of biomass. The fourth and fifth terms will be verified in later discussion.

The biofilm and suspended growth operate by a similar principle regarding biological phosphorus removal. Gonzalez-Martinez and Wilderer (1991) operated a single stage batch fixed film bioreactor that was periodically filled and drained with anaerobic/aerobic conditions provided in cycles for phosphorus removal and defined the system as the

Sequential Batch Biofilm Reactor (SBBR). Goncalves and Rogalla (1992) operated a dual fixed-film system consisting of an anaerobic biofilter followed by an aerobic biofilter operated in series. This study investigated the effect of organic loading on phosphorus release and reported that organic loading in the anaerobic phase would be a key condition for phosphorus release. Continuing these studies, Goncalves *et al*. (1994) tested a modified pilot-scale system consisting of five identical upflow floating biofilters. Of the five biofilters, one would be temporarily under anaerobic conditions. The five biofilters would be anaerobic by turns. The system could accomplish not only P removal but also total nitrogen removal.

Morgenroth and Wilderer (1998,1999) have investigated the features of phosphorus removal using a SBBR system. They evaluated the SBBR using mathematical models and obtained some important information. They indicated that the major limiting process for SBBR is the efficient removal of phosphorus rich biomass from the reactor and mass transfer of DO and that the substrate is only a minor effect. Furthermore, they also investigated the influence of influent COD and biofilm thickness on phosphorus removal using a modified IAWQ Model No. 2.

Many studies have focused on the development of processes using a series of biofilters. However, little research has been devoted to the biofilm characteristics, including polyphosphate in biofilm, PHAs accumulation and carbon source transformation. On the basis of Wilderer's models, this study further investigated phosphate incorporation and intercellular carbon storage and utilization in a biofilm system using a SBBR. The objective of this research is to unveil a useful biofilm characteristic to lead to the development of more effective biofilter processes for phosphorus removal. So, performance of this system, effect of cycle duration, effect of anaerobic/aerobic time ratio, and comparison of the P-removal feature between biofilm and suspended growth will be the four main points of the discussion.

Material and methods

Biofilter pilot plant

In this study, a phosphorus removal biofilm was grown in a lab-scale submerged biofilter, SBBR. Alternation between anaerobic and aerobic conditions was used to obtain an enriched culture of PAOs. The biofilter was packed with ceramic balls with a 0.5–0.8 cm diameter. The biofilter diameter was 15 cm and the packing height was 1.0 metre. The porosity of the packing was 0.4. Synthetic wastewater with a COD of 300mg/L, NH_3-N of 30mg/L, PO_4^{3-}-P of 5 mg/L and essential trace elements was supplied by using batch feeding. The pollutant strength was roughly the same as that of domestic wastewater in Taipei. In the aerobic phase the biofilter was aerated to maintain a DO concentration of 5.0mg/L.

The reactor was operated under the fill-and-drain procedure with 4 phases: Filling (2 minutes), anaerobic phase, aerobic phase and draining (2 minutes). The typical operating sequence for this reactor is shown in Figure 1. Various ratios of anaerobic/aerobic (An/Ox) time were carried out. In order to obtain homogeneous wastewater in the entire reactor, mixing was provided by a circulating pump at a rate of 350 mL/min. During the anaerobic and aerobic phase, the test solution pH was controlled at 7.0–7.2. During the aerobic phase, oxygen was provided with fine-bubble aeration and dissoluble oxygen was maintained at 5.0 mg/L.

Experimental design and analysis

The biofilter was operated at different HRTs (also cycle durations) of 4, 6 and 8 hours. An/Ox time ratios were 1/1, 1/2 and 1/3 every HRT run. In the SBBR system, the biofilm was developed until it reached a steady state. Generally, a time span of one month was nec-

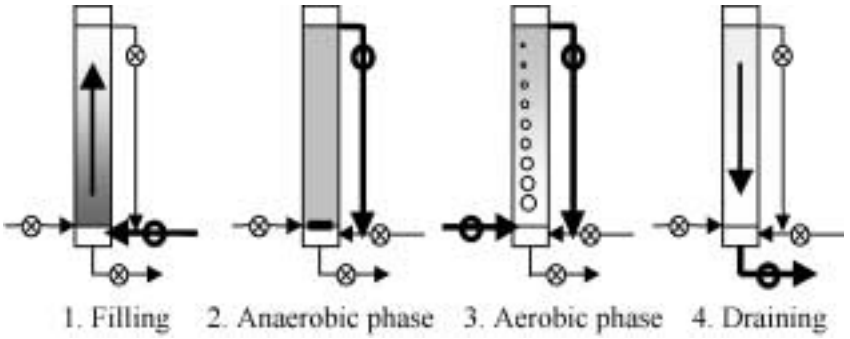

Figure 1 Operating sequence for the SBBR

essary to reach a steady state. Daily observations of the effluent quality (including COD, NH_3-N, NO_3^--N and phosphate concentrations) were made during the experimental period. In order to avoid biofilter clogging and remove the biomass containing excess phosphorus, a backwashing was necessary. Backwashing was executed at the end of the aerobic phase once a day. The waste sludge characteristics of the backwashing were measured, including quantity of waste sludge and the phosphorus content in the waste sludge. In general, the biofilter system could obtain a stable amount of biomass using a controlled headloss. When the effluent was stable, stability data was collected from the HRT run.

PHAs were determined by analysis of Poly-b-hydroxybutyrate (PHB) and Poly-b-hydroxyvalerate (PHV). Biofilm samples were at first centrifuged and lyophilized. PHAs of lyophilized biofilm samples were digested, methylated and chloroform extracted according to Brandl *et al*. (1988) and Comeau *et al*. (1988). Aliquots of 10 mg were extracted with acidified chloroform/methanol and the chloroform phase was washed by water re-extration. The composition of extracted methyl ester was assayed using a gas chromatograph (HP 6890) equipped with a capillary column (30 m*0.25 mm, HP-WAX), flame ionization detector and nitrogen carrier gas. 2 μL of the extract was injected and analyzed before split injection (split ratio, 1/20). The temperature of the injector and detector as well as temperature program used for separating the esters were the same as in Brandl *et al*. (1988). COD, orthophosphate, NH_3-N, NO_3^--N and suspended solid (SS) were determined according to standard procedures (refer to *Standard Methods*, APHA, 1995).

Results and discussion

Performance of the biofilter

After the biofilm was stable, the phosphate release under anaerobic conditions and phosphate uptake under the aerobic conditions would be significant. For instance, Figure 2 shows the variation in phosphate, COD and PHAs over the cycle time. In this typical run, HRT was 6 hours, including 2 hours of anaerobic conditions and 4 hours of aerobic conditions. More than 90% of the organic matter was eliminated and the soluble COD (sCOD) was under 50 mg/L after one hour. The addition of wastewater would result in carbon uptake, storage and simultaneous phosphorus release. The COD elimination mechanism included both physical adsorption and biological transformation. The COD biological transformation mechanism could be clearly shown in the accumulation of PHAs. In the accumulated PHAs, PHB was the major constituent and PHV was the minor constituent. PHAs accumulation would be rapid in the initial anaerobic conditions, while biofilm poly-P would be released into the bulk solution. Because the eliminated organic substrate mechanism included both physical adsorption and biological transformation in this biofilm system, the ratio of organic substrate decrease and phosphorus release was not clearly

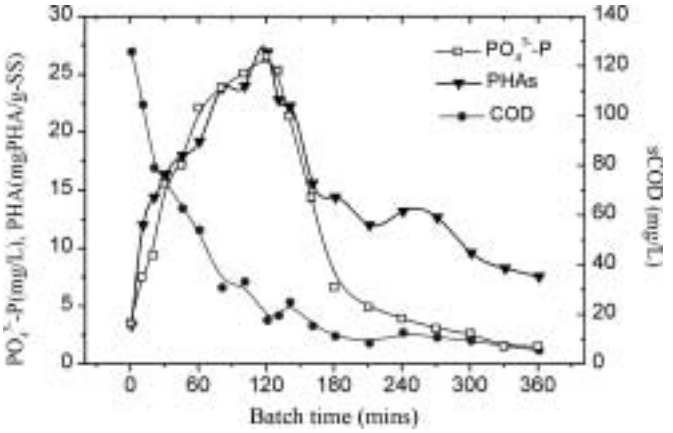

Figure 2 PO_4-P, PHA and sCOD concentration profiles through batch time with HRT

established. In the later anaerobic conditions, PHA accumulation and poly-P release would tend to be slow because the residual complex COD hydrolysis into SCFA would be difficult. Aeration was supplied in order to incorporate the phosphate. In the initial aerobic condition, biofilm PHAs would be rapidly utilized while the phosphate in the bulk solution would be rapidly incorporated. PHAs utilization and phosphate uptake would simultaneously tend to relax. Because the synthetic wastewater contained 45mg/L TKN, nitrification would occur in the late aerobic phase. Nitrification and low residual phosphate might influence the phosphate uptake. The above result shows that PAOs resulting in phosphorus release and uptake should exist in the SBBR system and the phosphorus removal mechanism was established.

The effect of cycle duration on phosphorus release and uptake and PHA accumulation

The cycle duration would determine the TP removal efficiency. The cycle duration effect involved the retention time and the carbon source supply. Figure 3(a) shows the variation in phosphate in the bulk solution over the cycle duration with the same anaerobic time of 2 hours. It seems that the phosphorus release might not show a significant difference when there was enough anaerobic time. However, the aerobic time would be insufficient for phosphorus uptake when the HRT was 4 hours with the aerobic time of 2 hours, as shown in Figure 3(a). The aerobic phosphorus uptake would be fast in the initial aerobic phase. However, the rate of phosphorus uptake would trend down when the residual phosphate was under 5 mg-P/L. This phenomenon seems to result from nitrification. So, it was necessary to extend the aerobic time to attain lower residual phosphate in bulk solution.

Figure 3(b) shows the variation in phosphate level with the same An/Ox time ratio of 1/2 in three cycles. Because there was not enough retention time for phosphorus release with a CD of 4 hours, the phosphate would not reach a saturated released phosphate concentration. In addition to phosphorus release, PHAs storage would be insufficient. This phenomenon would result in inactive PAOs that performed a slower phosphate uptake rate. When the An/Ox time ratio was 1/2 in the CD of 6 and 8 hours, the anaerobic retention time was sufficient for phosphorus release and the phosphate in the bulk solution could reach about 27 mg-P/L. In the last anaerobic phase, the phosphorus release would tend to stabilize. Although the biofilm still contained poly-P, insufficient SCFA would restrict the accumulation of PHAs and phosphorus release. Because of the mass transfer effect, the complex organic matter hydrolysis into the SCFA in the biofilm system would always be more incomplete than the suspended growth, although there was internal pump circulation. This

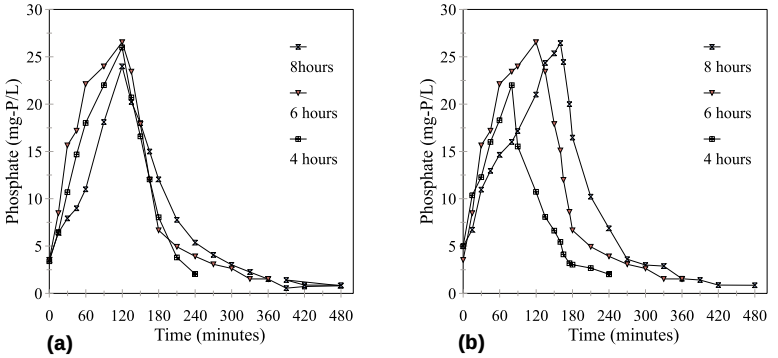

Figure 3 The profiles of phosphate level of bulk solution over cycle duration in different HRTs with the same anaerobic time of two hours (a), and with the same An/Ox time ratio of 1:2 (b)

would influence the saturated released phosphate level and phosphate uptake rate. Although it was difficult to establish the relationship between COD decay and phosphorus release in the anaerobic phase, the PHA content in the biofilm was closely related to the phosphorus release. The ratios of accumulated PHAs and released P could indicate the PAO growth condition in the anaerobic conditions. The PHAs/P ratios were between 0.8–1.2. The PHAs/P ratio should be maintained in the stable range to acquire a better P removal efficiency. Enough aerobic retention time was necessary for low residual phosphate in bulk solution and it was sufficient with a HRT of 6 to 8 hours.

The influence of simple carbon source on phosphorus release

As discussed above, because of the mass transfer and diffusion restriction, the organic substrate hydrolysis in the biofilm system was worse than the suspended growth. In batch experiments, acetate with 300 mg/L of COD was fed to release phosphorus sufficiently in the anaerobic phase. After draining and washing twice, the biofilm stored sufficient PHAs and was then filled with only $KH_2PO_{4(aq)}$ with PO_4^{3-}-P of 30 mg/L and aerated. Adding acetate could avoid the hydrolysis effect and benefit PHB accumulation. The control groups used milk as the carbon source in the anaerobic phase and $KH_2PO_{4(aq)}$ spiked NO_3^--N (30 mg-N/L) in the aerobic phase. All conditions were the same between the experimental and control groups except for the substrate type.

The PAO activity could be indicated by phosphorus release and uptake rates. A specific phosphorus release rate (SPRR) indicates the released phosphorus per gram of biomass per hour. This unit is mg-P/g-biomass.hr. Similarly, a specific phosphorus uptake rate (SPUR) indicates the incorporated phosphorus per gram of biomass per hour. Its unit is the same as above. A comparison of characteristics of biofilm between the experimental and control groups is shown in Table 1. Acetate is a simple substrate that is directly assembled for PHB by PAOs. SPRRmax always appeared in the initial 60 minutes. The SPRRmax in the experimental groups were always faster than those in the control groups. A faster SPRR should indicate more active PAOs.

Because specific P-released mass (SPRM) would always reach saturated conditions due to sufficient phosphorus release, the SPRMs of the experimental groups were still greater than that in the control groups. The saturated SPRMs of the experimental groups were about 15.0 mg-P/g-biomass and those of the control groups were about 8.0 mg-P/g-biomass. The PHB content of the experimental biofilm groups was still greater than that in the control groups. It seems that the increase in SPRM and PHA accumulation have the same tendency towards utilizing a simple carbon source. In the experimental groups, the larger proportion of PHAs was PHB. Because acetate was the carbon source in the experimental

Table 1 Comparison of the biofilm characteristics between the simple and complex carbon sources

HRT	4 hours		6 hours		8 hours	
experiment and control	Acetate	Milk	Acetate	Milk	Acetate	Milk
SPRRmax[a] (mg-P/g-biomass.hr)	17.50	11.07	13.89	10.60	11.96	7.00
SPRM (mg-P/g-biomass)	15.59	7.65	13.87	8.56	14.27	8.42
SPURmax (mg-P/g-biomass.hr)	7.52	5.22	7.01	5.07	6.22	4.86
PHB content of biofilm[b] (mg-PHAs/g-biomass)	35	21	41	27	38	24
The percent of PHB in PHAs (%)	92.0	71.3	88.2	75.4	88.1	71.4

[a]SPRRmax indicates the maximum SPRR during the anaerobic phase
[b]It indicates the final level of PHB content in the anaerobic phase

groups, acetyl-CoA could be formed directly from acetate. Two acetate-CoAs were then condensed into acetoacetyl-CoA. Acetoacetyl-CoA was reduced to form b-hydroxy-butyryl-CoA, which is a monomer of PHB. Acetate could therefore be assembled rapidly to PHB.

The higher specific P release rate would ensure that the biofilm had a higher specific P uptake rate. The phosphate uptake restrictions are insufficient phosphorus release, insufficient PHA accumulation, oxygen mass transfer limitation, more residual organic matter and nitrification, and so on. The SPURmax in the experimental groups were always faster than those in the control groups. The major factor resulting in the greater SPURmax of the experimental groups should be sufficient phosphorus release and the minor factors should be low residual COD and no NO_3^--N effect.

The effect of An/Ox time ratios on phosphorus release and uptake and PHA accumulation

The An/Ox time ratio in cycle duration influences the PAOs activity, as shown in rates of phosphate release and uptake. Figure 4 shows the phosphate level in the bulk solution and Figure 5 shows the PHA and phosphorus content of the biofilm at various An/Ox time ratios in a 6 hours cycle duration. The An/Ox time ratios were 1:1, 1:2 and 1:3. The corresponding anaerobic times were 180 min, 120 min and 90 min. An appropriate An/Ox time ratio would benefit TP removal. However, the TP potential removal might be depend on the phosphorus release, and a longer anaerobic time would not ensure higher released phosphorus. The fastest phosphorus release rate and maximum released phosphate level occurred in the 1:2 ratio, as shown in Figure 4. It seems that the PAO activity in the 1:2 ratio may be the maximum in three ratios. Although there was enough anaerobic time for phosphorus release to reach a high level in the 1:1 ratio, phosphorus release rate and released phosphate level in this 1:1 ratio would be still lower than in the 1:2 ratio. The excessive anaerobic time might lead to a decrease in the PAO activity. The decrease in the PAO activity seems to result from insufficient aerobic time for phosphorus uptake in the 1:1 ratio. When anaerobic time decreased to 90 minutes (An:Ox = 1:3), TP removal mechanism of PAOs was abnormal due to insufficient phosphorus release. Compared with the other two ratios, the phosphorus release and uptake rates would become slower and the TP removal would be lower. Figure 5 shows the phosphorus and PHA content of the biofilm. Both phosphorus and PHA are important intracellular energy materials. The ultimate anaerobic P content of the biofilm seems to be similar in the three ratios, which was about 1.8%. The ultimate aerobic PHAs content of the biofilm was also similar in the three ratios. However, the ultimate anaerobic PHA content and the ultimate aerobic P content of the biofilm in a time ratio of 1/2 would be

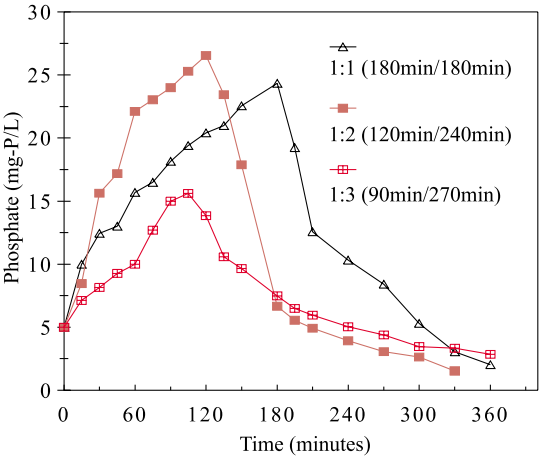

Figure 4 The phosphate level in the bulk solution at different An/Ox time ratios

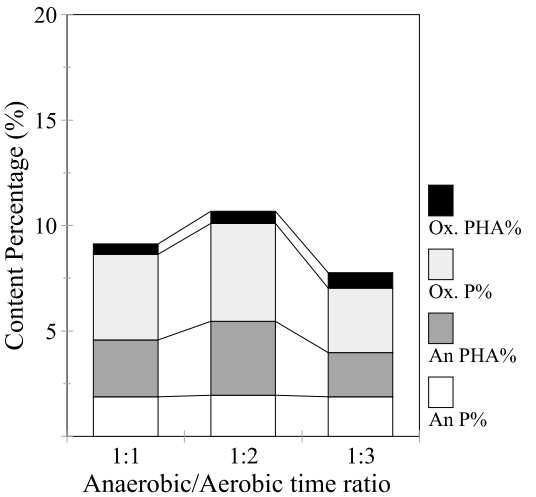

Figure 5 The PHA and P content of the biofilm at different An/Ox time ratios

the highest in the three time ratios. When PAOs with high activity exist in the system, they could accumulate a large amount of SCFA for the PHAs and release high phosphorus into the bulk solution. This result demonstrates again that accumulation of PHAs is closely related to phosphorus release and TP removal (see Table 2).

The relationship between phosphorus release and uptake per PHA and An/Ox time ratio is shown in Figure 6. It appears that P-release level per PHA accumulation (PR/PHA) would be closely related to the An/Ox time ratio. In other words, PR/PHA would determine the PAO activity and TP removal rate. The PR/PHA of An/Ox time ratio with 1:2 was the highest of the three ratios. The TP removal for this ratio was also the highest. However, the P-uptake level per PHA utilization (PU/PHA) would be independent of the An/Ox time ratio. This result shows that PU/PHAs would be at a fixed level of about 0.95. The fixed PU/PHA shows that the PAO activity would depend upon the P-release but not the P-uptake. An appropriate An/Ox time ratio would result in an increase in SPRR and PR/PHA, and the suggested ratio is 1/2.

Table 2 The comparison of the biofilm features at various An/Ox time ratios

An/Ox time ratio	1:1	1:2	1:3
An:Ox time (minutes)	180:180	120:240	90:270
$SPRR_{30}$ (mg-P/g-biofilm.hr)	4.8	6.9	2.6
SPUR (mg-P/g-biofilm.hr)	4.6	6.4	2.3
PHB of An. biofilm[(b)] (%)	2.7	3.5	2.1
P content of Ox. biofilm (%)	4.06	4.65	3.05
TP removal rate (%)	75.6	81.9	66.37

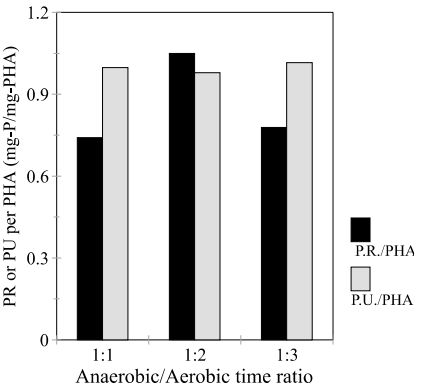

Figure 6 The P release and uptake level per PHA at various An/Ox time ratios

Comparison of phosphorus removal characteristics between biofilm and suspended growth

Previous research (Goncalves *et al*., 1994) has shown the advantages of using biofilm for phosphorus removal. These advantages include low waste sludge and high P content of biofilm. In this study, sludge features in the SBBR contrasted with the suspended growth (Anaerobic-anoxic-aerobic process, A_2O). The A_2O pilot plane has run for four years in the same lab. Table 3 shows the comparisons between suspended growth and biofilm. Both systems were grown under the same conditions. In run1 and run2, the substrate loading and removal efficiency were similar in both systems. The SBBR could dispose more wastewater because its HRT was shorter than A_2O. The observed yield coefficients (Y_{obs}) indicated that the SBBR biomass production was less than A_2O. Therefore, dry waste sludge production of the biofilm would decrease by about 30–40%, compared with suspended growth. In the SBBR, the efficiency of phosphorus uptake would be enhanced. Although there was only 5mg-P/L of influent, Poly-P content of sludge could reach to 4.2% or above. These results show the low waste sludge and high P content of waste sludge in the SBBR. From the production of dry waste sludge and P content of waste sludge, the mass balance of phosphorus would coincide in both systems.

Conclusions

The principle behind TP removal in a biofilm system is similar to that in a suspended growth system. This research investigated the biofilm features that could accumulate excess poly-P. There are many advantages using a biofilm system such as a SBBR, i.e., low sludge waste and high phosphorus content of biomass. These advantages have been established in this study. Among the factors of TP removal efficiency, both the released phosphorus rate and PHA accumulation are important. Both factors may affect one another. The important challenge in promoting TP removal efficiency is how to increase the SPRR and PHA accumulation in the biofilm system.

Table 3 Comparison of phosphorus removal performance between A_2O and SBBR

Process		MCRT	HRT	F/M	Y_{obs}	P content of waste sludge	TP removal	COD removal
		days	hours	kgCOD/ kg-SS.d		%	%	%
Run 1	A_2O	10	10	0.39±0.04	0.30	3.8	86.9	95.0
	SBBR	12.98	4	0.30±0.05	0.25	4.21	79.2	94.3
Run 2	A_2O	15	10	0.24±0.02	0.28	3.2	82.5	95.0
	SBBR	16.23	6	0.22±0.03	0.24	4.77	81.9	95.2

The exact HRT design and An/Ox time ratio would benefit the SPRR increase and PHA accumulation. Although the greater amount of wastewater could be disposed in a shorter HRT, insufficient released phosphorus resulting from insufficient anaerobic time would create a decline in the PAO activity. In general, an appropriate An/Ox time ratio is suggested as 1/2 and HRT is over 6 hours.

Mass transfer and hydrolysis are always the limitation step in the biofilm treatment processes. The substrate hydrolysis level influences the production of SCFAs, which are important components of PHAs. Simple substrates, like acetate, may be spiked into the wastewater to increase PHA formation. In the anaerobic phase, mixing was only due to internal circulation, the efficiency of mass transfer was therefore not always enough. The biofilm phosphorus removal process has many obstacles to overcome. The effect of mass transfer on PHA accumulation and phosphorus release is worth further research.

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