Effects of Solid Retention Time (SRT) on Sludge Characteristics in Enhanced Biological Phosphorus Removal (EBPR) Reactor

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This study investigated the effects of solid retention time (SRT) on sludge characteristics and operational performance in enhanced biological phosphorus removal (EBPR) reactor. The results showed that the reactor operated at SRT of τ = 8.3 d could achieve phosphate removal efficiency η > 90 % and SVI < 100 mL g^{-1}. In comparison, increasing SRT to τ = 16.6 d led to a decrease of phosphate removal (η < 85 %) and an increase of SVI value (160 mL g^{-1}), implying a performance degradation and worse settleability of the sludge. In both cases, chemical oxygen demand (COD) removal was observed stable in terms of overall efficiency of η = 90 %. Denaturing gradient gel electrophoresis (DGGE) profiles of sludge revealed a good phylogenetic relationship of sludge with different SRTs. However, the predominant population of community appeared to vary. Especially, the appearance of filamentous microorganisms at longer SRT may be responsible for worse performance and sludge settleability of EBPR system.

Key words:
EBPR, SRT, microbial community, filamentous microorganisms

Introduction

The solids retention time (SRT) is believed to be a significant operational parameter in enhanced biological phosphorus removal (EBPR) system. However, the relationship between SRT and phosphorus removal has not been sufficiently investigated in previously reported studies. It was demonstrated that the increase of SRT could lead to the decrease of biomass yield and excess sludge discharged, which reduced the P removed by discharging excess sludge.[1] Nevertheless, it has been addressed that phosphate-accumulating organisms (PAOs) take predominant roles in EBPR systems at a long SRT.[2,3] Whang *et al.*[4] found that the effects of SRT on EBPR performances largely depended on operating temperature. Additionally, Barnard[5] observed that SRT played an insignificant role during phosphorus removal. In summary, there was still a lot of apparent contradiction about the affect of SRT on performance.

At present, a great number of studies have been carried out regarding the effects of SRT on mechanisms for phosphorus removal in EBPR system. For example, Seviour *et al.*[6] reported that glycogen-accumulating organisms (GAOs) could successfully compete with PAOs at a long SRT, which resulted in the decrease of phosphorus removal in EBPR system.

In fact, another concern, i.e. the effects of long SRT on sludge bulking are rarely found. During the past decades, sludge bulking phenomena have been widely reported in biological nutrient removal process in wastewater treatment plants (WWTP).[7] It has been known that when EBPR was introduced into an activated sludge system, the problems related to worse sludge settling property might generally be encountered, which was indicated by an increase of SVI value.[8] SRT is considered a crucial parameter to control the settleability of activated sludge for its correlation to growth rate of filamentous microorganisms.[9,10] The kinetic selection theory is often used as an engineering method for control of filaments bulking in biological wastewater systems.[11] This theory is based on the Monod expression for microbial growth:

$$\mu_s = \mu_{s,\max} \frac{\gamma_s}{K_s + \gamma_s} \quad (1)$$

According to this theory, both SRT and organic loading rate are important factors affecting the settleability of activated sludge.[9] Filamentous bacteria are slowly growing organisms that can be characterized as lower maximum specific growth rates (μ_{max}) and affinity constant (K_s) than non-filamen-

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tous organisms. Therefore, filamentous bacteria fail to take dominance due to the inherent biases of long generation time under short SRT and high organic loading. Fig. 1 showed the competition of substrate between zoogloea and filamentous bacteria. Since filamentous bacteria have a high surface area-to-volume ratio (*A/V*) providing a high substrate uptake rate at low concentrations. If substrate concentration is below γ_s^{crit}, filamentous bacteria will take dominance.[12,13] Long SRT is responsible for the growing of microorganisms with slow specific growing rate such as filamentous bacteria, consequently, changing the microbial community structures and affecting the settleability of activated sludge. However, there has been no previous report of the dependence of phosphorus removal and bulking sludge on SRT in EBPR system.

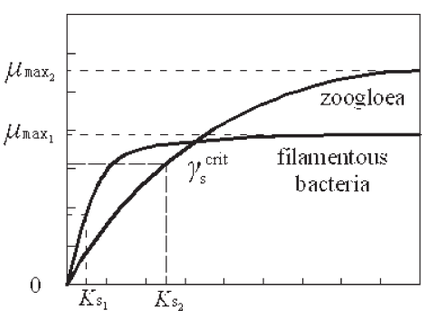


Fig. 1 – *Schematic of growth and competition between zoogloea and filamentous bacteria*

This study mainly deals with the relationships between the SRT and microbial community structure in the EBPR, aiming to examine the influences of SRT on phosphorus removal, COD removal and filamentous bulking.

Materials and methods

Sequencing batch reactors

The experiments were performed in two sequencing batch reactors (SBRs) with the effective volume of 2 L in each reactor (23 ± 1 °C) and the pH was controlled at 7.0. Strainer screen was equipped on each reactor to avoid sludge loss in effluent. Seed sludge was obtained from Taiping wastewater treatment plant operated stably with high efficiency of biological phosphorus removal. Mixed liquors with the volumes of 60 mL and 30 mL were taken out from SBR1 and SBR2 per cycle respectively. Since 4 cycles were performed everyday, the SRTs for the two reactors were 8.3 (SBR1) and 16.6 days (SBR2). The reactors were operated continuously in time interval of 6 h according to the phases described in Table 1. Volumes of 1 L synthetic wastewater were fed into the reactor during the first 8 min of the anaerobic stage. Nitrogen gas was bubbled into the SBRs during the anaerobic phase at a gas flow rate of Q_v = 0.2 L min^{-1} to keep the anaerobic condition. A gas flow rate of Q_v = 0.2 L min^{-1} air was bubbled during the aerobic stage. The SBR was mixed constantly with a magnetic stirrer (250 rpm) during the anaerobic and aerobic phases. At the end of the cycle 1 L of effluent was pumped out of the reactors, resulting in a volumetric exchange ratio (VER) of 0.5 and a hydraulic residence time (HRT) of 12 h.

Table 1 – *Operational stages and phases of the SBR reactor*

t/min	Operation
0–8	Fill
0–90	Anaerobic mix
90–330	Aerobic mix: DO > 2.0 mg L^{-1} O_2
330–332	Mix and pump mixed liquid
332–345	Settle
345–355	Draw
355–360	Idle

Mediums

The synthetic wastewater used as influent contained $CH_3COONa \cdot 3H_2O$ of 4.69 mmol L^{-1} (300 mg L^{-1} COD), NH_4Cl 1.79 mmol L^{-1} (25 mg L^{-1} N). $NaH_2PO_4 \cdot 2H_2O$ 0.32 mmol L^{-1} (10 mg L^{-1} P), and 0.5 mL L^{-1} influent of the following trace solution: EDTA 100 mg L^{-1}, $MgSO_4 \cdot 7H_2O$ 20.6 g L^{-1}, $CaCl_2$ 5.6 g L^{-1}, $MnSO_4 \cdot H_2O$ 0.14 g L^{-1}, $FeSO_4 \cdot 7H_2O$ 5.7 g L^{-1}, $CuCl_2 \cdot 2H_2O$ 0.19 mg L^{-1}, $ZnCl_2$ 0.05 g L^{-1}, H_3BO_3 0.05 g L^{-1}.

Analyses

COD, mixed liquor suspended solids (MLSS) and mixed volatile suspended solids (MLVSS) were determined according to standard methods for the examination of water and wastewater.[14]

The PO_4^{3-}, NO_3^- and NO_2^- concentrations of the supernatant were measured by ion chromatography (DIONEX ICS-3000) with an Ionpac AS11-HC column. High-purity helium was used as the carrier gas at a flow rate of Q_v = 1.2 mL min^{-1}.

SRT was determined taking into account the excess sludge and the suspended solids in the effluent, and the value was calculated according to $\tau = V/4Q$, with V the liquid volume of reactor; Q the volume of mixed liquid discharged from reactor

after aerobic stage per cycle; the cycle number is 4 per day. SVI was measured in an unstirred cylinder of 100 mL and periodically doubled checked by measuring the height of the sludge blanket directly in the reactor, after 30 min of settling. Sludge taken from the SBR at the end of aerobic stage was examined using a scanning electron microscope (SEM) (Hitachi S-4700). Samples were fixed in 2.5 % paraformaldehyde and 1.5 % glutaraldehyde in buffer (0.1 mol L^{-1} cacodylate, pH 7.4) overnight at 4 °C. And then samples were washed three times in buffer, dehydrated stepwise in a graded series of water/ethanol solutions (φ = 25, 50, 70, 85, 95, 100 %), and were dried (critical-point carbon dioxide). Samples were then sputter coated with Pt prior to SEM observation.

Sludge samples were collected from two reactors operated under different SRTs at day 58. Total DNA extraction and PCR amplification were profiled according to methods previously used.[15] Denaturing gradient gel electrophoresis (DGGE) was performed using a Bio-Rad Dcode Universal Mutation Detection System (Bio- Rad, Hercules, CA, USA). The denaturing gradient was ranged from 30 % to 55 %. Samples of PCR product were loaded on gels, which were then run for 6 h at 60 °C (150 V). The polyacrylamide gels were strained using silver before bands were observed by flatbed scanner (UMAX PowerLook 1000, China). DNA sequencing was carried out by ITT Biotech-Bioservice (Bielefeld, Germany). Sequence homology searches were conducted using the GenBank nucleotide sequence library and the BLAST program through the National Center for Biotechnology Information (NCBI) internet site (http://www.ncbi.nlm.nih.gov/BLAST).

Results and discussion

The effects of SRT on performance of EBPR reactor

Fig. 2 showed the operational performance of EBPR reactors at different SRT. SRT had a great influence on the lag time that the reactors needed to attain an efficient COD and phosphorus removal. The lag time was approximately 20 d (τ = 8.3 d, Fig. 2a) for SBR1 and 40 d for SBR2 (τ = 16.6 d, Fig. 2b), indicating that long SRT was not feasible for fast start-up of EBPR system. The concentrations of phosphate in effluent were observed $\gamma < 1$ mg L^{-1} and $\gamma <$ 2 mg L^{-1} for SBR1 and SBR2, respectively (Fig. 2). Therefore, a higher phosphorus removal rate was obtained in SBR1 (91 ± 2 %, n = 10) than that in SBR2 (85 ± 2 %, n = 10). However, the phosphate concentration in SBR2 was inversely higher than SBR1 at the end of anaerobic phase (Fig. 3).

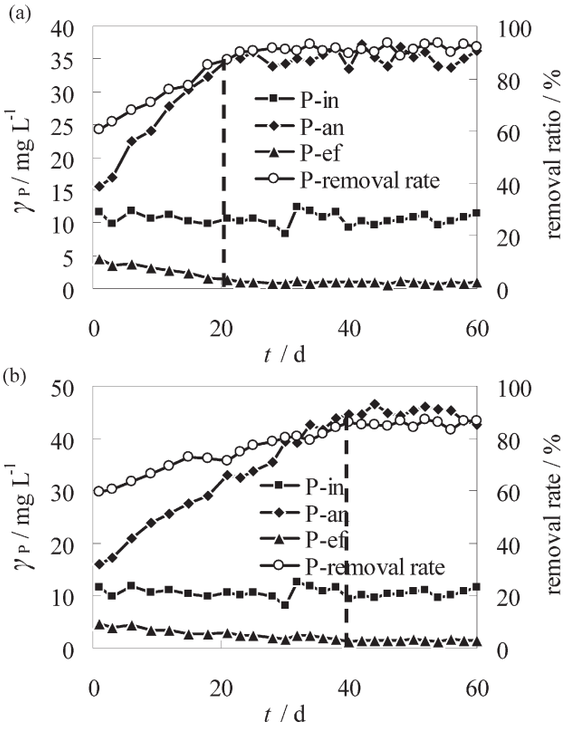


Fig. 2 – *Phosphate removal efficiency (P-re), phosphate concentration of influent (P-in), the end of anaerobic phase (P-an) and effluent (P-ef) in the SBR system for operated SRT of 8.3 d (a) and 16.6 d (b)*

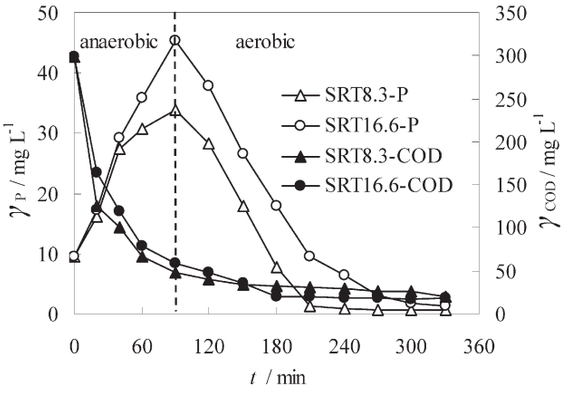


Fig. 3 – *Variation of COD and phosphate concentration in a typical cycle under SRTs of 8.3 d and 16.6 d*

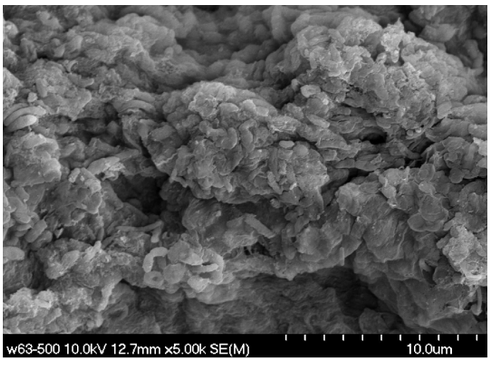
Normally, a great amount of phosphate is released in the anaerobic phased, indicating a predominance of PAOs, which will result in a good phosphate removal rate. However, when a SBR system was operated with a long SRT (e.g. SBR2), high phosphate concentration found at the end of anaerobic phase. As showed in Fig. 3, SBR2 exhibited a larger P-release ability accompanied with a lower COD consumption rate, especially at the end

of anaerobic phase, indicating that secondary P-release had taken place in SBR2. The secondary P-release could due to endogenous respiration of heterotrophic bacteria including PAOs.[8] Secondary P-release anaerobically does not lead to PHA production, and thus, aerobically, PAOs would not have more energy for P uptake as compared to the low SRT case.

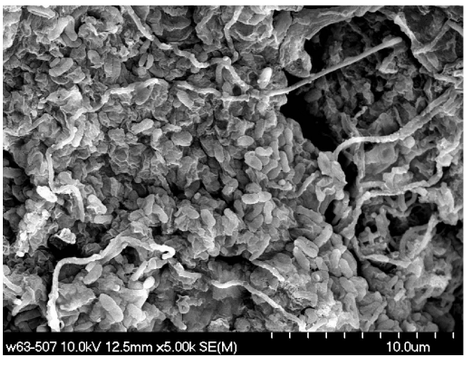
COD concentration in the effluent in SBR1 was found to be slightly higher than that in SBR2, whereas the COD removed in the anaerobic phase in SBR1 was greater than SBR2. Meanwhile, the distinction seemed to be not significant and the overall COD removal efficiencies in both reactors tended to be as high as η = 90 % after the lagging stage.

The effects of SRT on sludge characteristics

The SEM images of sludge samples taken from SBR1 and SBR2 confirmed the theoretical analysis of bulking. Short bacillus were the dominant microorganisms in both samples (Fig. 4), while filamentous microorganisms in SBR2 can be seen clearly in Fig. 4b.



(a)



(b)

Fig. 4 – *Observation of the microbial morphology of activated sludge for SRT of (a) 8.3 days and (b) 16.6 days*

The EBPR reactors operated at different SRTs (τ = 8.3 d and 16.6 d) were continuously monitored for a period of 60 days and the results were summarized in Table 2. Longer SRTs had a substantially negative impact on the settling properties of the sludge. After 30-day operation, the SVI in SBR2 increased to a value higher than 160 mL g^{-1} in contrast to that of < 100 mL g^{-1} in SBR1, suggesting the slight bulking in SBR2 with long SRT (16.6 d). The sludge concentration of SBR2 that was characterized by mixed liquor suspended solid (MLSS) was slightly higher than of SBR1 due to the slower rate of sludge wastage (Table 2). Derived from that, the organic loading of SBR2 was lower than SBR1 since similar feeding condition was provided for both of the reactors. The absence of substrate in SBR2 with organic loading of 0.21 kg COD kg^{-1} MLVSS d^{-1} was beneficial to the proliferation of filamentous bacteria. Since the filamentous bacteria have a higher *A/V* ratio than non-filamentous bacteria,[16] it is more facilitated for mass transfer to the cells when substrate was absent. Combined with discussion in context, the absence of substrate could lead to bacterial endogenous respiration, which further caused the secondary P-release in SBR2.

Table 2 – *Characteristics of sludge taken from reactors for different operated SRT*

	SBR1	SBR2
SRT, τ/d	8.3	16.6
MLSS, γ/g L^{-1}	2.64	3.29
MLVSS, γ/g L^{-1}	2.09	2.66
SVI/mL g^{-1}	89	160
organic loading, kg COD kg^{-1} MLVSS d^{-1}	0.30	0.21

The effects of SRT on microbial communities

In order to further understand the effects of SRT on the microbial community in the EBPR system, DGGE analysis was conducted. As shown in Fig. 5, the DGGE profiles revealed that sludge with different SRT had similar community structure. However, the predominant population of community was highly different. Two types of potential PAOs, band 10 and band 8, were observed in both systems. The pattern showed that *Gemmatimonas aurantiaca* (band 10)[17] decreased while *Tetrasphaera elongata* (band 8)[12] slight increased at longer SRT (16.6 d). However, SRT did not have a great affect on *Candidatus Competibacter phosphatis* clone

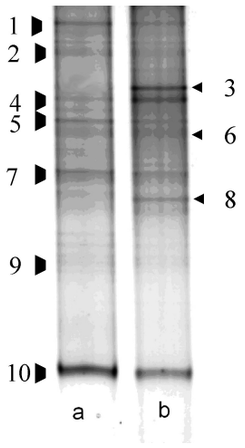


Fig. 5 – *DGGE profiles of sludge samples for SRT of (a) 8.3 days and (b) 16.6 days*

SBRQ22 (band 7),[18] which were considered as the GAOs. Especially, Type 0803 *filamentous bacterium* strain Ben05B (Band3)[19] which denoted to potential filamentous microorganism appeared in SBR2 (τ = 16.6 d).

While the use of acetate as a carbon source in EBPR systems has been often documented to yield efficient and stable P removal performance, there are also many reports related to the P removal deteriorated due to microbial competition of GAOs with PAOs and sludge bulking.[20–22] GAOs are the main competitors of PAOs in EBPR system since they can consume acetate and store intracellular polymers (glycogen) anaerobically. However, based on results shown in Fig. 3, the COD (particularly VFA), which had not been fully consumed anaerobically, was consecutively carry over to aerobic phase. Filamentous bacteria were able to survive in the system. So, in addition to GAOs, filamentous became another competitor of PAOs for substrates.

Conclusions

The results of this study provide an improved comprehension to the influence of SRTs on the sludge characteristics and performance of EBPR systems. Maintaining a shorter SRT (8.3 days) resulted in a good sludge settleability (SVI < 100 mL g^{-1}) and a higher phosphate removal efficiency (η > 90 %). In contrast, longer SRT (16.6 days) was shown to have a negative impact on the sludge settling property (SVI > 160 mL g^{-1}) and operation performance (phosphate removal efficiency η < 85 %). Phosphate concentration released in anaerobic phase in SBR2 was higher than that in SBR1, which was likely due to secondary P-release. In both cases, the system could stably degrade COD by 90 %. Similar communities were observed with different SRT using DGGE profiles. However, the predominant population of community was different, *i.e.* longer SRT favored the growths of filamentous bacteria.

Table 3 – *Alignment results of different bands in DGGE profile*

NO.	Most closely related bacterial sequence	Accession No.	Identify %
band 1	Uncultured bacterium clone SBRQ157	AF361092.1	96
band 2	Uncultured bacterium PHOS-HE28	AF314421.1	98
band 3	Type 0803 *filamentous bacterium* strain Ben05B	X86070.1	90
band 4	Uncultured bacterium FukuN108	AJ289984.1	94
band 5	*Dyella ginsengisoli* strain LA-4	EF191354.1	97
band 6	Uncultured actinobacterium clone GCP18	AF387313.1	98
band 7	*Candidatus Competibacter phosphatis* clone SBRQ22	AY172162.1	99
band 8	*Tetrasphaera elongata*	AB051430.1	98
band 9	Uncultured bacterium clone SBRQ191	AF361091.1	98
band 10	*Gemmatimonas aurantiaca*	AB072735.1	100

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List of symbols

A – surface area, m^2
μ_{max} – maximum specific growth rate, h^{-1}
K_s – affinity constant, mg L^{-1}
γ_s – substrate mass concentration, mg L^{-1}
γ_s^{crit} – critical substrate mass concentration, mg L^{-1}
V – liquid volume of reactor, m^3
Q – liquid volume of mixed liquid discharged from reactor after aerobic stage per cycle, $m^3\ d^{-1}$
Q_v – volume flow rate, L min^{-1}
η – removal efficiency, %
τ – solid retention time, d
φ – volume fraction, %

List of abbreviations

EBPR – enhanced biological phosphorus removal
PAOs – phosphate-accumulating organisms
GAOs – glycogen-accumulating organisms
DGGE – denaturing gradient gel electrophoresis
COD – chemical oxygen demand
WWTP – wastewater treatment plants
SVI – sludge volume index
SBRs – sequencing batch reactors
SRT – solid retention time
HRT – hydraulic retention time
VER – volumetric exchange ratio
MLSS – mixed liquid suspended solids
MLVSS – mixed volatile suspended solids
SEM – scanning electron microscope

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