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New Scenario for Enhancing Phosphorus Removal in SBR_S-AA and SBR_S-AO Systems during Winter Season

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ABSTRACT: This paper deals with new scenario for enhancing phosphorus removal in pilot plant SBRs (AA) and SBRs (AO) through improvement of PAOs and DNPAOs during winter season when temperature range from 8C to 10C .It is observed that the SBRs (AO) can get acceptable phosphorus removal efficiency (PRE) after 39 day of its operation. This acceptable phosphorus removal efficiency (PRE) can be achieved in the SBRs (AA) after 79 days. This means that the improvement of phosphorus accumulating organisms PAOs can be reached with short time period than that of denitrifying phosphorus accumulating organisms at winter Season. The experimental results showed that denitrifying phosphorus accumulating organisms DNPAOs can immediately get a good phosphorus removal during aerobic condition more than anoxic condition. Phosphorus accumulating organisms PAOs can get low phosphorus removal during anoxic condition. Therefore, there are two different Accumulibacter enriched in SBRs (AA) and SBRs (AO). Scanning electron micrograph (SEM) is used in this study whereas SEM analysis showed that Accumulibacter is prevailing in SBRs (AA) and SBRs (AO) whereas there is long-rod morphology. The scenario proposed in this study was confirmed to be effective in increasing the enrichment of Accumulibacter at winter season.

1 INTRODUCTION

As phosphorus is the limiting nutrient in algal blooms, phosphorus removal from wastewater, hence, has become an important need to protect public health and reduce ecological risk. In order to meet a stringent limit for phosphorus, enhanced biological phosphorus removal (EBPR) is generally regarded as an economical and environmentally friendly technology for the removal of phosphorus from wastewater due to its advantages relative to conventional chemical precipitation method such as ferric chloride and aluminium oxide. The discharge of nutrient materials (i.e., nitrogen and phosphorus) from wastewater to soil and waters may adversely affect water resources in some ways, especially potential contributions to eutrophication. Nitrogen and phosphorus polluted surface waters often need to be pretreated prior to use in drinking water systems, such as Tigers River, Euphrates River, and Shatt AL-Arab, Iraq. In EBPR process, a group of bacteria are generally enriched through sequential anaerobic-aerobic conditions, known as polyphosphate accumulating organisms (PAO) responsible for phosphorus removal. During the anaerobic period, PAO take up carbon sources, particularly volatile fatty acids (VFA) such as acetate and propionate, stored them as poly-β-hydroxyalkanoates (PHA), supplied with energy from the hydrolysis of polyphosphate (poly-P) (resulting in the release of phosphorus from the cells of PAO) and glycolysis of glycogen. In the subsequent aerobic period, PAO take up phosphorus in excess of the anaerobic release to store them as poly-P, usually called luxury phosphorus uptake, simultaneously accompanying the growth of biomass and the regeneration of glycogen, with the required energy from the oxidation of PHA stored in cells of PAO under the anaerobic condition [1, 2]. There is no information available on the running performance of EBPR system at even lower temperature such as around 10 C^o, often presenting in winter. Thus, a new strategy for obtaining microorganisms responsible for phosphorus removal (i.e., PAO and denitrifying phosphorus accumulating organisms at 8-11C^o was developed in this study. This proposed strategy was performed using two lab-scale EBPR reactors, which was operated under both anaerobic-aerobic and anaerobic-anoxic conditions.This results obtained from this study, linking running performance with microbial population, may serve as a new suggestion for the design and operation of EBPR system, especially during winter season.

2 MATERIAL AND METHODS

2.1 *Experimental reactor*

Two lab-scale sequencing batch reactors (SBR) with a working volume of 3.3 L (see Fig.1) were conducted for phosphorus removal, one operated with a sequence of anaerobic-aerobic conditions and anaerobic-anoxic. The cycle time consisted of a 0.5h filling period, a 2h anaerobic period, a 4h aerobic or anoxic period, a 1h settling period and a 0.5h decant period. In each cycle, 1.9 L of synthetic wastewater (composition detailed in Table.1) was fed to the reactor during the filling phase, resulting in a 13.9 h of hydraulic retention time (HRT) and an effluent of the same amount as influent (1.9 L) was discharged at the end of one cycle. In the AA reactor, sodium nitrate solution was pumped in the first 1 min of the anoxic period to provide anoxic condition. Volumes of 330 mL and 115 mL mixed liquor were removed per day from AO reactor and AA reactor, to maintain the solids retention time (SRT) at 10 and 20 days, respectively. Air was supplied at a flow rate of 1.5 L/min to maintain the dissolved oxygen (DO) at greater than 2 mg/L during the aerobic period. The pH in two reactors was maintained at 7.0±0.2, with the addition of 0.5 M HCl or 0.5 M NaOH when the pH was above or below this setpoint. Two reactors were operated at room temperature, ranging from 8℃ to 11 ℃. Two reactors responsible for the PAO and denitrifying phosphorus accumulating organisms' enrichment were operated for 80 days.

2.2 *Batch tests*

For the batch tests, 0.5 L of activated sludge was taken from both the AA and AO reactor at the end of aerobic and anoxic conditions at 80 days, and was immediately washed twice with the nutrient solution (see Table1) not containing the basic medium. The activated sludge treated from AA reactor responsible for anoxic phosphorus removal was divided into two parts and filled into two 1L test devices, One part was operated with an anaerobic-aerobic mode and the other in the form of anaerobic-anoxic condition.

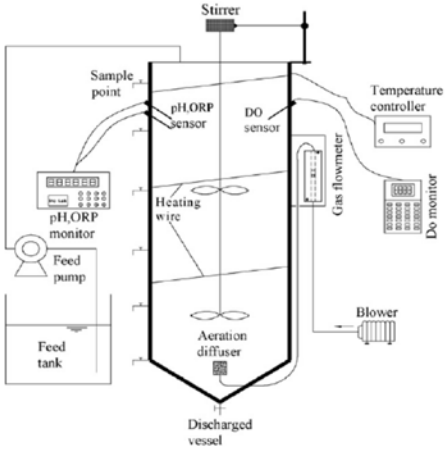


Fig.1 Schematic diagram of SBR system

Table 1. composition of inlet wastewater

Feeds	Weight g/L	nutrient	Weight g/L
CH_3COO	41.00	$Fecl_3 \cdot 6H_2O$	1.50
KH_2PO_4	7.04	H_3BO_3	0.15
$(NH_4)_2S$	18.84	$CuSO_4 \cdot 5H_2$	0.03
$Cacl_2$	0.85	KI	0.18
$MgSO_4 \cdot 7$	7.20	$Mncl_2 \cdot 4H_2$	0.12
Nutrient	0.60mL/L	$Na_2MoO_4 \cdot 2$	0.06
Notes: COD:P=20:1; pH:7.0±0.2.		$ZnSO_4 \cdot 7H_2$	0.12
		$Cocl_2 \cdot 6H_2O$	0.15
		EDTA	10.00

3 RESULTS

The EBPR performance of anaerobic-aerobic and anaerobic-anoxic systems was investigated throughout the enrichment to PAO and denitrifying phosphorus accumulating organisms during winter seasons.

3.1 *Performance of AA and AO reactor*

The phosphorus removal performance throughout the microorganisms acclimatization process in the both EBPR reactors, namely AA and AO proposed in this study, at low temperature (8~11C) over the 80 days' time period is shown in Fig.2. During the AO reactor operation under anaerobic-aerobic condition, a stable phosphorus removal performance was presented after 40 days, as given by the variation of phosphorus concentration in the effluent, while the AA reactor reached the similar stable-state phase after approximately 80 days running under anaerobic-anoxic condition. At the same operation parameters, each reactor responded differently throughout the PAO and denitrifying phosphorus accumulating organisms enrichment experiments, which denitrifying phosphorus accumulating organisms acclimatization to attain stable state required one times time more than PAO, indicating the higher activities of PAO at low temperature than denitrifying phosphorus accumulating organisms . At the stable-state phase, both the AA and AO reactors exhibited a good phosphorus removing performance and the effluent phosphorus concentrations were both less than 0.5 mg P/L. Consistently, the stable concentrations of MLSS, MLVSS, phosphorus release and uptake and the constant ratios of MLVSS to MLSS and phosphorus release to phosphorus uptake were also observed both in the AA and AO reactors.The amount of phosphorus stored in the microorganisms such as PAO and denitrifying phosphorus accumulating organisms can be generally implied according to the ratio of MLVSS to MLSS, and the lower the ratio, the greater amount of phosphorus may be stored in microbes responsible for phosphorus removal. At the end of acclimatization of PAO and denitrifying phosphorus accumulating organisms in the two reactors, the average MLVSS and MLSS concentrations were 2.6 g/L and 3.7 g/L, 3.5 g/L and 4.5g/L, respectively, exhibiting that their ratios were

0.70 and 0.78, respectively. These results implied that a higher amount of phosphorus was stored in the AO sludge than AA sludge per gram of biomass.A typical key phosphorus biochemical transformation responsible for EBPR was observed both in the AA and AO reactors through a cycle batch test performed at the end of acclimatization study, as given in Fig2, Fig.3, Fig4 and Fig.5, strongly suggesting that PAO and denitrifying phosphorus accumulating organisms were predominant in their respective reactors proposed in this study. However, a significant difference in the amount of phosphorus release and uptake per MLSS between AO sludge and AA sludge was monitored (see Fig.2), probably due to the fact that Accumulibacter exhibits a different metabolic process based on the different running modes, namely anaerobic-aerobic and anaerobic-anoxic. For AO sludge, the anaerobic phosphorus release rate and aerobic phosphorus uptake rate were 19.46 mg P/ (g MLSS) and 24.74 mg P/ (g MLSS), respectively, both higher than the phosphorus release rate and anoxic phosphorus uptake rate of AA sludge, which were 13.56 mg P/ (g MLSS) and 17.33 mg P/ (g MLSS), respectively. These results demonstrated the PAO and denitrifying phosphorus accumulating organisms phenotypes responsible for phosphorus removal from wastewater. Although the significant difference existing in the amount of phosphorus release/uptake between PAO and denitrifying phosphorus accumulating organisms was observed, the ratio of the phosphorus release to the phosphorus uptake (0.786) in AO sludge was quite consistent with that in AA sludge (0.782), further suggesting that both PAO and denitrifying phosphorus accumulating organisms were dominant in their respective reactor at the end of enrichment period. This explanation was also demonstrated by the linear relationship between the amount of COD consumption and that of phosphorus release under anaerobic condition, as given in Fig.3 (discussed later).

3.2 *Anaerobic-Aerobic batch test with denitrifying phosphorus accumulating organisms sludge*

The phosphorus release and uptake capacities of denitrifying phosphorus accumulating organisms during two different cycles (anaerobic-aerobic and anaerobic-anoxic) at the end of acclimatization phase were investigated through two batch tests proposed here. Typical profiles (variation of carbon, nitrogen and phosphorus with time) monitored in these batch tests are shown in Fig.4. Under the anaerobic conditions, sodium acetate was mostly taken up, which was accompanied by phosphorus release, additionally showing a good correlation between sodium acetate uptake and phosphorus release here (see Fig.3). The phosphorus anaerobic release rate of denitrifying phosphorus accumulating organisms obtained here was 13.56 mg P / g MLSS lower than that of PAO (19.46 mg P / g MLSS), likely due to the less amount of Accumulibacter enriched in AA reactor compared to AO reactor (see Fig.6). After a two hours anaerobic phase, denitrifying phosphorus accumulating organisms sludge exhibited a good phosphorus uptake performance both under anoxic and aerobic conditions. The phosphorus uptake rates obtained in these batch tests were 17.33 mg P / g MLSS in anoxic mode and 17.76 mg P / g MLSS under aerobic condition, indicating that denitrifying phosphorus accumulating organisms was able to immediately used oxygen as the electron acceptor when exposed to aerobic condition, as evidenced by the rapidly phosphorus uptake rate (see Fig.4). Concurrently, residual sodium acetate from anaerobic phase was completely consumed by the denitrifying bacteria or by the heterotrophic bacteria. Obviously, nitrate added in the anoxic phase was removed from wastewater by the denitrifying phosphorus removing bacteria, namely Accumulibacter, with the function of simultaneous denitrificaiton and phosphorus removal.

3.3 *Anaerobic-anoxic batch test with PAO sludge*

Two batch tests similar to those conducted in section 3.2 were performed to compare the phosphorus uptake capacity of PAO from AO reactor in anaerobic-anoxic and anaerobic-aerobic modes at the end of acclimatization phase. This result obtained in these batch tests are shown in Fig.5. During a 2 h anaerobic phase, a good performance of both phosphorus release and sodium acetate uptake was present for PAO sludge from the AO reactor, as also illustrated in Fig.3, where the phosphorus release rate was 19.46 mg P / g MLSS and the sodium acetate uptake rate was 61.49 mg COD / g MLSS, both higher than that of denitrifying phosphorus accumulating organisms (13.56 mg P / g MLSS, 47.56 mg COD / g MLSS, as shown in Fig.4). In contrast with denitrifying phosphorus accumulating organisms (see Fig.4), however, a significant difference of phosphorus uptake performance of PAO between aerobic and anoxic was clearly present in Fig.5. Here, the aerobic phosphorus uptake rate was 24.74 mg P / g MLSS, while that was 4.86 mg P / g MLSS in anoxic condition, suggesting that the phosphorus uptake ability of PAO sludge was inhibited when exposed to anoxic condition, as also evidenced by the less nitrate reduction in this batch test.

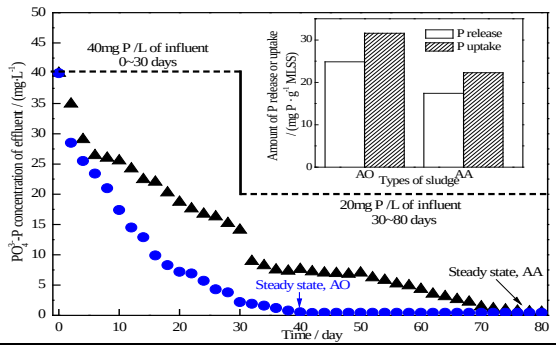


Fig.2 Phosphorus removal with respect to AA& AO

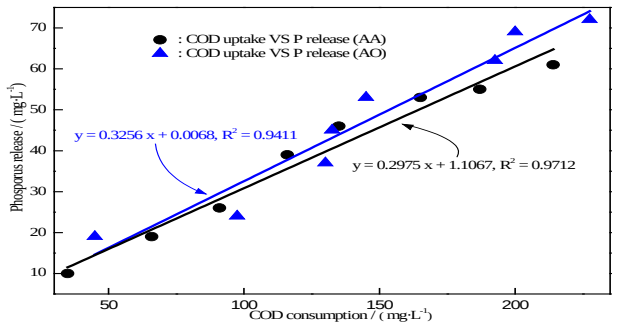


Fig.3 COD consumption versus phosphorus release

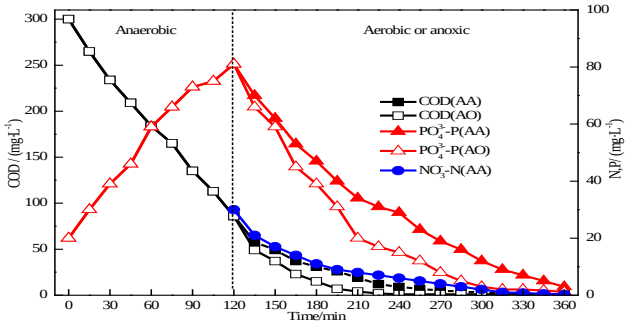


Fig.4 Nutrient profiles of DNPAOs

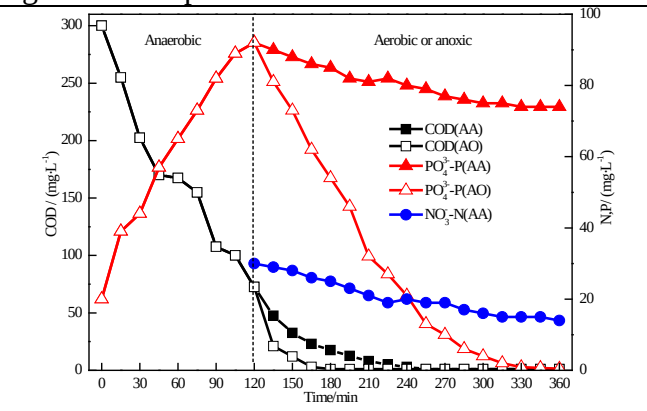
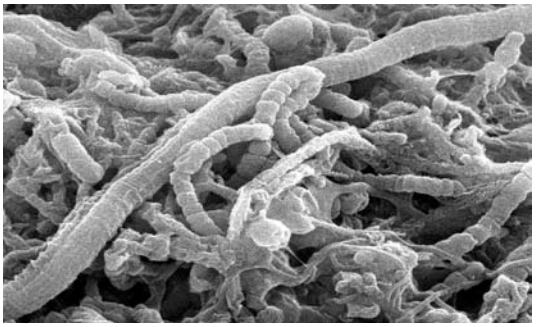


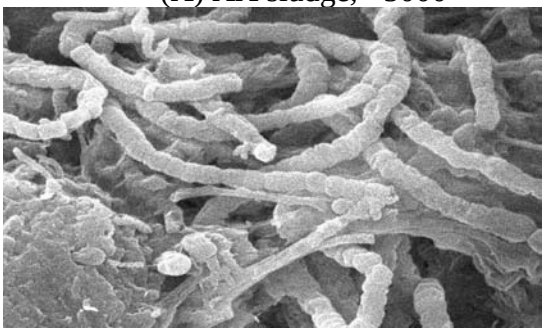
Fig.5 Nutrient of PAO sludge exposed AA or AO

3.4 *SEM analysis*

Throughout the entire start-up period of AA and AO reactors, the different phosphorus removal performance observed in the two reactors could be due to the variations in the microbial population. For this, the techniques of scanning electron micrograph (SEM) was adopted here to obtain a better understanding of the microbial community shift both in AA and AO reactors during the acclimatization phase. From SEM images (see Fig.6), it is clear that significant differences in microbial morphologies were observed between the seed sludge from A2O and the AA or AO sludge from EBPR systems studied here. Long-rod morphology microbes were abundantly enriched both in the AA and AO reactors, while seed sludge has a higher proportion of cocci or short-rod morphology microorganisms. Similar microbes were enriched in the two reactors during the acclimatization process suggested that the long-rod morphology of Accumulibacter responsible for phosphorus removal may preferably take up sodium acetate, as supplied in the influent in this study, regardless of the types of electron acceptors.



(A) AA sludge, ×5000



(B) AO sludge, ×5000

Fig.6 SEM images of activated sludge at the end of activation period

4 DISCUSSION

4.1 *Operational Strategy of Phosphorus Organisms*

The strategy of enrichment PAO and denitrifying phosphorus accumulating organisms under anaerobic-aerobic and anaerobic-anoxic mode respectively was developed based on the previous reports that Accumulibacter, a known PAO, contains two different types: one is capable of not only aerobic phosphorus uptake by using oxygen as the electron acceptor, but also anoxic phosphorus uptake by using nitrate, namely denitrifying phosphorus accumulating organisms , and the other only using oxygen instead of nitrate as the electron acceptor for phosphorus removal [3, 4], and that temperature seems to be one of the most important influence factors on wastewater systems containing EBPR in practical operation, particularly at low temperature [panswad et al.2003].It can be observed from Fig.1 that both AO and AA reactors operated in anaerobic-aerobic and anaerobic-anoxic conditions respectively confirmed the phenotypes of PAO and denitrifying phosphorus accumulating organisms responsible for phosphorus removal and reached the similar stable-state, as evidenced by the effluent phosphorus concentrations, MLSS, MLVSS, the ratio of MLVSS/MLSS, phosphorus release rate and uptake rate and their ratio. The AO and AA reactors reached the stable-state after 40 days and 80 days respective-

ly, suggesting the higher activities of PAO to low temperature than denitrifying phosphorus accumulating organisms , probably due to the fact that the amount energy generated from the oxidative phosphorylation with NO3- is about 40% lower than that with O2 [Ahn et al.2002]. The ratio of MLVSS to MLSS from 0.86 (3.6/4.2) of the start-up phase (namely, seed sludge collected from an aerobic basin within an A2O process) decreased to 0.70 (2.6/3.7) of stable-state phase in AO reactor and to 0.78 (3.5/4.5) in AA reactor, which are in agreement with the reports [Lu et al.2006], indicating that the higher amount of phosphorus was stored in PAO or denitrifying phosphorus accumulating organisms than in seed sludge, suggesting that this strategy studied here dramatically promoted the growth of PAO and denitrifying phosphorus accumulating organisms in their respective reactor.The specific phosphorus release and uptake rates for PAO were estimated to be 19.46 and 24.74 mg P/ g MLSS both higher than that for denitrifying phosphorus accumulating organisms , 13.56 and 17.33 mg P/ g MLSS, respectively. This was likely due to the following two reasons: (1) the energy produced by PAO with oxygen was higher than that of denitrifying phosphorus accumulating organisms with nitrate [Ahn et al.2002]; (2) the size of denitrifying phosphorus accumulating organisms was bigger than that of PAO, causing the limited transfer of carbon, nitrogen and phosphorus to the active biomass [Zeng et al.2003]. Indeed, SEM conducted in this study showed that denitrifying phosphorus accumulating organisms grow with the aggregation of biomass into similar granules, while PAO grow with flocs. Overall, these results obtained here demonstrated that the operational strategy at low temperature proposed in this study is rather effective in acclimatization of PAO and denitrifying phosphorus accumulating organisms in EBPR systems, therefore providing a practical strategy for stable-state operation of this process at low temperature such as in winter.

4.2 *Phosphorus Organisms during Switching Tests*

Fig.4 and Fig.5 show the correlation between the types of electron acceptor and the phosphorus removal performance. For denitrifying phosphorus accumulating organisms , no considerable difference in phosphorus uptake rate with nitrate and oxygen as electron acceptors was observed by switching the mode from normal anoxic to aerobic, namely that when denitrifying phosphorus accumulating organisms exposed to aerobic condition it can take up phosphorus immediately, which agrees well with the report [Gebremariam et al.2011]. However, the phosphorus uptake performance of PAO was obviously inhibited when it exposed to anoxic rather than aerobic condition. These results obtained through the switching batch tests suggested that denitrifying phosphorus accumulating organisms can readily produce the quality of enzymes for aerobic metabolisms similar to anoxic metabolisms, while PAO lacks the enzymes required for anoxic metabolisms using nitrate instead of oxygen as an electron acceptor [Martin et al.2011]. From Fig.5, the phosphorus uptake rate of PAO was very low in anoxic condition as compared with the aerobic condition. Interestingly, some studies [7, 10] have demonstrated that when the anoxic phase was extended to 30h rather than 4h, the phosphorus uptake rate of PAO can be obviously improved, suggesting that a several hours lag phase may be existence in phosphorus uptake when PAO exposed to anoxic condition. From these studies, we hypothesize that PAO could gradually develop the required amount of enzymes for anoxic metabolisms during the lag time, which may be agreement with the explanation mentioned above (PAO lacks the enzymes required for anoxic metabolisms using nitrate).Through four batch tests, comparison of the phosphorus removal performance of PAO between aerobic and anoxic, and similar comparison to denitrifying phosphorus accumulating organisms were conducted, demonstrating that Accumulibacter has, at least, two different types, which supports the reports [3, 4] described above. Nevertheless, based on Carvalho et al. findings [Carvalho et al.2007].

4.3 *Identification of Accumulibacter in AA and AO*

Analysis of SEM showed that identical microbial morphologies (long-rod microbes) were present in the two reactors at the end of acclimatization period, suggesting that this kind of Accumulibacter may display good affinities for sodium acetate as the carbon source. Indeed, two different types (rods or cocci) of Accumulibacter were found by Martin et al. in two EBPR systems, where one was feed with sodium acetate and the other with propionate. Similarly, He et al. [He et al.2011] also found the distribution of the different types of Accumulibacter in one lab-scale reactor and six full-scale reactors both presenting good phosphorus performance. These studies may support the hypothesis that different carbon sources feed to the phosphorus removal microorganisms could promote the growth of different types of Accumulibacter, probably regardless of electron acceptors, which is also partially supported by the results obtained in this study. Overall, the combination of chemical analysis with microbial analysis suggested that Accumulibacter, both PAO and DNPAOs , with a long-rod morphology was more preferably enriched with sodium acetate as compared with other carbon sources such as sodium acetate.

5 CONCLUSIONS

A new scenario for enhancing phosphorus removal during winter season is investigated in this study. The results showed the following points;

COD / P ratio of two EBPR systems operated in anaerobic-aerobic (AA) and anaerobic-anoxic (AO)

modes respectively, from 20:1 to 15:1; with the decrease of concentration COD, PO43--P and NO3--N in the influent from 800 mg/L, 40mg/L and 50mg/L to 300 mg/L, 20mg/L and 30mg/L, respectively.

Adopting different solids retention time (SRT) for enrichment of different types of Accumulibacter; no excess of activated sludge was wasted at the beginning of start-up, and then 10 days SRT for PAO and 20 days SRT for denitrifying phosphorus accumulating organisms .

Maintaining a high and different MLSS concentrations in AA and AO reactors, based on the metabolic characteristics of Accumulibacter supplied the different electron acceptors, 4.5 g/L MLSS for AA reactor and 3.7 g/L MLSS for AO reactor.

This proposed strategy here was shown to be effective in achieving a very high enrichment of Accumulibacter at low temperature by linking chemical analysis with microbial observation. Chemical analysis of batch tests indicated the existence of two types of Accumulibacter, which one uses oxygen as the electron acceptor and the other uses nitrate. Through microbial observation, a high abundance of Accumulibacter was present in both AA and AO reactors. Although the strategy may not be the unique method for the enrichment of phosphorus removal microorganisms at winter, It is recommendable for the future studies in practical application.

REFERENCES

Carvalho G, Lemos P C, Oehmen A, Reis M A M. 2007 .Denitrifying phosphorus removal: linking the process performance with the microbial community structure. Water Research, 41(19): 4383-4396.

Lu H B, Oehmen A, Virdis B, Keller J, Yuan Z G. , 2006.Obtaining highly enriched cultures of Candidatus Accumulibacter phosphates through alternating carbon sources. Water Research, 40(20): 3838-3848.

Panswad T, Doungchai A, Anotai J.2003. Temperature effect on microbial community of enhanced biological phosphorus removal system. Water Research, 37(2): 409-415.

Ahn J, Daidou T, Tsuneda S, Hirata A.2002. Characterization of denitrifying phosphate-accumulating organisms cultivated under different electron acceptor conditions using polymerase chain reaction-denaturing gradient gel electrophoresis assay. Water Research, 36(2): 403-412.

Zeng R J, Saunders A M, Yuan Z, Blackall L L, Keller J.2003 .Identification and comparison of aerobic and denitrifying polyphosphate accumulating organisms. Biotechnology and Bioengineering, 83(2): 140-148.

Gebremariam S Y, Beutel M W, Christian D, Hess T F. , 2011.Research advances and challenges in the microbiology of enhanced biological phosphorus removalA critical review. Water Environment Research, 83(3): 195-219.

Martín H G, Ivanova N, Kunin V, Warnecke F, Barry K W, Mchardy A C, Yeates C, He S, Salamov A A, Szeto E. , 2006. Metagenomic analysis of two enhanced biological phosphorus removal (EBPR) sludge communities. Nature Biotechnology, 24(10): 1263-1269.

He S, Gu A Z, Mcmahon K D. , 2006. Fine-scale differences between Accumulibacter-like bacteria in enhanced biological phosphorus removal activated sludge. Water Science and Technology, 54(1): 111-118.