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The biomass fraction of phosphate-accumulating organisms grown in anoxic environment in an enhanced biological phosphorus removal (EBPR) system

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ABSTRACT

To improve the design and performance of anoxic phosphorus uptake process, the biomass fractions of PAOs grown in anoxic environment and denitrification rate, with PHAs as carbon source, was investigated in a pilot scale study of EBPR system which was based on stoichiometry and mass balance. The system, containing Modified University of Cape Town (MUCT) reactor, was maintained at steady state with COD loading of 0.25 kg COD/(kg MLVSS d), and solid retention time (SRT) of 12 d. Based on mass balance calculation, 46.74% of organic matter, 50.95% of nitrogen, and 42.03% of phosphorus in the influent were converted to carbon dioxide, nitrogen gas, and poly-phosphate, respectively. In the second anoxic stage, the biomass yield of PAOs with nitrate as electron acceptor was 4.90 g$C_5H_7O_2N$/d, and its yield fraction was 20.63%. For the reaction to proceed properly, 10.48 g PHA/d of PHAs was oxidized, and 3.68 g NO_3^--N/d of nitrate was reduced. The denitrification rate with PHAs as carbon source was 0.058 kg NO_3^--N/(kg MLVSS·d). These results showed that a higher amount of PAO growth under anoxic condition was observed in the MUCT system, and 0.058 kg NO_3^--N/(kg MLVSS·d) of the denitrification rate with PHAs could be used as the reference values for calculation of anoxic stage volume.

Keywords: Biomass; Anoxic phosphorus uptake; Phosphate-accumulating organisms; Stoichiometry; Mass balance

1. Introduction

Human population growth and resources consumption have placed increasing demands on aquatic ecosystems, and have affected global biogeochemical cycles of carbon, nitrogen, and phosphorus [1]. Nowadays, one of the greatest problems of continental water resources is eutrophication that is mainly caused by a higher concentration of nutrients, especially phosphorus and nitrogen compounds. The EBPR system is most commonly used process for eutrophication control among all kinds of chemical–physical or biological treatment processes, owing to the lower operation cost comparing with other chemical–physical processes.

Typically, the EBPR process comprises anaerobic, anoxic, and aerobic stages; the activated sludge goes through these stages in sequence and circles. In anaerobic stage, activated sludge releases phosphorus

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which was stored as poly-phosphate in aerobic stage, and accumulates to form poly-β-hydroxy-alkanoates (PHAs) when the carbon substrate is sufficient [2,3]. In aerobic stage, the released phosphorus has been taken excessively by PAOs and removed from the system by sludge waste [4]. Moreover, ammonification, nitrification, and carbon substrate oxidation occurs simultaneously. In anoxic stage, nitrate has been removed by denitrification. In the early studies of biological nutrient removal processes, it was assumed that the PAOs could not use nitrate as an electron acceptor; however, since then, there have been many reports claiming that a significant fraction of PAOs could take up phosphate in the anoxic stage [5–9]. The occurrence of PAOs grown in anoxic condition has been clearly demonstrated through batch tests with real activated sludge of full-scale wastewater treatment plants. It was shown that the denitrification–dephosphatation has similar capacities and characteristics, as phosphorus removal functioned in conventional anaerobic–aerobic process [10–12]. It was also indicated that anoxic phosphorus uptake played a significant role for the comprehensive function of an EBPR system [13–15]. The merits of enhanced denitrification–dephosphatation activity were saving energy (resulting from aeration), reducing COD consumption, and lowering sludge production.

The above reactions consequently formed a more complex community of microorganism than conventional activated sludge process with a single stage. To simplify, the *IAWA Task Group on Mathematical Modeling for Design and Operation of Biological Wastewater Treatment Process* suggests that only three groups of microorganisms, i.e. *heterotrophs*, *autotrophs,* and PAOs, should subsist in the EBPR system, representing organic matter, nitrogen, and phosphorus removal, respectively. More details about reaction kinetics of these microorganisms were described in the Activated Sludge Model No.2d [16] with some assumptions of microorganisms classification. Heterotrophic biomass includes *heterotrophs* and PAOs. The autotrophic biomass represented as *autotrophs*. Specifically, *heterotrophs* are assumed to be the 'allrounder,' which may grow aerobically, anoxically, and be active anaerobically. PAOs are assumed to be the representative for all types of PAOs, which may grow on stored PHAs in anoxic as well as aerobic environments. *Autotrophs* are responsible for nitrification, which means *autotrophs* oxidize ammonium/ammonia directly to nitrite and nitrate.

The anoxic phosphorus uptake process was not included in existing prescriptions for designing EBPR systems [17]. For improving the design and performance of EBPR systems, it is important to estimate the biomass fractions of PAOs grown in anoxic environment and denitrification rate with PHAs as carbon source. Batch test or activated sludge model to determine the biomass composition of the sludge were developed [4–6,8,12], and denitrification activity of PAOs was evaluated accordingly. The phosphorus uptake rate under anoxic condition was compared with the aerobic uptake rate, and the proportion of anoxic growth of PAOs was calculated. However, the measured value is higher than the calculated value that owes to eliminate all factors affecting the reaction rate during the tests. In addition, the application of math model is limited since the parameters calibration/validation is very complicated [18]. Thus, stoichiometry and mass balance was used to estimate the biomass fractions of PAOs grown in anoxic environment in this study, and denitrification rate with PHAs as carbon source was also investigated to improve the design of anoxic stage in EBPR system.

2. Materials and methods

2.1. Experimental setup

The setup of pilot scale study was located at laboratory and consisted of a 280 L synthetic wastewater tank, a MUCT reactor with an effective volume of 90 L, and a 36 L settler (Fig. 1). The synthetic wastewater tank was equipped with a mechanical mixer and temperature control system for maintaining constant influent quality. The MUCT reactor consists of an 18 L anaerobic stage of: two stages of anoxic zone with effective volume of 9 L and 18 L, respectively, plus a 45 L aerobic stage. Anaerobic stage and the two anoxic stages were equipped with mechanical mixers to keep the biomass in suspension. Compressed air was supplied for aeration and mixing in the aerobic stage, on-line pH, and DO monitor system was installed. Also, the pH was automatically controlled through the addition of 0.1 M $NaHCO_3$ solution. The influent flow, mixed liquor recycle flow, nitrified liquor recycle flow, and sludge recycle flow were controlled by peristaltic pumps. A slowly rotating scraper was installed in the settler.

2.2. Wastewater and sludge

Synthetic wastewater was prepared to simulate bioreactor influent of Changchun Municipal Wastewater Treatment Plant, (Changchun, China) as feeding solution for this study. The synthetic municipal wastewater consists of brewage, stable and consistent properties (ethanol content 3.5%, COD 114 g/L) 2 mL/L, peptone 0.1 g/L, NH_4Cl 0.19 g/L, KH_2PO_4 0.026 g/L, $NaHCO_3$

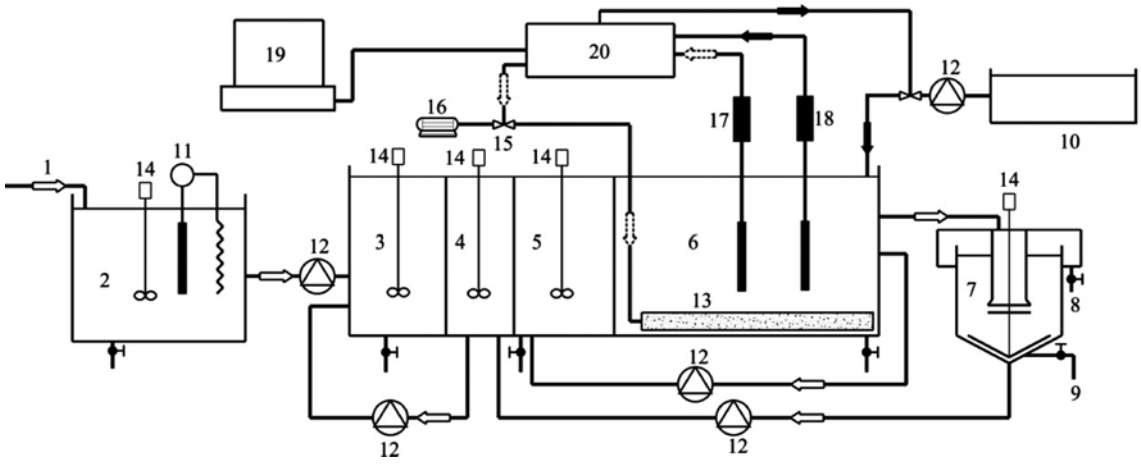

Fig. 1. Schematic diagram of pilot scale study setup.
Notes: 1 synthetic wastewater; 2 wastewater tank; 3 anaerobic stage; 4 anoxic stage; 5 anoxic stage; 6 aerobic stage; 7 settler; 8 effluent; 9 waste sludge; 10 reservoir $NaHCO_3$; 11 thermostat; 12 peristaltic pump; 13 diffuser; 14 mixer; 15 solenoid valve; 16 air pressure; 17 DO sensor; 18 pH sensor; 19 computer; 20 logic controller.

0.16 g/L, $CaCl_2$ 0.01 g/L, $MgSO_4$ 0.05 g/L, and trace mineral solution 0.6 mL/L ($FeCl_3$ 0.9 g/L, H_3BO_4 0.15 g/L, $CoCl_2{\cdot}7H_2O$ 0.15 g/L, $CuSO_4{\cdot}5H_2O$ 0.03 g/L, KI 0.18 g/L, $MnCl_2{\cdot}4H_2O$ 0.06 g/L, $Na_2Mo{\cdot}2H_2O$ 0.06 g/L, $ZnSO_4{\cdot}7H_2O$ 0.12 g/L). The temperature in feed tank was remained at 20˚C throughout the whole study. The corresponding COD, total nitrogen (TN), and total phosphorus (TP) concentration were 295 ± 16.16, 49.5 ± 3.21, and 5.9 ± 0.57 mg/L, respectively, which was expressed as means ±1.03 standard deviation ($n = 20$, $p = 0.683$).

Seeding sludge was collected from Changchun Sewage Treatment Plant (Changchun, China), which performs phosphorus control standard with anaerobic/aerobic process.

2.3. Experimental procedure

The influent flow rate was controlled at 240 L/day during the experiments, resulting in a hydraulic retention time in the MUCT reactor of 9 h. MLVSS and COD loading were maintained at 1.76 g/L (in anaerobic stage) and 0.25 kg COD/(kg MLVSS·d), respectively. DO concentration and pH in the aerobic stage were automatically controlled by programmable logic controller with set value of 2.0 mg/L and 7.0, respectively. The sludge was wasted to control SRT at 12 d and discharge rate of waste sludge was calculated by formula (1) [19].

$$Q_{exc} = \frac{VQ_{rec}}{(Q + Q_{rec})\mathrm{SRT} - V} \quad (1)$$

where Q_{exc} was the discharge rate of waste sludge (m^3/d), V was the total volume of MUCT reactor (m^3), Q_{rec} was the sludge recycle flow rate (m^3/d), and Q was the influent flow rate (m^3/d).

The mixed liquor recycle flow rate, nitrified liquor recycle flow rate, and sludge recycle flow rate were controlled at 1.0Q, 3.0Q and 0.5Q, respectively. All the operating conditions were kept constant during the experiment period.

Steady state was reached after 2 months acclimation. The characteristics of the influent wastewater, the biological sludge, and the treated wastewater were daily recorded and the statistical analysis results are presented here for discussion.

2.4. Analytical methods

Samples were collected regularly from different stages of the reactor as well as the feed solution for offline measurement of ammonia, nitrate, TN, TP, COD, and PHAs. For ammonia, nitrate, TN, TP, and COD, samples were centrifuged at 4,500 r/min for 7 min and measured according to standard methods. After re-centrifugation, the pellets were collected and stored frozen for later analysis of PHAs as described below. The PHAs of lyophilized sludge samples were digested, methylated, and extracted according to the publication of Barndl et al. [20].

3. Assumptions of mass balance calculation

In calculating the biomass distribution of the nitrogen and phosphorus removal system, the assumptions

in ASM2d were included [16]. The activated sludge model employed in this study bears the following assumptions: ① The *heterotrophs*, PAOs, and *autotrophs* are homogenous and do not undergo changes with time. ② The growth of *heterotrophs*, PAOs, and *autotrophs* represent biomass production. ③ PAOs can grow at the nearly same yield constant under both aerobic and anoxic environments. ④ Carbonaceous matter is removed in the form of carbon dioxide, except for biomass production. ⑤ Organic nitrogen and ammonia are removed in the form of nitrogen gas through nitrification and denitrification. ⑥ Phosphorus is removed as poly-phosphate in the waste sludge.

In addition, the compounds for microbial cell synthesis were calculated based on empirical formula $C_5H_7O_2N$, which represents the composition of *heterotrophs*, PAOs, and *autotrophs* [21]. The theoretical oxidation equation of the microorganisms was as follows (Eq. (2)):

$$C_5H_7O_2N + 5O_2 + H^+ \rightarrow 5CO_2 + 2H_2O + NH_4^+ \quad (2)$$

Based on Eq. (2), the oxygen stoichiometric coefficient was 1.42 $gCOD/gC_5H_7O_2$ N and the nitrogen content constituted 12.4% biomass. The phosphorus content of the biomass was considered as 3% for biomass balance calculation [22]. This value excluded the mass of accumulated poly-phosphate by PAOs [16].

4. Results and discussion

4.1. COD, TN, and TP removal

The comprehensive performance of the pilot scale setup during the whole study period is summarized in Figs. 2–6, which shows the average values of concentration of COD, TP, TN, NH_4^+-N, and NO_3^--N, and PHAs content in the sludge along with the anaerobic, first anoxic, second anoxic, and aerobic stages. The pilot scale plant exhibited good performances in organic matter, nitrogen, and phosphorus removal. The average effluent concentrations of COD, TP, TN, NH_4^+-N were 16.55, 0.46, 12.40, and 1.2 mg/L, respectively. Figs. 2 and 3 illustrate that large quantity of COD was adsorbed on activated sludge and/or uptaken by PAOs and stored as PHAs in anaerobic stage, which could suppress inhibitory effect of COD on the denitrification–dephosphatation and aerobic nitrification. Fig. 4 illustrates that TP concentration of influent varies from 5.33 to 6.47 mg/L with an average value of 5.9 ± 0.57 mg/L; the corresponding phosphate concentration effluent was below 0.5 mg/L. A recent study addressed the significance of stable operating condition and influent concentration in good

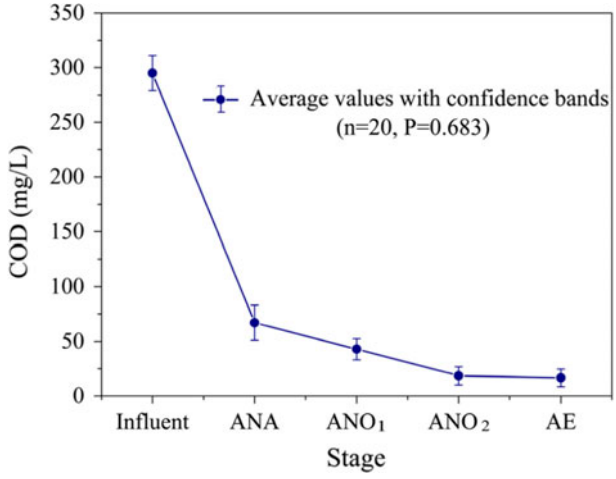

Fig. 2. The COD profile of pilot study. ANA, anaerobic stage; ANO_1, first anoxic stage; ANO_2, second anoxic stage; and AE, aerobic stage.

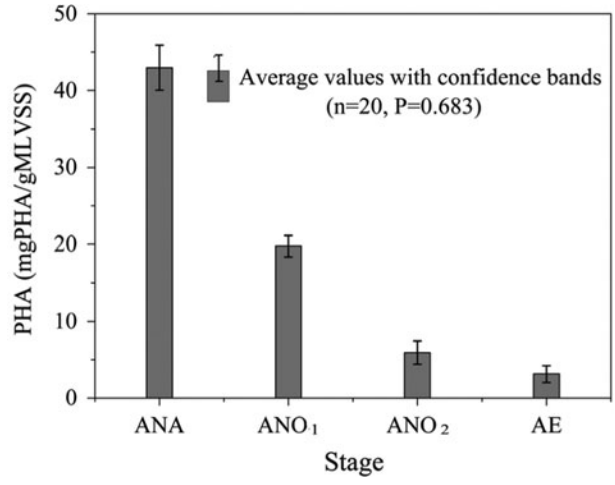

Fig. 3. The PHAs profile of pilot study. ANA, anaerobic stage; ANO_1, first anoxic stage; ANO_2, second anoxic stage; and AE, aerobic stage.

phosphorus removal performance [23]. Figs. 5 and 6 illustrate that high NO_3^--N concentration in effluent combined with high TN concentration, which owing to the unit configuration. The aerobic stage was the last stage of the system and was responsible for phosphate accumulation, also produce nitrate which was inevitable.

4.2. Metabolism of organic matter, nitrogen, and phosphorus

Based on the experimental results and the above mentioned assumptions, the mass distributions of

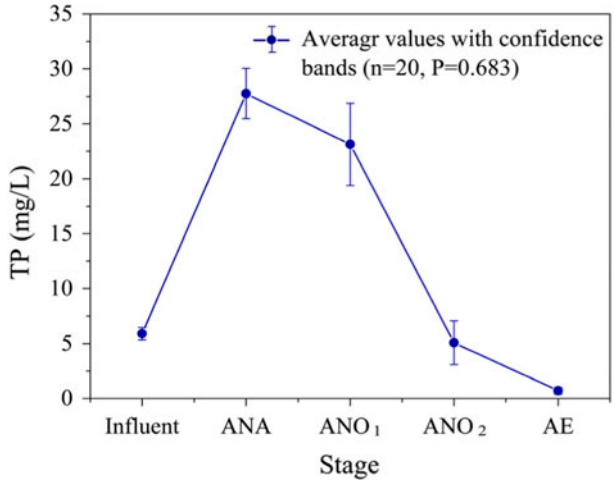

Fig. 4. The TP profile of pilot study. ANA, anaerobic stage; ANO_1, first anoxic stage; ANO_2, second anoxic stage; and AE, aerobic stage.

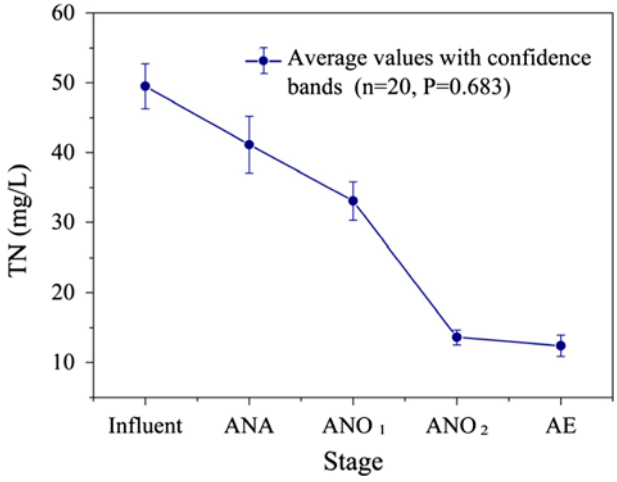

Fig. 5. The TP profile of pilot study. ANA, anaerobic stage; ANO_1, first anoxic stage; ANO_2, second anoxic stage; and AE, aerobic stage.

organic maters, nitrogen, and phosphorus were calculated around the entire treatment system and the results are summarized in Table 1. Table 1 shows that approximately 46.74% of the organic matters are removed in the form of carbon dioxide; 50.95% of the total nitrogen is removed in the form of nitrogen gas, and 42.03% of the total phosphorus is removed as poly-phosphate. Moreover, about 47.65% of the organic matters, 24.00% of the nitrogen, and 50.34% of the phosphorus of the influent were transferred and/or converted into biomass and removed from system as sludge waste.

The mass flow of PHAs, phosphorus, and nitrogen were calculated around each reaction stage and the

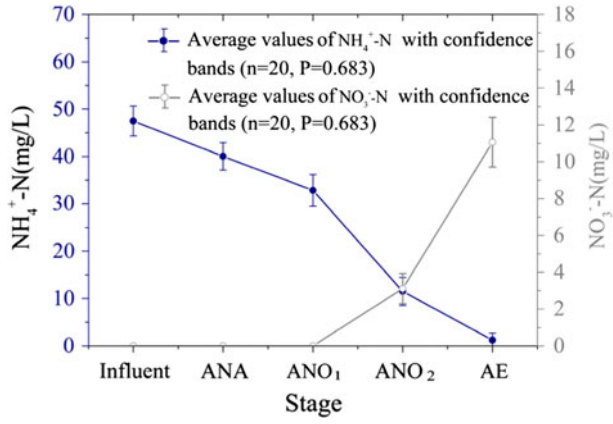

Fig. 6. The NH_4^+-N and NO_3^--N profile of pilot study. ANA, anaerobic stage; ANO_1, first anoxic stage; ANO_2, second anoxic stage; and AE, aerobic stage.

results are illustrated in Fig. 7. The poly-phosphate hydrolysis and PHAs synthesis of PAOs were taken as main reactions in anaerobic stage. According to the experimental results and mass balance calculation, the mass of synthesized PHAs was 19.62 gPHA/d. The needed energy was derived from hydrolysis of intracellular poly-phosphate and glycogen glycolysis followed by the release of ortho-phosphate to the bulk solution. The total mass of released phosphorus was 6.35 g/d.

In the first anoxic stage, denitrification competed with phosphorus release severely for limited organic substrate. Chuang et al. found that denitrification can capture substrate better than phosphorus release [24]. The mixed liquor in anaerobic stage flowed into ANO_1 with relatively small amount of readily biodegradable organic substrate, which affected the simultaneous denitrification and phosphorus release process. Once nitrate had been depleted through denitrification, the residual organic substrates were consumed through phosphorus release process, also utilized by PAOs for PHAs synthesis. According to the experimental results and mass balance calculation, the mass of synthesized PHAs and released phosphorus were 1.51 gPHA/d and 0.48 g/d in the first anoxic stage, respectively.

In the second anoxic stage, the phosphorus uptake and denitrification occurred simultaneously, as illustrated in Fig. 7. In the second anoxic and aerobic stages, PAOs used PHAs to generate energy for growth and multiplication, glycogen synthesis, and phosphorus uptake. Phosphorus in the wastewater was converted into biomass through PAOs metabolism, and this biomass containing high poly-phosphate content would be wasted as waste sludge from the

Table 1
Mass distributions of organic matters, nitrogen, and phosphorus in treatment system

	Influent			Effluent			Removal			Sludge waste		
Items	COD	N	P	COD	N	P	COD	N	P	COD	N	P
Mass flow (g/d)	70.80	11.88	1.42	3.97	2.98	0.11	33.09	6.05	0.60	33.74	2.85	0.71
Mass distribution (%)	100	100	100	5.61	25.05	7.62	46.74	50.95	42.03	47.65	24.00	50.34

settler. According to the experimental results and mass balance calculation, the total mass of oxidized PHAs and uptaken phosphorus with nitrate as electron acceptor were 10.48 gPHA/d and 3.17 g/d, respectively, and that with oxygen as electron acceptor were 10.58 gPHA/d and 5.00 g/d, respectively. It clearly showed that denitrification during anoxic process had constituted a substantial part in total phosphorus removal; the contribution could achieve 38.80%. This result also indicated that significant amounts of intracellular PHAs were oxidized by nitrate (products of aerobic stage), that is to say, higher phosphorus removal efficiency largely depended on the performance of anoxic PHAs oxidation and phosphorus uptake.

In the aerobic stage, the mass of oxidized NH_4^+-N was 11.14 g/d Fig. (7). NH_4^+-N removal efficiency was high at the steady state, since a high quantity of COD adsorbed on and/or uptaken by activated sludge in the anaerobic stage results in lower COD/NH_4^+-N ratio of around 1.74 ± 0.27 entering the AE. According to Nam et al. if the COD/NH_4^+-N is below 5, *autotrophs* like nitrosomonas and nitrobacter had a better capability of grabbing C and N substrate than *heterotrophs* in aerobic condition [25].

4.3. Calculation of the biomass distribution

4.3.1. The biomass fractions of phosphate-accumulating organisms

PHAs in PAOs have the following approximate chemical composition: $CH_{1.5}O_{0.5}$; the Eq. (3) was used for converting PHAs to COD value [21]:

$$CH_{1.5}O_{0.5} + 1.125O_2 \rightarrow CO_2 + 0.75H_2O \quad (3)$$

Based on Eq. (3), the stoichiometric coefficient of the PHAs–COD was 1.67 gCOD/gPHA. Thus the synthesized PHAs in anaerobic stage could be expressed as 19.62 gPHA/d × 1.67 gCOD/gPHA = 32.76 gCOD, i.e. the equivalent value of synthesized PHAs was 32.76 gCOD/d. Ignoring other reactions, the COD mass flow into ANO_1 was 38.04 g/d. The equivalent value of synthesized PHAs was 2.53 gCOD/d in the ANO_1.

According to Henze, the assumptions for the evaluated expression are the observed yield constant, $Y_{obs} = 0.4$ kg COD/kg COD [21]. Therefore, under anoxic condition, the expression looks as follows (Eq. (4)) [21]:

$$2CH_{1.5}O_{0.5} + 0.18NH_4^+ + 1.08NO_3^- + 0.21PO_4^{3-} \rightarrow 0.18C_5H_7O_2N + 0.21(HPO_3) + 1.1CO_2 + 0.36H_2O + 1.53OH^- + 0.54N_2 \quad (4)$$

Or under aerobic condition, the expression was as follows (Eq.(5)) [21]:

$$2CH_{1.5}O_{0.5} + 0.18NH_4^+ + 1.35O_2 + 0.21PO_4^{3-} \rightarrow 0.18C_5H_7O_2N + 0.21(HPO_3) + 1.1CO_2 + 0.9H_2O + 0.45OH^- \quad (5)$$

Based on Eqs. (4) and (5), the stoichiometric coefficient of the PHAs–COD biomass was 0.28 $gC_5H_7O_2N$/gCOD. Using Eq. (3), the equivalent value of oxidized PHAs in ANO_2 could be expressed as 17.51 gCOD/d, and that in AE could be expressed as 17.67 gCOD/d.

Hence, the biomass yield of PAOs ($M_{PAO,\ ANO_2}$) with nitrate as electron acceptor was 4.90 $gC_5H_7O_2N$/d, and this value was approximately 20.63% to the total yield biomass (23.76 $gC_5H_7O_2N$/d). The COD mass for the yield of PAOs growth of denitrification was 6.96 gCOD/d by Eq. (2). These results showed that a high amount of PAOs grown on stored PHAs in anoxic condition was present in this EBPR system for synthetic municipal wastewater treatment. The proportion of denitrifying PAOs was estimated to be 49.75% of total PAOs.

Meanwhile, the yield biomass of PAOs growth ($M_{PAO,AE}$) with oxygen as the electron acceptor was 4.94 $gC_5H_7O_2N$/d. This value was approximately 20.82% to the total yield biomass. The COD mass for the yield of PAOs growth in AE was 7.02 gCOD/d by Eq. (2).

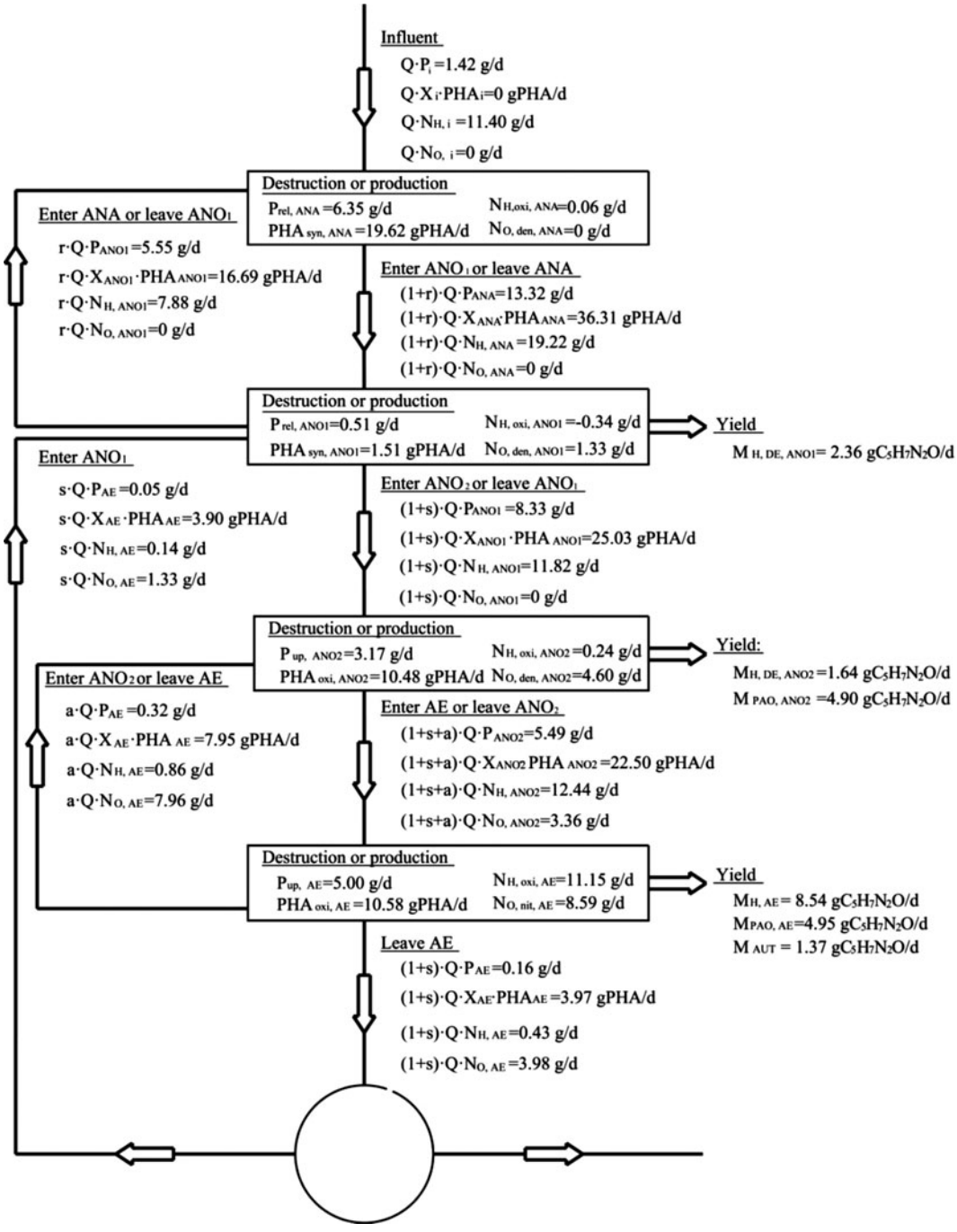

Fig. 7. The mass flow of PHAs, phosphorus, and nitrogen in anaerobic, first anoxic, second anoxic, and aerobic stages.

The proportion of PAOs was estimated to be 41.45% of total waste sludge, which was in accordance with the results obtained by Kuba et al. [26].

Based on Eq. (4), the stoichiometric coefficient of nitrate nitrogen–PHAs–COD was 0.21 g NO_3^--N/gCOD. The mass of nitrate nitrogen removed by PAOs in ANO_2 was obtained by multiplying the oxidized PHAs (gCOD/d) with 0.21 gNO_3^--N/gCOD, i.e. 17.51 gCOD/d × 0.21 g NO_3^--N/gCOD=3.68 g NO_3^--N/d.

The MUCT is a pre-denitrification process, where the nitrified wastewater with high concentration of nitrate in aerobic stage is returned to the second anoxic stage. The total input mass of nitrate was

7.96 g/d and the degraded mass was 4.60 g/d, where the mass of nitrate reduced by PAOs with PHAs as carbon source was 3.68 g/d. It is clearly illustrated that the sludge entering the ANO_2 has maximal PHAs content allowing for denitrification and phosphorus uptake in the ANO_2, which favors the priority use of PHAs–COD for denitrification–dephosphatation and minimizes aerobic PHAs oxidation in MUCT system.

Design of the activated sludge plant for C, N, and P removal could be derived from bioprocess stoichiometry and microbial kinetics, and expressed as mass balance [17]. From the point of the engineering, for EBPR system, only denitrification rate with influent COD as electron donor were included for anoxic stage volume design. However, in this study, 80% of nitrate as an electron acceptor for PAOs were reduced and mass fraction of denitrifying PAOs in active sludge was around 20.63%. These results clearly indicated that denitrifying PAOs played a significant role for denitrification and phosphorus uptake. Thus, it is suggested that denitrification rate with PHAs as carbon source should be considered for anoxic stage design to enhance anoxic phosphorus uptake process. The denitrification rate with PHAs as carbon source was 0.058 kg NO_3^--N/(kg MLVSS·d) in this study, which could be used as the reference value for design calculation of anoxic reactor.

4.3.2. The biomass fractions of heterotrophs with nitrate as an electron acceptor.

Fig. 7 showed that the amounts of nitrate nitrogen removed were around 1.33 g NO_3^--N/d in ANO_1 and 3.68 g NO_3^--N/d in ANO_2.

In ANO_2, the amount of nitrate nitrogen, therein removed by *heterotrophs*, was 0.92 g NO_3^--N/d. Provided that the organism assimilates ammonium acting as an energy and carbon source, the following Eq. (6) could be used to calculate the *heterotrophs* yield by denitrification in ANO_1 and ANO_2 [21]:

$$0.61C_{18}H_{19}O_9N + 4.54NO_3^-\text{-}N + 0.39NH_4^+\text{-}N + 4.15H^+ \rightarrow C_5H_7O_2N + 2.27N_2 + 5.98CO_2 + 5.15H_2O \quad (6)$$

Based on Eq. (6), the stoichiometric coefficient of the nitrate nitrogen biomass was 1.78 $gC_5H_7O_2N/gNO_3^-$-N. Hence, the *heterotrophs* yield in ANO_1 ($M_{H,DE,1}$) could be converted to 2.37 $gC_5H_7O_2N$/d, and that in ANO_2 ($M_{H,DE,2}$) could be converted to 1.64 $gC_5H_7O_2N$/d. The COD mass for the yield of $M_{H,DE,1}$ and $M_{H,DE,2}$ were 3.35 gCOD/d and 2.33 gCOD/d by Eq. (2), respectively. These values were approximately 9.94 and 6.90% of the total yield biomass, respectively.

Meanwhile, based on Eq. (6), the stoichiometric coefficient of nitrate nitrogen substrate was 3.77 $gC_{18}H_{19}O_9N/g$ NO_3^--N. Assuming that $C_{18}H_{19}O_9N$ could be oxidized to carbon dioxide, it may result in the following expression (Eq. (7)) [21]:

$$C_{18}H_{19}O_9N + 17.5O_2 + H^+ \rightarrow 18CO_2 + 8H_2O + NH_4^+ \quad (7)$$

The microbiological oxygen consumption in Eq. (7) was calculated at 1.42 gCOD/g $C_{18}H_{19}O_9N$. Therefore, the nitrate nitrogen–COD coefficient was 5.35 gCOD/g NO_3^--N. This meant that 5.35 gCOD was consumed in denitrification of 1.0 g of nitrate nitrogen. Hence, the COD consumed by *heterotrophs* denitrification in the ANO_1 and ANO_2 were 7.10 and 5.61 gCOD/d, respectively.

Regardless of other reactions, the mass of COD entering the ANO_2 was 28.41 gCOD/d, and the mass of COD entering the AE was 22.80 gCOD/d.

In MUCT system, sludge is returned to the first anoxic stage and not to the anaerobic stage (where COD is rich). Therefore, in anaerobic stage, an amount of biodegradable COD was absorbed by PAOs and stored in the form of PHAs. With further polishing of COD by denitrification in the first anoxic stage, COD transferred into the second anoxic stage had only low strength, and 80% nitrate was reduced by PAOs with PHAs as carbon source, resulting low production of *heterotrophs*.

4.3.3. The biomass fractions of autotrophs

Fig. 7 showed that the amount of nitrate produced was approximately 8.59 g NO_3^--N/d. The oxidation of ammonia under aerobic conditions was described in a simplified manner as follows (Eq. (8)) [21]:

$$NH_4^+ + 1.86O_2 + 1.98HCO_3^- \rightarrow 0.020C_5H_7O_2N + 0.98NO_3^- + 1.88H_2CO_3 + 1.04H_2O \quad (8)$$

Based on Eq. (8), the stoichiometric coefficient of the ammonia biomass was 0.16 $gC_5H_7O_2N/g$ NO_3^--N. Hence, the *autotrophs* yield (M_{AUT}) could be calculated as 1.37 $gC_5H_7O_2N$/d and the COD amount for *autotrophs* yield was 2.53 gCOD/d by Eq. (2). This value was approximately 5.78% of the total yield biomass.

The aerobic yield of *heterotrophs* ($M_{H,OX}$) was about 8.54 $gC_5H_7O_2N$/d and the COD amount for

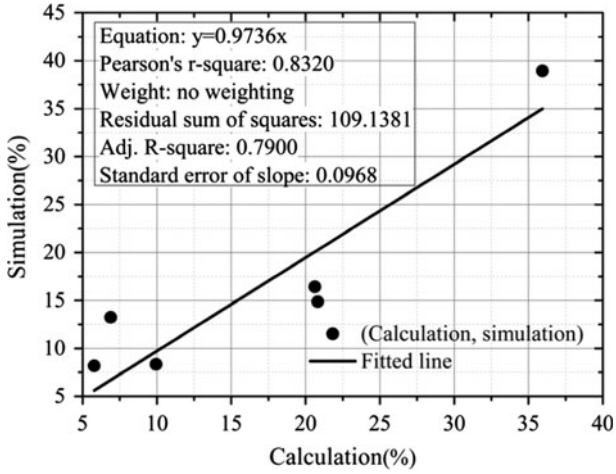

Fig. 8. Simulated vs. calculated results for the biomass fraction of microorganisms.

heterotrophs yield was 12.12 gCOD/d by Eq.(2). This value accounted for 35.94% of the total yield biomass. COD consumption in anaerobic and first anoxic stage reduced the impact to aerobic nitrification process, which favor *autotrophs* growth in the aerobic environment.

The difference of COD between the influent and effluent was considered as the removal amount of *heterotrophs* oxidation in AE, i.e. the amount of COD removed in AE was 18.83 gCOD/d.

It was significant that *heterotrophs* and PAOs were the major community. Moreover, the denitrification yield of PAOs also played an essential role in the system.

4.3.4. Verification of the biomass fractions

Wang et al. (2007) established the MUCT process kinetics model based on ASM2d, and dynamics and stoichiometry parameters were checked and verified with data collected from a yearlong pilot scale study of EBPR system [27]. The established model was run to simulate biomass distribution, and the simulated vs. calculated biomass fractions are shown in Fig. 8. The simulation value was highly consistent with calculated value based on stoichiometry and mass balance, which proved the dependability of computing method for biomass distribution based on stoichiometry and mass balance.

5. Conclusions

(1) EBPR system showed that, 46.74% of organic matter, 50.15% of nitrogen, and 37.80% of phosphorus in the influent were converted to carbon dioxide, nitrogen gas, poly-phosphate, respectively, and removed from the system. Significant amounts of intracellular PHAs were oxidized by PAOs using nitrate as an electron acceptor.

(2) Based on stoichiometry and mass balance, the yield fractions of *heterotrophs* growth in the first anoxic, second anoxic, and aerobic stages were 9.94, 6.90, and 35.94%, respectively; the yield fractions of PAOs growth in second anoxic and aerobic stages were 20.63 and 20.82%, respectively; the yield fraction of *autotrophs* growth in aerobic stage was 5.78%. These results showed that a high amount of PAOs growth on stored PHAs in anoxic condition was present in this EBPR system for municipal wastewater treatment.

(3) In the second anoxic stage, mass of nitrate reduced with PHAs as carbon source was 3.68, and 10.48 g/d of PHAs need to be supplemented for the reaction to proceed properly. The production of PAOs is 4.90 $gC_5H_7NO_2$/d. The relative enrichment of denitrifying PAOs highly depends on the availability of NO_3^--N in the second anoxic stage.

(4) For EBPR system design, it is suggested that calculation of anoxic stage volume should include denitrification rate with PHAs as carbon source. The research results in this study indicated that denitrification rate with PHAs as carbon source was 0.058 kg NO_3^--N/ (kg MLVSS·d), which could be used as the reference value to improve the design of anoxic stage in EBPR system.

(5) The computing method for biomass distribution based on stoichiometry and mass balance should be feasible. Since the synthetic municipal wastewater was used in this study, the established model should be further verified with actual wastewater in the near future.

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Nomenclatures

Q	—	influent flow rate, m^3/d
a	—	nitrified liquor recycle ratio
r	—	mixed liquor recycle ratio
s	—	sludge recycle ratio

P_i — average influent TP concentration, mg/L
P_{ANA} — average TP concentration in ANA, mg/L
$P_{rel,\ ANA}$ — mass of phosphorus release in ANA, g/d
P_{ANO_1} — average TP concentration in ANO_1, mg/L
$P_{rel,\ ANO_1}$ — mass of phosphorus release in ANO_1, g/d
P_{ANO_2} — average TP concentration in ANO_2, mg/L
$P_{up,\ ANO_2}$ — mass of phosphorus uptake in ANO_2, g/d
P_{AE} — average TP concentration in AE, mg/L
$P_{up,\ AE}$ — mass of phosphorus uptake in AE, g/d
PHA_{ANA} — average PHAs content of the sludge in ANA, mg/g MLVSS
$PHA_{syn,ANA}$ — mass synthesized PHAs in ANA, g/d
PHA_{ANO_1} — average PHAs content of the sludge in ANO_1, mg/g MLVSS
PHA_{syn,ANO_1} — mass synthesized PHAs in ANO_1, g/d
PHA_{ANO_2} — average PHAs content of the sludge in ANO_2, mg/g MLVSS
PHA_{oxi,ANO_2} — mass oxidized PHAs in ANO_2, g/d
PHA_{AE} — average PHAs content of the sludge in AE, mg/g MLVSS
$PHA_{oxi,AE}$ — mass oxidized PHAs in AE, g/d
$N_{H,i}$ — average influent NH_4^+-N concentration, mg/L
$N_{H,ANA}$ — average NH_4^+-N concentration in ANA, mg/L
$N_{H,oxi,\ ANA}$ — mass of oxidized NH_4^+-N in ANA, g/d
N_{H,ANO_1} — average NH_4^+-N concentration in ANO_1, mg/L
N_{H,oxi,ANO_1} — mass oxidized NH_4^+-N in ANO_1, g/d
N_{H,ANO_2} — average NH_4^+-N concentration in ANO_2, mg/L
N_{H,oxi,ANO_2} — mass oxidized NH_4^+-N in ANO_2, g/d
$N_{H,AE}$ — average NH_4^+-N concentration in AE, mg/L
$N_{H,oxi,\ AE}$ — mass oxidized NH_4^+-N in AE, g/d
$N_{O,\ den,\ ANA}$ — mass denitrified NH_4^+-N in ANA, g/d
N_{O,ANO_1} — average NH_4^+-N concentration in ANO_1, mg/L
N_{O,den,ANO_1} — mass denitrified NH_4^+-N in ANO_1, g/d
N_{O,ANO_2} — average NO_3^--N concentration in ANO_2, mg/L
N_{O,den,ANO_2} — mass denitrified NO_3^--N in ANO_2, g/d
$N_{O,AE}$ — average NO_3^--N concentration in AE, mg/L
$N_{O,nit,\ AE}$ — mass produced NO_3^--N in AE, g/d
X_i — MLVSS in influent, mg/L
X_{ANA} — MLVSS in ANA, mg/L
X_{ANO_1} — MLVSS in ANO_1, mg/L
X_{ANO_2} — MLVSS in ANO_2, mg/L
X_{AE} — MLVSS in AE, mg/L
M_{H,DE,ANO_1} — yield of *heterotrophs* growth in first anoxic stage, $gC_5H_7O_2N/d$
M_{H,DE,ANO_2} — yield of *heterotrophs* growth in second anoxic stage, $gC_5H_7O_2N/d$
M_{PAO,ANO_2} — yield of PAOs growth in second anoxic stage, $gC_5H_7O_2N/d$
$M_{PAO,AE}$ — yield of PAOs growth in aerobic stage, $gC_5H_7O_2N/d$
M_{AUT} — yield of *autotrophs* growth in aerobic stage, $gC_5H_7O_2N/d$
$M_{H,AE}$ — yield of *heterotrophs* growth in aerobic stage, $gC_5H_7O_2N/d$

References

[1] G. Friedl, A. Wüest, Disrupting biogeochemical cycles-consequences of damming, Aquat. Sci. 64 (2002) 55–65.
[2] Y.M. Choi, K.H. Kwon, S.W. Kim, S. Lee, K.Y. Min, Optimization of phosphorus reduction in BNR process for urban watershed management, Desalin. Water Treat. 38 (2012) 216–221.
[3] T. Mino, V. Arun, Y. Tsuzuki, T. Matsuo, Effect of phosphorus accumulation on acetate metabolism in the biological phosphorus removal process, Proceedings of the IWAPRC International conference on biological phosphate removal from wastewaters, Rome, Adv. Water Pollut. Pergamon Press, Oxford, CO (1987) 27–28.
[4] M.C. Wentzel, G.A. Ekama, R.E. Loewenthal, P.L. Dold, G.V.R. Marais, Enhanced polyphosphate organisms cultures in activated sludge systems. Part II: Experimental behavior, Water SA 15 (1989) 71–81.
[5] G.J.F. Vlekke, Y. Comeau, W.K. Oldham, Biological phosphate removal from wastewater with oxygen or nitrate in sequencing batch reactors, Environ. Technol. Lett. 9 (1988) 791–796.
[6] J.P. Kerrn-Jesperson, M. Henze, Biological phosphorus uptake under anoxic and aerobic conditions, Water Res. 27 (1994) 617–624.
[7] T. Kuba, G.J.F. Smolders, M.C.M. Van Loosdrecht, J.J. Heijnen, Biological phosphorus removal from wastewater by anaerobic-anoxic sequencing batch reactor, Water Sci. Technol. 27 (1993) 241–252.
[8] Q.Y. Yuan, J. Oleszkiewicz, Selection and enrichment of denitrifying phosphorus accumulating organisms in activated sludge, Desalin. Water Treat. 22 (2010) 72–77.
[9] Z.R. Hu, M.C. Wentzel, G.A. Ekama, Anoxic growth of phosphate-accumulating organisms (PAOs) in biological nutrient removal activated sludge systems, Water Res. 36 (2002) 4927–4937.
[10] P.S. Barker, P.L. Dold, Denitrification behaviour in biological excess phosphorus removal activated sludge systems, Water Res. 30 (1996) 769–780.
[11] T. Mino, M.C.M. van Loosdrecht, J.J. Heijnen, Microbiology and biochemistry of the enhanced biological phosphate removal process, Water Res. 32 (1998) 3193–3207.
[12] T. Kuba, E. Murnleitner, M.C.M. Van Loosdrecht, J.J. Heijnen, A metabolic model for biological phosphorus

removal by denitrifying organisms, Biotechnol. Bioeng. 52 (1996) 685–695.
[13] J.M. Duan, W. Li, K. Zhao, J. Krampe, Variation of organics, nitrogen and phosphorus within a cycle of a Bio-Denipho system, Desalin. Water Treat. 40 (2012) 24–32.
[14] S.M. Souza, O.Q.F. Araújo, M.A.Z. Coelho, Model-based optimization of a sequencing batch reactor for biological nitrogen removal, Bioresour. Technol. 99 (2008) 3213–3223.
[15] A.S. Çığgın, K. Özlem, O. Derin, Effect of high nitrate concentration on PHB storage in sequencing batch reactor under anoxic conditions, Bioresour. Technol. 100 (2009) 1376–1382.
[16] H. Mogens, G. Willi, T. Mino, M. Tomonori, M.C. Wentzel, G.V.R. Marais, M.C.M. Van Loosdrecht, Activated sludge model NO. 2 D, ASM2D, Water Sci. Technol. 39 (1999) 165–182.
[17] G. Insel, E. Dulekgurgen, S. Sözen, D. Orhon, Conceptual basis for the appropriate design of biological wastewater treatment systems: Drawbacks of existing prescriptions, Desalin. Water Treat. 26 (2011) 104–110.
[18] G.A. Ekama, Using bioprocess stoichiometry to build a plant-wide mass balance based steady-state WWTP model, Water Res. 43 (2009) 2101–2120.
[19] X.D. Hao, M.C.M. Van Loosdrecht, S.C.F. Meijer, Y. Qian, Model-based evaluation of two BNR processes —UCT and A_2 N, Water Res. 35 (2001) 2851–2860.
[20] H. Barndl, R.A. Gross, R.W. Lenz, R.C. Fuller, *Pseudomonas oleovorans* as a source of poly-β-Hydroxyalkanoates for potential applications as biodegradable polyesters, Appl. Environ. Microb. 54 (1988) 1977–1982.
[21] M. Henze, P. Harremoes, J.L.C. Jansens, E. Arvin, Wastewater Treatment Biological and Chemical Processes, Springer-Verlag, Berlin, 2001.
[22] X. Jiang, X.C. Jin, Y. Yao, L. Li, F.C. Wu, Effects of oxygen on the release and distribution of phosphorus in the sediments under the light condition, Environ. Pollut. 141 (2006) 482–487.
[23] E. Vaiopoulou, A. Aivasidis, A modified UCT method for biological nutrient removal: Configuration and performance, Chemosphere 141 (2008) 1062–1068.
[24] S.H. Chuang, C.F. Ouyang, Y. Wang, Kinetic competition between phosphorus release and denitrification on sludge under anoxic condition, Water Res. 30 (1996) 2961–2968.
[25] H.U. Nam, J.H. Lee, C.W. Kim, T.J. Papk, Enhanced biological nutrients removal using the combined fixed-film reactor with bypass flow, Water Res. 34 (2000) 1570–1576.
[26] T. Kuba, M.C.M. Van Loosdrecht, F.A. Brandse, J.J. Heijnen, Occurrence of denitrifying phosphorus removing bacteria in modified UCT-type wastewater treatment plants, Water Res. 31 (1997) 777–786.
[27] X.L. Wang, J. Yin, S.K. Li, X.D. Wei, S. Gao, Methodological study on calibrating and validating model parameters of MUCT process at low C/N ratio, Environ. Sci. 32 (2011) 3412–3418.