

Original Research

Effect of the Return Activated Sludge on the Start-Up of a Lab-Scale Continuous Flow EBPR Reactor

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Abstract

Since it was difficult in the atmosphere to obtain a deeper anaerobic environment, most previous investigations for drawing conclusions to further study denitrification and phosphorous removal, especially biological P removal, were based on the operations of SBR or full-scale reactors. In this study, a 4 L/h of continuous flow enhanced biological phosphorus removal (EBPR) reactor was constructed and started up, and activated sludge and EBPR performances were monitored for 365 days. The analysis of suspended solids (SS) and flow velocity at different temperature stages, indicated that the poor return activated sludge (RAS) was caused by the large dynamic friction of sludge in the larger specific surface area flow field. The start-up time of the bio-reactor feeding 5 mg-P/L and the $\geq 88\%$ removal efficiency was extended to 200–365 days, with the relative abundance of polyphosphate accumulating organisms (PAOs) + polyphosphate accumulating organisms (GAOs) above 12.2% at the genus level after its stabilization. The results of the start-up phase showed that the key to a lab-scale continuous flow reactor was smooth RAS. If the problem was better settled, it would provide a promising and practical alternative for further study on N and P removal.

Keywords: lab-scale continuous flow reactor, start-up phase, return activated sludge, suspended solid, enhanced biological phosphorus removal (EBPR)

Introduction

The effluent discharged from wastewater treatment plants (WWTPs) was an important source of nitrogen (N) and phosphorus (P) in many surfaces' waters, it

was the critical factor leading to eutrophication, too, so denitrification and phosphorous removal were one of the main purposes for wastewater treatment [1-3]. Many studies had revealed that one main challenge remaining with the biological nutrient removal (BNR) processes, was how to improve their efficiency, reliability, and stability since many WWTPs experienced unpredicted upsets and performance fluctuations [4-7]. In the past several decades, the most promising biotechnologies

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[8-10] had played an important role in controlling N and P emissions (e.g., anaerobic ammonium oxidation (Anammox), enhanced biological phosphorus removal (EBPR), etc.). Most of the nutrient removal performances were limited by the difficult enrichment of the functionally relevant organisms [11-14]. What's more, EBPR processes often suffer from upsets, deterioration, and failures because of secondary P release and so on, and as such must rely on costly backup chemical precipitation systems [2, 6, 15]. In order to understand the underlying biological mechanisms and solve the above-mentioned problems for P removal, most of the conclusions of the EBPR system were drawn through operating sequencing batch reactors (SBRs) [16-19] or continuous flow full-scale WWTPs [2, 13, 20, 21]. There had been fewer previous studies for drawing statistically reliable conclusions based on the operation of a lab-scale continuous flow EBPR reactor.

Alternating anaerobic and aerobic phases in the EBPR process enriched two important population groups that affect the function of the system. Compared with the pilot and full-scale WWTPs, there was a more difficult problem in obtaining a deeper and more stable anaerobic environment for the lab-scale reactors in the atmosphere, because their larger specific surfaces are easy to mass transfer and heat transfer [22-24]. In order to obtain the anaerobic phases needed by the EBPR process, bubbling nitrogen or argon as required into the reactor to create well anaerobic conditions was a successful method for dealing with this problem in the lab-scale SBRs [25, 26]. However, in the continuous flow bioreactors, there were many methods (intermittent aeration [27, 28], carrier biofilms [29], high COD load [5], the remainder of the aerobic period proceeded without aeration [19], and so on) to be used to create anaerobic conditions. During the aeration-off period of intermittent aeration, the necessary mixing was affected [30]; biofilm in the carrier was predominately good at TN removal activity, but not perfect on the biological P removal [4]; high COD could provide sufficient carbon sources for nitrogen and phosphorus removal without the promising biotechnologies. Therefore, it was clear that one of the simplest and most valid ways was for the aerobic period to proceed without aeration to allow depletion of residual DO and ensure anaerobic conditions during the operation of a lab-scale continuous flow EBPR reactor.

The aim of this paper was to show a 4 L/h lab-scale continuous flow EBPR reactor started up and had demonstrated to allow depletion of residual DO to ensure anaerobic conditions during operation. The anoxic-anaerobic-oxic reactor was set up to guarantee the acquisition of anaerobic conditions and the smoothness of RAS, through (I) the shape of an anaerobic tank should be designed in order to decrease the SSA in a small-scale bioreactor; (II) designing a swing zone (its air distributor device was

turned off /on during operational time) to prolong the time of anaerobic retention; (III) turning down the revs of the latter stirrer on the anaerobic zone, or even turn it off; (IV) bigger radius of the cross-section of flow was chosen. And the contradiction was analyzed between the difficulty of deeper anaerobic condition acquisition and easiness of transfer mass and heat, through MLSS of the RAS and SS of the effluent were analyzed and polyphosphate accumulating organisms (PAOs) were enriched.

With the lab-scale continuous flow bioreactor successful and perfect startup, (1) the EBPR and Anammox technologies which both required a well-distributed and stable DO environment could be researched better [9, 10, 31, 32]; (2) the low flow rate wastewater could be handled better, too.

Material and Methods

Bioreactor Setup

The schematic flow diagram of the laboratory-scale continuous flow bioreactor was shown in Fig. 1. The working volume of the pre-anoxic tank, anoxic tank, anaerobic tank, swing tank, aerobic tank, and secondary clarifier was 2.40 L, 4.00 L, 8.00 L, 6.68 L, 18.92 L, and 10.00 L, respectively. The flow rates in the bioreactor were controlled at 4 L/h (96 L/d) by a peristaltic pump.

The cube anaerobic zone shape was designed to the 20.0 × 20.0 × 25.0 cm (working volume 8 L) and a removable cover on it with stirrers fixed. In front of it, a 6.0 × 20.0 × 25.0 cm pre-anoxic zone and a 10.0 × 20.0 × 25.0 cm anoxic tank were designed with the same cover to avoid the influence on the anaerobic environment because of the presence of nitrates in the RAS. And behind it, a 16.7 × 20 × 25 cm swing tank (Fig. 1a1) was designed with the same removable cover, too. The porous stone diffuser (Fig. 1a1) as same as the aerobic tank was located at its bottom with an independent valve (the valve was turned off while operating). And a 47.3 × 20 × 25 cm aerobic tank, whose valve was open during that time, was continuously running as same as the several tanks in front of the aerobic tank in the hope of getting certain DO and ORP (Fig. 1(a2, a3)).

The size of the secondary clarifier was designed as a cuboid of 35 × 20 × 10 cm bonding an inverted cone with a height of 10 cm (Fig. 1(b, c)) to ensure a hydraulic retention time (HRT) of 2 h. In this smaller rate of flow bioreactor, it was designed to be a horizontal flow sedimentation tank (because of secondary P release) fixing several of the baffles without a moving mud scraper, and its floor gradient was designed as steep as possible. The pumps were installed by means of flexible pipe with ID equal to 1 cm, and to ensure artesian the reactor was linked with the clarifier by means of flexible pipe with ID equal to 2 cm (Fig. 1c).

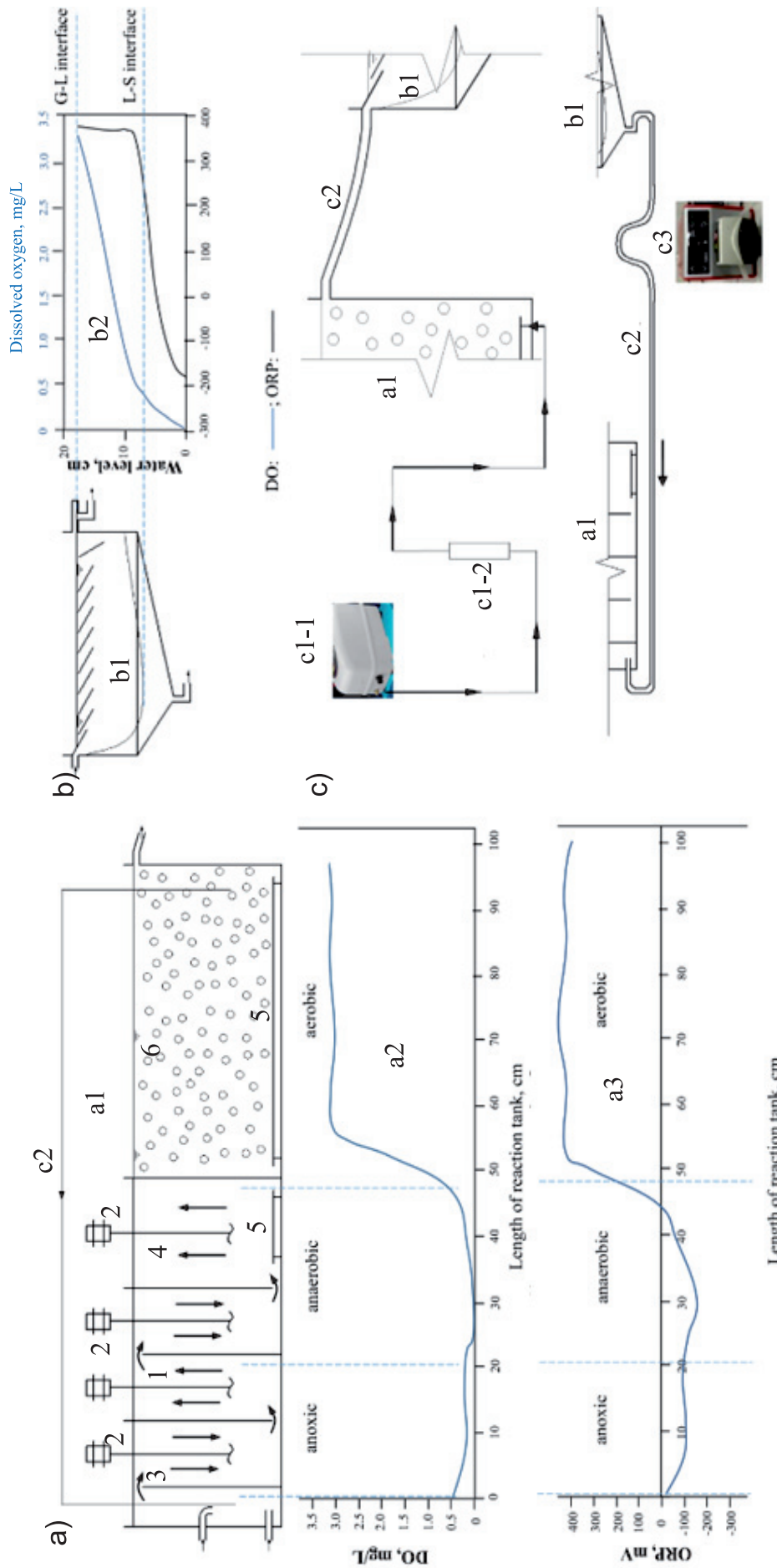


Fig. 1. The schematic and illustrated figure of the lab-scale continuous flow EBPR reactor. a) The model of an operating bio-reactor tank DO and ORP distribution: a1. bio-reactor tank (1. anaerobic tank, 2. removable cover with stirrer(s), 3. pre-anoxic and anoxic tank, 4. swing tank, 5. porous stone diffuser, 6. aerobic tank), a2. the dissolved oxygen distribution model, a3. the oxidation-reduction potential model; b) The model of the secondary clarifier DO and ORP distribution: b1. secondary clarifier, b2. the DO and ORP distribution model; c) Experimental setup apparatus installation instruction: c1-1 air pump, c1-2 airflow meter, c2. flexible pipe, c3. peristaltic pump.

Reactor Operation and Experimental Procedures

The reactor was operated for 365 days (Day 0 on Aug. 28st 2018) at indoor temperature (-4~33.5°C), monitored daily for water temperature and room temperature. The synthetic feed was prepared daily (or every 4 days), and the wasted sludge was discharged through a known volume of the return activated sludge (RAS) under the secondary clarifier tank every 2~4 days. COD and TP were analyzed every 4 days, containing influent and effluent, and the TP of P-rich liquid (filtering liquid of the waste sludge after 24 h anaerobic fermentation at approximately 25°C). Mixed liquid suspended solids (MLSS) concentration in the reactor was maintained at 1536~2671 mg/L. The sludge retention time (SRT), hydraulic retention time (HRT), sludge recycle ratio, and internal recycle ratio was set at 10~20 days, 12 h, 50%, and 200%, respectively.

The suspended solids concentration (SS) of RAS, mixed liquid (ML), and effluent were evaluated every 12 h during Days 4~13 and Days 33~46. After the manual "return activated sludge" 12 h, the mixed liquid suspended solids (MLSS) concentrations in the RAS pipe were analyzed on operational days 70, 102, 120, 164, 180, 188, and 196; when waste sludge was discharged, the sludge discharge velocity was calculated according to the volume, the time of sludge discharge and the diameter of sludge discharge pipe on operational days 70, 120, 188, and 196. The twice SBR batch tests were performed using the mixed liquid from the aerobic tank on days 220 and 360. The microbial community structure was analyzed for activated sludge with high-throughput sequencing technology on day 216.

The DO, ORP, MLSS, $\text{NH}_4^+\text{-N}$, and $\text{NO}_2^-\text{-N}$ variations were analyzed at a typical cycle on day 256. The DO, ORP, and temperature were monitored using a multi-parameter controller (HQ30d, HACH, United States). The SS, MLSS, $\text{PO}_4^{3-}\text{-P}$, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, COD, and TP concentrations were analyzed according to Standard Methods [33].

Synthetic Wastewater and Inoculated Sludge

The lab-scale continuous flow bioreactor was fed synthetic wastewater, which was composed of $\text{C}_6\text{H}_{12}\text{O}_6$ (carbon source), NH_4Cl , KH_2PO_4 , CaCl_2 , and $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, tap water, trace elements, and so on. The low N level supplied to the reactor limited the nitrification metabolism to maintain an adequate amount of N for biomass assimilation and discharged ≤ 15 mg/L TN concentrations in the effluent (Chinese National Sewage Discharge Standard Class I (grade A) level (GB18918-2002)) [34]. COD/N/P ratio in the influent was kept as 400/20/5 such that it simulated actual medium-strength domestic wastewater [35]. The composition of trace elements and others in the synthetic feed is detailed in Schuler and Jenkins, 2003 [36].

The reactor was initially seeded with 12.5 L of activated sludge, which was taken from Xi'an Third Wastewater Treatment Plant, Shaanxi Province, China. After seeding with activated sludge, continuous feeding of the reactor was started.

Batch SBR Tests and Microbial Sequencing

The twice anaerobic-aerobic cycle studies were performed in a separate batch reactor with 0.5 L of mixed liquid from the aerobic tank using acetate. The phosphate and acetate feed concentrations were 10 mg-P/L and 100 mg-COD/L, respectively. The nitrogen concentration was 8 mg $\text{NH}_4\text{-N/L}$ with nitrification inhibited. All other feeds and operational conditions were like the other batch SBR tests [16, 37]. The bioreactor was well operated before the batch tests were performed.

The biomass of mixed liquid was collected from the anaerobic, anoxic, aerobic tank and secondary clarifier to verify the connectivity of sludge in the reactor. The biomass in the mixed liquid was sent for inspection after it was centrifuged, dehydrated, and frozen. The microbial community and statistics were performed using our previous approach [38].

Results and Discussion

Performances of the Operation in this Reactor

At the initial stage of reactor operation, the RAS pipe color often turned shallow to the color of mixed liquid pipe, or even to the effluent and influent pipe color. And Fig. 2 showed the variation of temperature during the operation of the reactor, but several data on the water temperature were missing on operational days 156~163 because of Spring Festival (The synthetic feed was prepared every 4 days). Compared with SBR which retained sludge used as return activated sludge (RAS), the RAS in the pipe of this reactor was coming from the secondary clarifier. The MLSS of this operating reactor tank was decreasing with the MLSS of RAS decreasing. Results exhibited markedly it needed to add manual reflow of sludge because of its poor return activated sludge. It could be noticed that exhibited a similar cosine pattern water temperature with the range of 2.0~30.9°C in this reactor, which scope was larger than Xi'an Third Wastewater Plant (108°E, 34°N) of 13.0~25.0°C, also larger than the Nine Springs WWTP (Madison, WI, USA, 89°W, 43°N) of 10~22°C [20], and the change of water temperature varied with the change of room temperature. It suggested that the lab-scale EBPR reactor was more likely to heat transfer than full-scale wastewater plants because of its small volume and large specific surface area [22].

As shown in Fig. 3a) and Fig. 3b), the variation of COD and TP were monitored in the influent and effluent

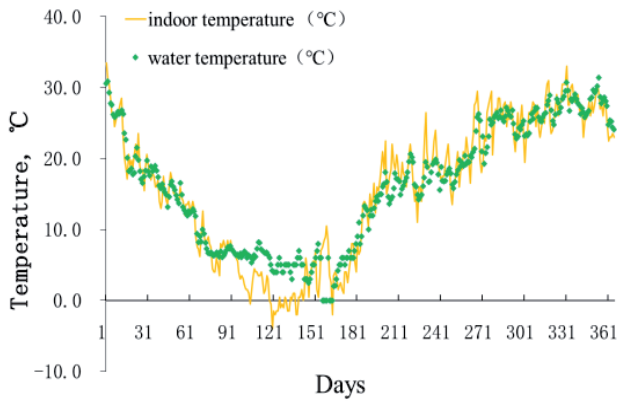


Fig. 2. Variations of temperature during the operation.

every 4 days during the start-up phase. Except for days 135~200 when the water temperature experienced a baptism below 10°C (Fig. 2), the steady COD and TP removal efficiency of 85~96% was observed and the effluent reached the Chinese National Sewage Discharge Standard Class I (grade A) level. The COD ($\geq 86\%$) and TP ($\geq 88\%$) removal efficiencies suggested that the activated sludge process (as a classical water treatment method) has a very stable removal effect on COD and TP at low-ratios P/C in wastewater, and its start-up time was not beyond 20 days [35, 39].

This reactor, which only provided the nitrogen of anabolism, was started as an EBPR system to need strict conditions and plenty of time to get acclimatize and become functionally established. And in Fig. 3b),

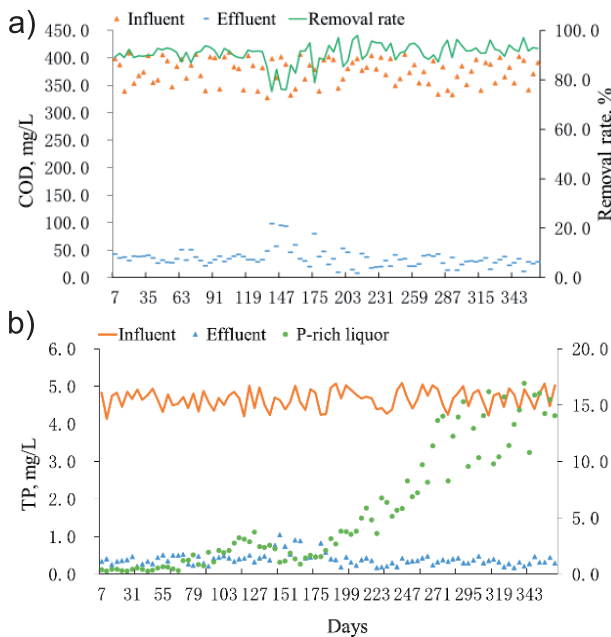


Fig. 3. Variation of performances with the increasing operating time for a year in the reactor. a) Variation of chemical oxygen demand (COD); b) Variation of total phosphorus (TP).

a slow but steady increase in the TP of the so-called P-rich liquid, coming from anaerobic fermentation of the waste sludge for 24 h, was observed and eventually stabilized to 15 mg P/L during days 270~365. The TP dates of P-rich liquid proved that the efficiency and robustness of EBPR systems were affected by several key operational and environmental factors (e.g., low temperature, which the classical activated sludge performances were even affected, too (Fig. 2; Fig. 3), etc.) [6, 40]. Therefore, the start-up time of the lab-scale continuous flow EBPR reactor was extended to 200~365 days because of the poor return of activated sludge and the large water temperature scope (especially part days of the low temperature).

Impact Analyses of the Poor RAS

To assess how the return activated sludge (RAS) was affected by the small-scale volume pipe and tank, the suspended solids (SS) and flow velocity were performed at different temperature stages. Fig. 4a) and Fig. 4b) showed the variations of SS in RAS, mixed liquid, and effluent during Days 4~13 and 33~46 when the water temperature had dropped approximately 10°C. While Fig. 4c) showed the variation of the sludge discharge flow velocity and the MLSS of RAS with the variation in water temperatures. The results showed that the SS periodically reduced to certain values after every manual reflow of sludge: the effluent SS tended to decrease first and then increase, the SS value of RAS was reduced by almost half, and the SS value of mix liquid by one-third. Simultaneously, the cycle of manual reflow sludge needed was obviously shortened with the water temperature down. Additionally, the value of MLSS in the RAS and flow velocity decreased with temperature decreased. It suggested that the lower the water temperature, the worse the reflux of activated sludge, except the RAS when its water temperature was 6.0°C on Day 102 was better than it when its water temperature was 8.0°C on Day 180. The opposite variation was likely because the former water got cooling and the latter began warming.

As was well known, the coefficient of kinetic viscosity of the return activated sludge increased with the temperature decreased and sludge had a greater coefficient of kinetic viscosity than water as a non-Newtonian body of Bingham plastic fluid. So, the effect of dynamic friction on the efficiency of RAS was enhanced when it had an increased coefficient of kinetic viscosity, and reflux sludge efficiency became worse. The lab-scale continuous flow EBPR reactor had a poorer RAS efficiency than full-scale WWTPs in this study [13] because of its smaller volume, and the sludge was flowing along the wall of the pipe or the tank with its greater dynamic friction [41]. When the water in RAS was pumped back, the real return activated sludge needed was left in the second clarifier [42] and still needed manual reflow sludge.

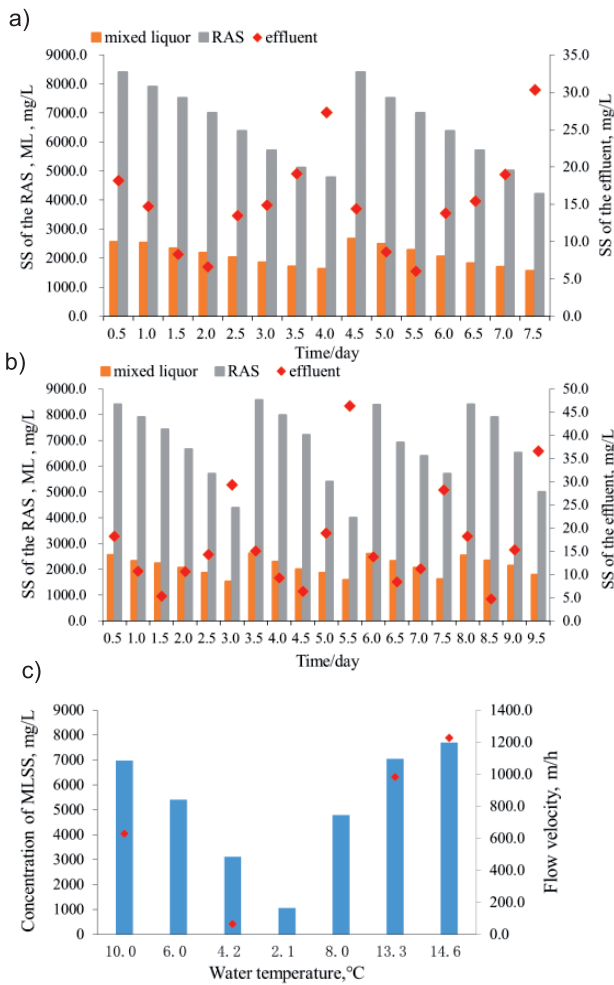


Fig. 4. Variations of the suspended solid in RAS, mix liquid and effluent. a) During days 4~13 (water temperature was 28.6~24.5°C); b) During days 33~46 (water temperature was 19.0~13.2°C); c) Variations of discharge sludge and RAS at different temperatures.

Performances of the Reactor at a Typical Cycle

From the pre-anoxic tank to the secondary clarifier shown in Fig 1, the variations of DO and ORP were also

observed in the various tanks (Table 1), even containing the sludge fermented to produce methane. And Fig. 5a) Variations of NO₂⁻-N with DO and ORP at a cycle on day 256; b) Nutrient removal at the typical cycle at a different phase. By and large, the distribution of DO and ORP at a typical cycle (Table 1) was successful after the reactor design efforts. The DO and ORP variations in Fig. 5a) and Fig. 1 were similar, and NO₂⁻-N appeared more in the measured anoxic environment in Tanks 1, 2, and 5 (Fig 5a).

Nutrient removal and MLSS variation were investigated at the typical cycle (Fig 5b) to further verify the distributions of DO and ORP in the continuous flow bioreactor. In the aerobic phase, the DO and ORP were above 3 mg/L, and 200 mV, respectively [43]; in the anaerobic phase, the DO was less than 0.2 mg/L [5, 6, 44], but the ORP was not below -200 mV, even above -180 mV [9] 10%. These imperfections might be the result that the MLSS value being below 3000 mg/L [13, 17] and obviously moving backward in different tanks (Fig. 5b). NH₄⁺-N concentration dropped substantially from 19.3 mg/L (influent) to 4.235mg/L (Tank 1) as a result of dilution and denitrification, and PO₄³⁻-P concentration conversion here as this result contained anaerobic P release, which peaked at Tank 4. And NH₄⁺-N and PO₄³⁻-P concentration obviously dropped from Tank4 to Tank6 (Fig. 6b) as a result of nitrification and enhanced biological P uptake [19, 44]. In addition, sludge-water separation could be completed before anaerobic P release in the secondary clarifier; but this condition was obviously achieved in the sludge, especially return sludge accumulates here. The transformation law of the MLSS, NH₄⁺-N, and PO₄³⁻-P proved that under the premise of sludge concentration was guaranteed, DO and ORP could reach the value range for nitrogen and phosphorus removal after the stabilization of the reactor [5, 9, 14, 16].

Analysis on PAOs and Microbial Community

Fig. 6a) P release and uptake profiles in batch tests performed with sludge from this reactor on days 220

Table 1. Measured values of DO and ORP in the reactor at a typical cycle on day 25.

Position	Model coordinate	≤0 cm	0~10 cm	10~20 cm	20~30 cm	30~47 cm	≥47 cm
	Number	Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6
Dissolved oxygen (DO), mg/L		0.33	0.26	0.10	0.07	0.14	3.68
Oxidation-reduction potential (ORP), mV		-57.5	-70.1	-138.6	-161.9	-124.8	276.7
Position	Secondary clarifier	Liquid supernatant ⁽¹⁾	Near the L-S interface ⁽²⁾		Activated sludge ⁽³⁾	Sludge of producing methane*	
Dissolved oxygen (DO), mg/L		3.70	0.41		0.16	0.02	
Oxidation-reduction potential (ORP), mV		224.1	-9.9		-92.9	-259.9	

⁽¹⁾⁻⁽³⁾ The values of the different locations were observed in the secondary clarifier, but they were homogeneous in other tanks.

* The waste-activated sludge was kept in a sealed plastic bucket to produce methane.

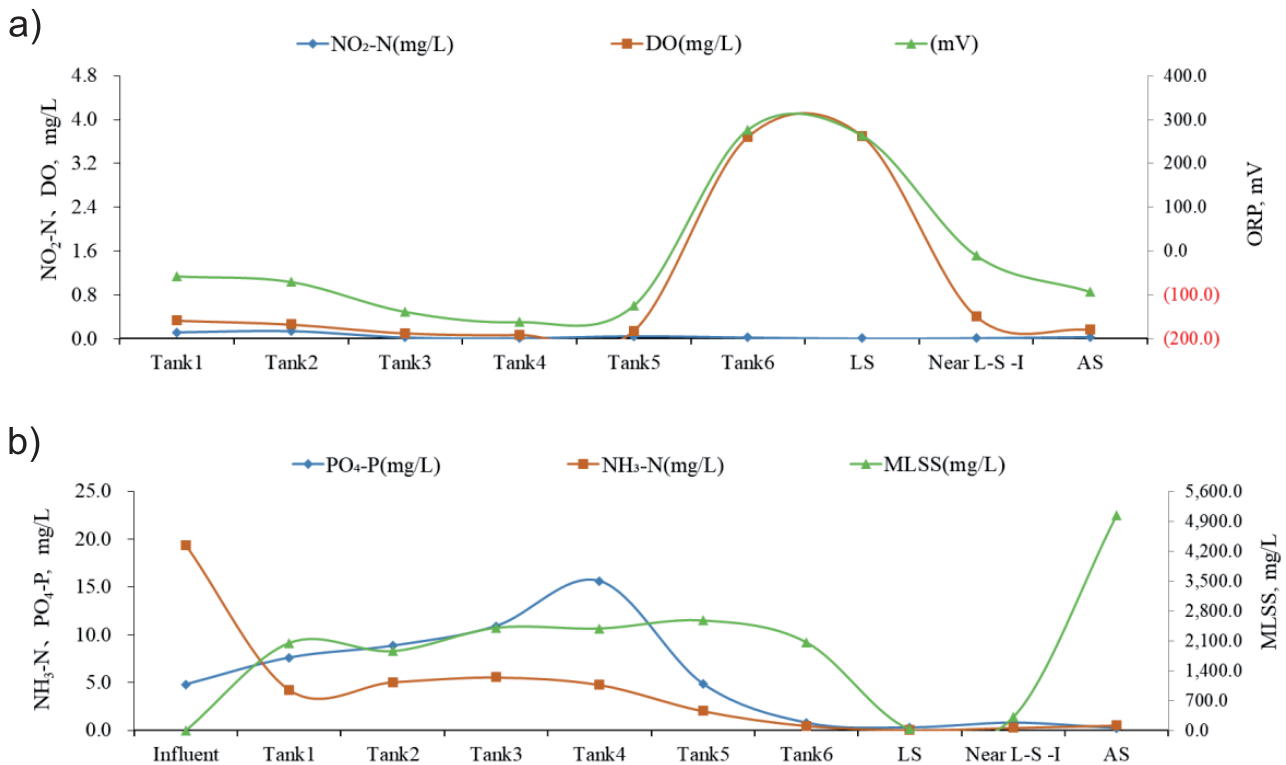


Fig. 5. Variation of performances at a typical cycle on day 256.

a) Variation of nitrate-nitrogen ($\text{NO}_2\text{-N}$), dissolved oxygen (DO), and oxidation-reduction potential (ORP); b) Nutrient removal and Variation of MLSS (or SS) conversion;

The LS, Near L-S-I, and AS stands for Liquid supernatant, Near the L-S interface, and Activated sludge, respectively.

and 360; Fig. 6b) the genus level characteristics of community distribution in biomass of different phase tanks on day 216.

Perfect phenomena of anaerobic P release and aerobic P uptake [45] both were observed in the Batch SBR tests. The 4~5 mg P/L of anaerobic P release was obviously at a low level, but its net aerobic P uptake was not low, compared with the EBPR reports [13, 16, 21, 39]. The top 7 microorganisms, which accounted for 70.2~80.6% of the top 20 genera, showed little difference in each phase tank, and the PAOs (*Candidatus Accumulibacter*, *Tetrasphaera* and *Dechloromonas*) plus GAOs (*Candidatus Competibacter*) accounted for 7.8~12.2 % of the all genera detected (Fig. 6b). The results of PAOs analysis suggested that the lab-scale continuous flow EBPR reactor was gradually started up for a low C/P synthetic wastewater using $\text{C}_6\text{H}_{12}\text{O}_6$ as carbon source, and the *Candidatus Competibacter* Accounting (GAOs) for a large proportion.

In this reactor, the *Candidatus Accumulibacter* genus PAOs were enriched less than *Tetrasphaera*, and P release/substrate cons of *Tetrasphaera* were lower [17, 25] which also explained the low P release in batch tests (Fig. 6a). And the high relative abundance of *Dechloromonas* was a characteristic of the EBPR reactor [46, 47]. Compared with the lab-scale SBRs and full-scale WWTPs [43, 44, 48], there were some differences in the lab-scale continuous flow EBPR reactor of this

study. Because of its poor RAS, its connectivity was also determined by analyzing microbial communities from different tanks, rather than just taking one of them. The relative abundance of genera varied greatly in different tanks due to the different MLSS values and dead zones [7, 19], and part of them varied by order of magnitude, which included autotrophic, fermentation, denitrification, and other genera (Fig. 6b).

Among the above-mentioned genera with the relative abundance varied greatly in different tanks, it was in the secondary clarifier that the relative abundance of nitrifying and denitrifying bacteria was both high. And the "Red bacteria" appeared (Fig. 7) in the secondary clarifier during the operation days 35~60. Various studies had shown that the abundance of anammox, commonly known as "Red bacteria", was low and sensitive to environmental conditions [10, 12]. Therefore, with the manual reflow sludge cycles discovered and shortened the anammox bacteria did not appear once more in this reactor. The unstable and unknown operation of the secondary clarifier had allowed the emergence of dissolved oxygen bands with different concentrations, which increased the probability of the emergence of dissolved oxygen suitable for the growth of Anammox [49, 50]. The result indicated that the operation of those discontinuous reactors could produce more complex nitrifying, which increased the possibility of nitrous oxide (N_2O) as a very

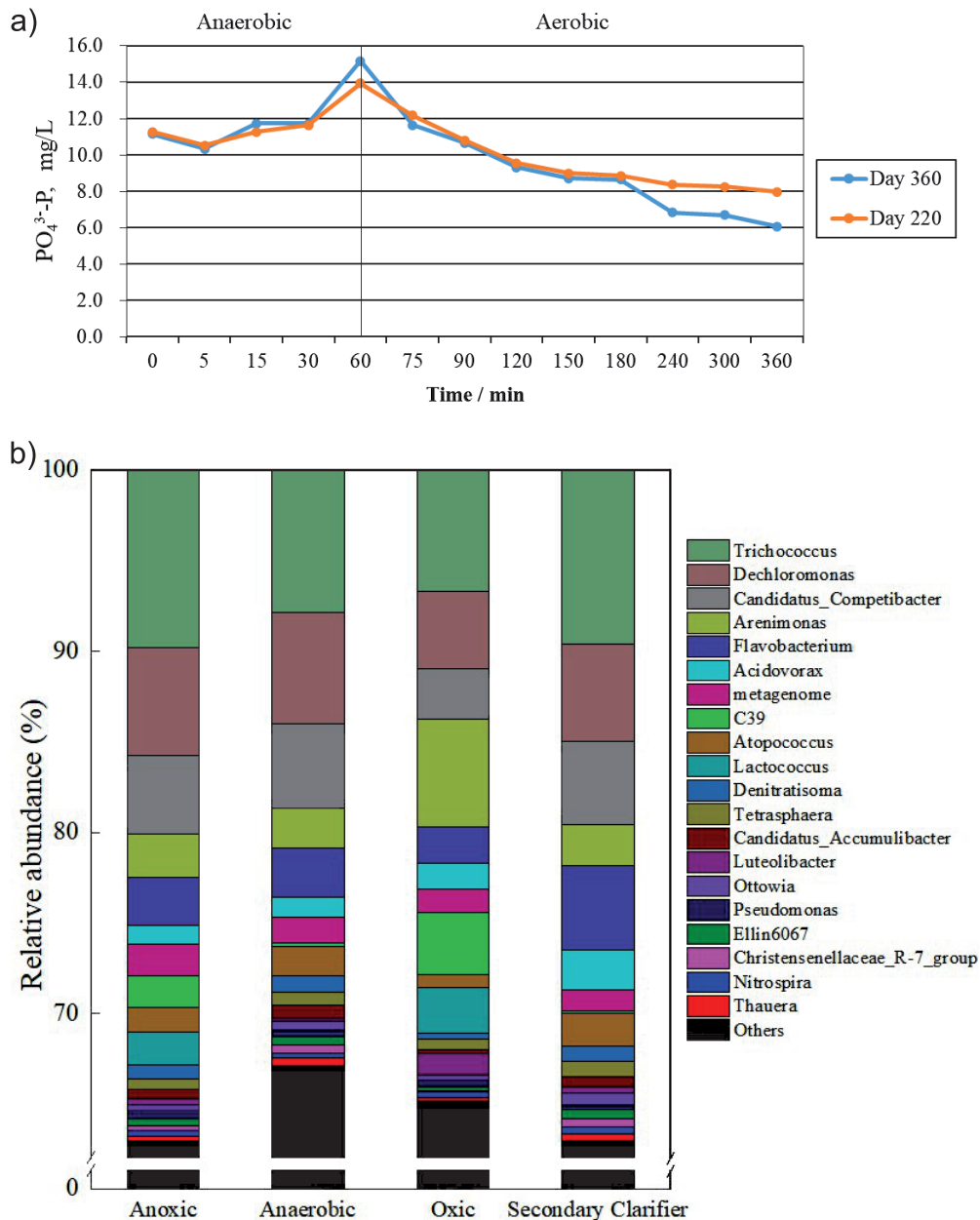


Fig. 6. Characteristics of PAOs and microbial community. a) Anaerobic P release and aerobic P uptake profiles of PAOs in the activated sludge on operational days; b) Characteristics of community distribution at genus level on operational day 216.



Fig. 7. Photo of anammox in the secondary clarifier.

strong greenhouse gas, >300 times stronger than CO₂) production [51, 52]. It also proved that the operation of a continuous flow reactor in the laboratory was also necessary for the future study of nitrogen removal and other aspects.

Conclusions

The key to the lab-scale anoxic – anaerobic - oxic reactor was found during the start-up of a 4 L/h continuous flow EBPR reactor for 365 days. The analysis of the contradiction which between the difficulty of deeper anaerobic condition acquisition

and easiness of transfer mass and heat, indicated that the smoothness of return activated sludge (RAS) was the key to solve the problem that most previous investigations were based on the operation of SBR or full-scale reactors but only a few studies on a lab-scale continuous flow reactor. When the concentration of MLSS of the lab-scale continuous flow reactor was sufficient, the most promising biotechnologies which required well-distributed and stable dissolved oxygen (DO) environment that can be studied in a laboratory by obtaining statistically reliable conclusions.

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Conflict of Interest

The authors declare no conflict of interest.

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