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Enhanced Biological Nutrients Removal in Modified Step-feed Anaerobic/Anoxic/Oxic Process*

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Abstract In order to enhance phosphorus removal in traditional step-feed anoxic/oxic nitrogen removal process, a modified pilot-scale step-feed anaerobic/anoxic/oxic (SFA^2/O) system was developed, which combined a reactor similar to UCT-type configuration and two-stage anoxic/oxic process. The simultaneous nitrogen and phosphorus removal capacities and the potential of denitrifying phosphorus removal, in particular, were investigated with four different feeding patterns using real municipal wastewater. The results showed that the feeding ratios (Q_1) in the first stage determined the nutrient removal performance in the SFA^2/O system. The average phosphorus removal efficiency increased from 19.17% to 96.25% as Q_1 was gradually increased from run 1 to run 4, but the nitrogen removal efficiency exhibited a different tendency, which attained a maximum 73.61% in run 3 and then decreased to 59.62% in run 4. As a compromise between nitrogen and phosphorus removal, run 3 ($Q_1 = 0.45Q_{total}$) was identified as the optimal and stable case with the maximum anoxic phosphorus uptake rate of 1.58 $mg\cdot(g\ MLSS)^{-1}\cdot h^{-1}$. The results of batch tests showed that ratio of the anoxic phosphate uptake capacity to the aerobic phosphate uptake capacity increased from 11.96% to 36.85% with the optimal influent feeding ratio to the system in run 3, which demonstrated that the denitrifying polyP accumulating organisms could be accumulated and contributed more to the total phosphorus removal by optimizing the inflow ratio distribution. However, the nitrate recirculation to anoxic zone and influent feeding ratios should be carefully controlled for carbon source saving.
Keywords nutrients removal, nitrogen, phosphorus, anaerobic/anoxic/oxic, step-feed

1 INTRODUCTION

To prevent the eutrophication in enclosed water system, biological nitrogen and phosphorus removal from wastewater has been extensively investigated and employed [1–3]. For simultaneous nitrogen and phosphorus removal, wastewater treatment system is designed to provide anaerobic, anoxic and aerobic environment for phosphorus release, denitrification and nitrification/phosphorus uptake, respectively [4]. In principle, oxygen is used as an electron acceptor for phosphorus uptake and nitrification in these processes. Nitrate, the product of nitrification and the electron acceptor for denitrification, is generally recognized as an inhibiting component for the biological phosphorus removal process [5, 6]. However, many studies have verified that in the absence of any exogenous carbon sources in the anaerobic zone, phosphorus removal may occur in the presence of nitrate [7–10]. It is presumed that the denitrifying polyP accumulating organisms (DNPAOs) use intracellular storage compounds poly-hydroxyl-alkanoates (PHA) as carbon and energy sources to assimilate P and synthesize polyP, as polyP accumulating organisms (PAOs) do, and use nitrate as terminal electron acceptor [11]. If the DNPAOs take up and store phosphate using nitrate as electron acceptor, then the organic carbon substrate can be used simultaneously for both phosphorus and nitrogen removal. This is of significance since organic carbon content in most municipal wastewater is often limited for phosphorus and nitrogen removal. The accumulation of DNPAOs has been investigated extensively in laboratory scale reactors and it has been shown that phosphorus removal by DNPAOs has similar capacities and characteristics as PAOs in anaerobic processes [9]. The main advantages of applying denitrifying phosphorus removal are energy saving [aeration and external carbon source] and less sludge production [12]. It has been demonstrated that numerous treatment processes, such as dephanox process, the anaerobic-anoxic-oxic (A^2/O) process and the University of Cape Town (UCT) process developed for simultaneous nitrogen and phosphorus removal, are well suitable for denitrifying phosphorus removal [13–15].

The step-feed anoxic/oxic activated sludge process is characterized by multi-stages of anoxic/oxic (A/O) reactor in series, which eliminates the need for internal recycling and optimizes organic carbon utilization for denitrification [16, 17]. A pilot-scale step-feed A/O nitrogen removal system has been used to treat low COD/TN municipal wastewater with deep nitrogen removal by making full use of carbon source in raw wastewater, but it is unfavorable for optimal phosphorus removal. To accumulate the phosphorus removing organisms, especially the DNPAOs, a modified step-feed A/O system, defined as SFA^2/O (step-feed anaerobic/anoxic/oxic), is developed and adopted in

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this study. An anaerobic zone is introduced before the first anoxic zone for phosphorus release. The returned activated sludge from the settler is recirculated to the beginning of the anoxic zone of first stage, and the mixed liquor in this anoxic zone is recirculated to the beginning of the anaerobic zone. The first stage is similar to the UCT-type configuration, which is recognized as an alternative denitrifying phosphorus removal process with single sludge [13, 18]. The modified process combines the advantages of pre-denitrifying UCT-type system and the step-feed A/O system. It is expected to enhance the phosphorus removal and use the carbon source to a best extent. Due to the coexistence and/or competition behavior between microorganisms in the simultaneous nitrogen and phosphorus removal process, however, the operation of this system is complicated especially when the step-feed control strategy is adopted. The stability of the system and effective N and P removal are affected by several factors, such as the internal recycling ratio, influent flow distribution, C/N and C/P. To make use of the available organic substrate for N and P removal in low COD/N municipal wastewater treatment system, the optimization of feeding ratios is a key issue in the step-feed A^2/O system.

In this study, the technical feasibility of simultaneous phosphorus and nitrogen removal is investigated in the modified step-feed nutrients removal system, SFA^2/O. The objectives are as follows. (1) Enhance the phosphorus removal by introducing the anaerobic reactor and reduce the external carbon source dosage by strategic step-feed scenario. (2) Investigate the effects of influent flow distribution ratio on operation and provide the optimal feeding ratio for N and P removal in municipal wastewater, especially at low C/N ratio. (3) Evaluate the possibility of denitrifying phosphorus removal and the contribution of denitrifying dephosphatation to the phosphorus removal. Based on the operation of the pilot-scale step-feed A^2/O system, we expect to provide some fundamental data for upgrading the existing wastewater treatment plants and solve the conflictive problems in the operation of conventional nutrient removal process.

2 MATERIALS AND METHODS

2.1 Pilot-scale step-feed A^2/O system

2.1.1 *Experimental conditions and setup*

Figure 1 shows the schematic diagram of the pilot-scale SFA^2/O system. The notations A, Di(i = 1,2,3) and Nij(i = 1,2,3; j = 1,2,3) indicate the anaerobic zone, anoxic zone of stage i, and aerobic zone j of stage i, respectively. The total influent flow (Q_{total}) from the primary settler is divided into three streams: Q_1, Q_2 and Q_3, which are the feeding flows into the anaerobic zone (A), the second anoxic zone D2, and the third one D3, respectively. Two recycle flows compose the recirculation system: one is the returned activated sludge Q_r ($Q_r = 0.6Q_{\text{total}}$) from the secondary settler to the beginning of the first anoxic zone (D1) and the other is the internal recycle flow Q_c ($Q_c = 0.3Q_{\text{total}}$) from the end of first anoxic zone (D1) to the beginning of the anaerobic zone (A). The total working volume is 320 L in the reactor. The effective volumes of the anaerobic reactor, anoxic1, aerobic1, anoxic2, aerobic2, anoxic3 and aerobic3 are 32 L, 32 L, 64 L, 32 L, 64 L, 32 L and 64 L, respectively.

The stream Q_1 provides the necessary substrate for phosphorus release in the anaerobic zone. The intracellular PHA accumulated by phosphorus removal organisms may, thus, serve as a carbon source for denitrification and phosphorus uptake when nitrate is available in the subsequent anoxic zone (D1). Residual phosphorus is absorbed in the subsequent aerobic zone (N11−N13). It is assumed that no phosphorus is released in the anoxic zone D2 and D3 with a low C/N feeding influent.

During the start-up period of this study, the dissolved oxygen (DO) concentration in aerobic zones was controlled at above 2.5 mg·L^{-1} so that the nitrifying bacteria grew prosperously. In order to evaluate the effect of feeding ratio on nitrogen and phosphorus removal, once the steady-state condition was achieved, the influent flow distribution ratio was changed to 0.2∶0.3∶0.5 (run 1), 0.3∶0.4∶0.3 (run 2), 0.45∶0.35∶0.2 (run 3) and 0.6∶0.25∶0.15 (run 4). Each experimental circle lasted for 20 or 30 days (*i.e*, 2 or 3 times of solid retention time, SRT) for data collection. The influent flow rate and hydraulic retention time (HRT) of each stage under different runs are shown in Table 1. The other operational parameters of A^2/O step-feed process are shown in Table 2.

2.1.2 *Wastewater and sludge*

The wastewater used in this study was collected from the sewers of campus residential area. The characteristics of the wastewater are summarized in Table 3. The activated sludge was seeded from a full-scale wastewater treatment plant in Beijing, China (200000 m^3·d^{-1}), with regular operation and nutrient removal. The sludge was fed to an anaerobic/aerobic operating sequencing batch reactor (SBR) to accumulate phosphorus removal organisms. After the operation for one month, a maximum specific P release rate 10.98 mg·g^{-1}·h^{-1} and uptake rate 12.94 mg·g^{-1}·h^{-1} were achieved with 100% P removal. Then the sludge was inoculated to the pilot-scale SFA^2/O system and different operating conditions were carried out.

2.2 Batch test for sludge characterization assay

To evaluate the sludge performance on phosphorus release and uptake when the system was operated at different feeding patterns, batch experiments were carried out at the same time. The activated sludge at the end of anaerobic stage was transferred to three paralleled SBR reactors, each with a volume of 10 L. The temperature was kept at 20°C. NaAc and K_2HPO_4 were fed and the initial COD and PO_4^{3-}-P concentration were maintained at 200 mg·L^{-1} and 6 mg·L^{-1},

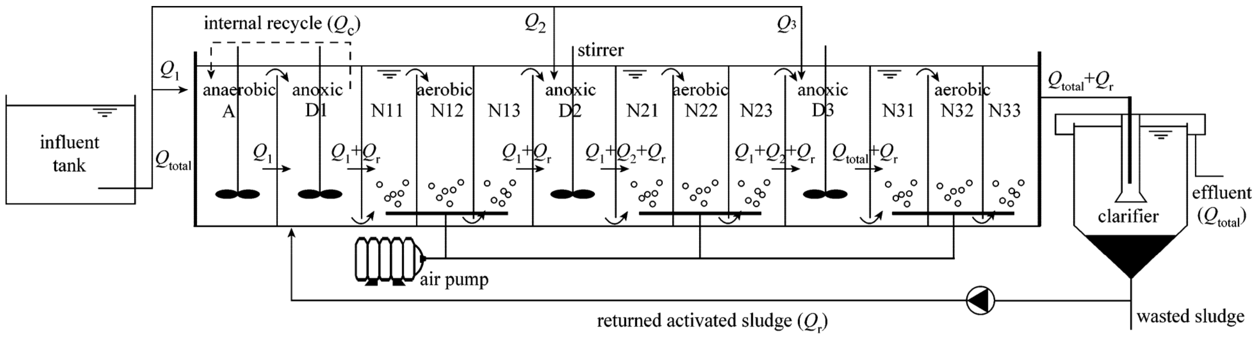


Figure 1 Schematic diagram of the modified SFA²/O process

Table 1 The influent flow rate and hydraulic retention time under different runs

Run	Time/d	$Q_1:Q_2:Q_3$	Hydraulic retention time (HRT)/h						
			A	D1	N11−N13	D2	N21−N23	D3	N31−N33
1	0−20	0.2 ∶ 0.3 ∶ 0.5	4.0	4.0	8.0	2.67	5.33	1.6	3.2
2	20−50	0.3 ∶ 0.4 ∶ 0.3	2.67	2.67	5.33	2.0	4.0	2.67	5.33
3	51−80	0.45 ∶ 0.35 ∶ 0.2	1.78	1.78	3.56	2.29	4.58	4.0	8.0
4	81−99	0.6 ∶ 0.25 ∶ 0.15	1.33	1.33	2.66	3.2	6.4	5.33	10.67

Table 2 The operational parameters of SFA²/O process

Q_{total}/L·d^{-1}	Q_r/L·d^{-1}	Q_c/L·d^{-1}	SRT/d	T/°C	pH	DO/mg·L^{-1}	MLSS$_{ave}$/g·L^{-1}
960	576	288	10	20±1	7.14−7.67	2.2−4.16	3.8

respectively. The anaerobic condition was controlled at 180 min for complete P release and adequate PHB (poly-β-hydroxybutyrate) synthesis in the sludge. The aerobic or anoxic condition was kept for 300 min for full phosphorus uptake. Samples were collected at 30 min interval.

2.3 Analyses

Samples for dynamic studies were collected regularly from different zones of the reactors. The conventional parameters including NH_4^+-N, NO_2^--N, NO_3^--N, COD, BOD_5, MLSS (mixed liquid suspended solids) and SVI (sludge volume index) were routinely analyzed according to the Standard Method [19]. Multi-3000 N/C analyzer (jena, Germany) was used for TN analysis. DO, pH and temperature were determined on-line using pH/Oxi 340i sensors (WTW, Germany).

3 RESULTS AND DISCUSSION

3.1 Phosphorus removal: P release and P uptake

In the modified step-feed A²/O system, P release and P uptake mainly occurred in the first stage. The factors influencing P removal were evaluated for the optimal operation of the first stage. Figs. 2 and 3 show the TP removal efficiency of runs 1−4 and the concentrations of phosphorus and nitrate in different phases, respectively. During the first period (run 1), P removal efficiency is only 19.17% even though the PAOs are enriched in the seeding sludge. The poor phosphorus removal efficiency mainly results from the lower influent feeding ratio of Q_1. Less organic compounds in Q_1 has a negative effect on the PHA synthesis [20], decreasing the phosphorus release in the anaerobic zone. In the absence of any exogenous carbon source or intracellular compounds (PHA) in the aerobic zone, PAOs will not grow aerobically and polyP synthesis efficiency will be decreased greatly. As a result, obviously excessive P uptake phenomenon is not observed in the aerobic zone. In addition, due to the higher nitrate in

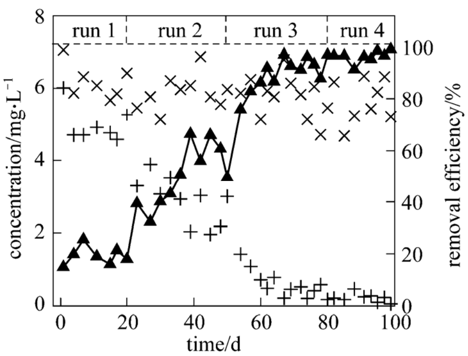


Figure 2 TP removal efficiency in different phases
× influent; + effluent; ▲ removal efficiency

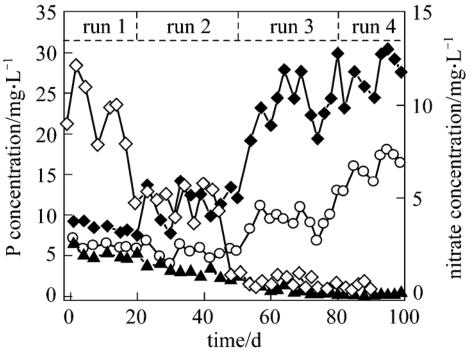


Figure 3 Concentration of phosphorus and nitrate in different phases
○ anoxic (D1) effluent P; ▲ aerobic (N13) effluent P;
◆ anaerobic (A) effluent P; ◇ anoxic (D1) effluent nitrate

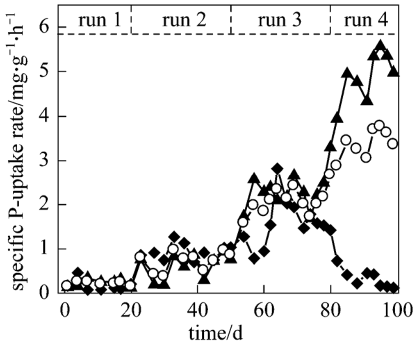


Figure 4 Comparison of specific anoxic and aerobic P-uptake rate
◆ anoxic (D1) P uptake; ▲ aerobic (first stage) P uptake;
○ average P uptake rate of system

Q_r and lower organic substrate in feeding flows (Q_1), nitrate concentration is higher, up to 10.36 mg·L^{-1} in the effluent of anoxic zone (D1) (see Fig. 3). Nitrate is introduced to the "anaerobic" zone with internal recycling flow (Q_r), results in the competition between denitrifying bacteria and PAOs for available substrate, so that P release is inhibited [6, 21, 22]. Therefore, only an average phosphorus concentration 8.95 mg·L^{-1} and a maximum specific P release rate 0.28 mg·g^{-1}·h^{-1} (see Fig. 3) are obtained in the anaerobic zone. However, in runs 2, 3 and 4, P release and uptake rate increase as Q_1 is increased to 0.6(Q_1/Q_{total}), phosphorus concentration in effluent is lower than 0.5 mg·L^{-1}, and 96.25% TP removal efficiency is obtained in run 4, indicating that the complete P removal is achieved. Fig. 3 shows that when feeding ratio Q_1 increases (from run 1 to run 4), the specific maximum P release increases from 0.28 to 7.35 mg·g^{-1}·h^{-1}. In runs 3 and 4, higher P release rates are obtained. The maximum phosphorus release rates are 5.29 and 7.35 mg·g^{-1}·h^{-1} and the average phosphorus concentrations in the anaerobic zone are 24.01 and 27.9 mg·L^{-1}, accordingly. In run 4, however, the P release rate is not improved compared with run 3, which mainly ascribes to the higher carbon source concentration induced by influent in run 4, so that the PAOs do not have enough anaerobic time to take all organic substrate.

P uptake in the anoxic reactor and aerobic zone was evaluated. Fig. 4 shows the specific P uptake rate for different feeding ratios. Similar to P release rate in anaerobic zone, aerobic P uptake in the first stage and the average P uptake of the whole system increase significantly from run 1 to run 4. The average phosphorus concentration in aerobic zone (N13) is 5.45, 5.08, 5.14 and 1.84 mg·L^{-1}, and the average P uptake of the system is 0.22, 0.72, 2.08 and 3.39 mg·g^{-1}·h^{-1} (see Table 4). A general view is that, under anaerobic conditions, PAOs assimilate organic substrate rapidly to synthesize PHA using polyP stored in cells as energy source, and the orthophosphate generated from polyP degradation is released into the bulk liquid. In the absence of any organic compound in the aerobic zone, organisms with PHA stored are able to use the endogentic carbon as energy source to grow and assimilate phosphate to synthesize polyP. Therefore, the P uptake rate is decided by the energy source of PHA stored in the anaerobic zone. On the other hand, the P uptake has significant effect on P release. The more phosphorus is untaken, the more poly-P is stored, and the P release in the anaerobic zone will be further

Table 3 The characteristics of feeding wastewater (mg·L^{-1})

	COD	BOD_5	NH_4^+ - N	TN	NO_2^- - N	NO_3^- - N	TP	Alkalinity
min/max	228.5/307.6	110.7/150.5	53.42/70.7	56.37/74.87	0.08/0.19	0.05/1.42	5.14/7.30	289/376
average	273.37	124.30	62.13	65.58	0.14	0.74	6.74	341.5

Table 4 Summary of P removal in the different phases

	Run 1	Run 2	Run 3	Run 4
average TP removal efficiency/%	19.17	50.41	89.81	96.25
specific anaerobic P release rate of anaerobic zone/mg·g^{-1}·h^{-1}	0.13	0.49	2.26	3.6
specific anoxic P uptake rate of stage 1/mg·g^{-1}·h^{-1}	0.19	0.93	1.58	0.36
specific aerobic P uptake of stage 1/mg·g^{-1}·h^{-1}	0.23	0.62	2.32	4.91
specific P uptake rate of system/mg·g^{-1}·h^{-1}	0.22	0.72	2.08	3.39

promoted. In this study, it seems reasonable that the P removal efficiency of the system shows the same tendency with anaerobic P release rate because different Q_1 results in different PHA content of phosphorus removal organisms. In addition, P uptake is not complete in N13 until the end of system, which is typical in runs 3 and 4 (see Table 5). It suggests that the anoxic and aerobic retention time in the first stage is not enough for P uptake, and the intracellular storage compounds PHA may be used in the following stages to finish the P uptake.

Denitrifying phosphorus removal was also studied for different feeding ratios. Compared to the aerobic P uptake, the curve of specific anoxic P uptake rate is different. Fig. 4 shows that the maximum anoxic P uptake rate (q_a) of 1.58 mg·g^{-1}·h^{-1} occurs in run 3 with $0.45Q_{\text{total}}$ fed to the anaerobic zone, and the ratio of specific anoxic P uptake rate (q_a) to aerobic P uptake rate (q_o) is 0.68 accordingly. The higher q_a/q_o ratio is resulted from the UCT-type configuration, in which lots of PHA (COD) are available in the anoxic reactor for denitrifying before entering the aerobic reactor, so the anoxic P uptake rate is kept at a higher level and denitrifying P removal is enhanced gradually. The lower COD concentration of anaerobic effluent ensures the intracellular compounds, like PHA, as a carbon source with reducing power to assimilate P and synthesize polyP, at the same time, using nitrate as terminal electron acceptor. This reducing power provided by intracellular compounds guarantees the DNPAOs a dominative position in the competition with ordinary heterotrophic denitrifying bacteria. When Q_1 is increased from $0.45Q_{\text{total}}$ to $0.60Q_{\text{total}}$, q_a decreases from 1.58 to 0.36 mg·g^{-1}·h^{-1} (see Table 5). It suggests that more exogenous carbon sources in anaerobic effluent in run 4 stimulate the ordinary heterotrophic organisms (OHOs) to use nitrate as electron acceptor for heterotrophic denitrification in the anoxic zone, and anoxic phosphorus uptake occurs after available exogenous carbon sources is depleted. However, due to the limited returned activated sludge, nitrate becomes a limiting factor for anoxic P uptake. The lower nitrate concentration in the anoxic zone (D1), below 0.4 mg·L^{-1} (Fig. 3), verifies the above analysis furthermore. Summarize the above results, it is noted that the extent of anoxic phosphorus uptake is closely related to the presence of nitrate in the anoxic zone. If the system is operated such that the nitrate loading in the anoxic zone exceeds its denitrification potential, anoxic phosphorus uptake will be enhanced and aerobic P uptake is reduced correspondingly in the aerobic zone, and *vice versa*, when returned nitrate is not sufficient. In the further investigations on denitrifying P removal in SFA^2/O systems, the effect of nitrate loading

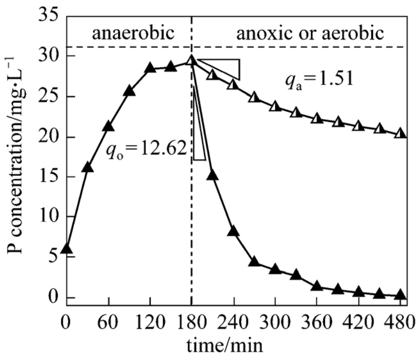
Table 5 Summary of nutrient and organic substrate removal in different phases

	Parameters	Influent	Stage 1					Stage 2				Stage 3				Effluent	Removal efficiency/%
			A	D1	N11	N12	N13	D2	N21	N22	N23	D3	N31	N32	N33		
Run 1	TN	74.4	27.75	26.52	25.06	25.40	25.7	29.84	26.60	25.77	27.95	30.34	29.90	27.82	26.95	27.09	63.49
	TP	6.47	8.95	6.38	6.10	5.66	5.45	6.79	6.07	5.76	5.45	5.96	5.67	5.55	5.35	5.23	19.17
	NH_4^+ - N	73.2	22.88	16.17	10.07	7.02	1.83	20.13	10.68	6.41	4.88	27.26	17.39	10.04	5.88	5.94	91.89
	NO_3^- - N	1.22	4.87	10.36	15.00	18.38	23.87	9.71	15.93	19.36	23.07	2.59	12.52	17.78	21.07	21.15	–
	COD	253	79.8	31.58	35.59	31.08	25.58	39.42	30.58	28.59	30.58	60.46	42.13	31.58	25.59	24.64	90.26
Run 2	TN	68.7	40.34	29.87	29.10	29.80	29.00	29.36	27.78	27.47	27.33	19.08	19.38	18.74	18.35	19.00	72.35
	TP	6.19	11.24	6.88	6.04	5.66	5.08	6.78	4.14	4.08	3.98	3.36	3.02	3.06	3.18	3.07	50.41
	NH_4^+ - N	67.7	39.98	25.41	12.07	4.68	1.69	19.26	11.36	6.12	2.35	10.05	7.51	4.98	1.33	1.34	98.01
	NO_3^- - N	1.02	0.36	4.46	17.03	25.12	27.31	10.10	16.42	21.35	24.98	9.03	11.87	13.76	17.02	17.66	–
	COD	259	109.8	47.58	39.12	30.08	36.59	42.13	35.58	28.76	25.58	35.58	28.56	30.08	27.58	31.58	87.80
Run 3	TN	61.8	44.46	31.51	27.44	28.59	29.04	25.16	25.73	24.16	24.06	19.16	17.67	16.66	16.24	16.31	73.61
	TP	6.86	24.01	11.74	7.5	6.43	5.14	2.04	1.93	1.49	1.29	1.86	0.92	0.93	0.98	0.63	89.81
	NH_4^+ - N	61.1	43.71	31.11	24.10	15.86	7.62	18.60	14.33	9.46	4.27	11.28	7.02	4.80	0.92	1.22	98.0
	NO_3^- - N	0.79	0.75	0.40	3.34	12.73	21.41	6.56	11.39	14.70	19.79	7.87	10.66	11.87	15.32	15.09	–
	COD	267	132.5	46.73	41.98	37.58	35.58	45.14	35.58	25.76	28.58	37.58	28.86	28.58	27.76	35.74	86.59
Run 4	TN	71.1	57.18	43.74	41.00	37.65	35.97	37.64	33.92	31.89	29.24	28.09	27.74	28.55	28.03	28.73	59.62
	TP	5.86	27.9	18.84	10.29	5.14	1.84	1.33	0.80	0.62	0.48	0.43	0.36	0.29	0.24	0.22	96.25
	NH_4^+ - N	70.2	56.73	43.62	28.89	22.27	15.86	28.06	17.69	10.37	6.71	14.03	10.07	8.85	3.66	2.86	95.92
	NO_3^- - N	0.99	0.45	0.12	11.1	15.39	19.20	9.58	16.23	21.52	22.53	14.06	17.67	19.71	22.21	22.59	–
	COD	254	158.9	66.68	46.52	39.12	35.58	39.58	31.08	35.58	29.12	30.08	25.56	24.08	22.08	25.26	90.05

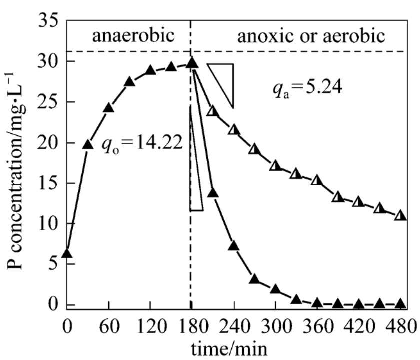
on anoxic P removal will be further substantiated in more detail.

3.2 Characterization analysis of sludge

To further investigate the effect of feeding patterns on the accumulation of PAOs and DNPAOs, batch tests were carried out in this study. Phosphate uptake rates under anoxic and aerobic conditions were measured in separate batch reactors. The activated sludge samples were obtained from the pilot scale SF A^2/O system when the system had been operated for 70 days. After confirming the complete phosphorous release in anaerobic phase, the anaerobic sludge was removed and divided into two parts equally. One was exposed to anoxic condition, and the other to aerobic condition. The results are compared to that from the seeding sludge (day 0), as showed in Fig. 5. The rates of anoxic (q_a) and aerobic phosphate uptake (q_o) on day 0 are 1.51 and 12.62 $mg\cdot g^{-1}\cdot h^{-1}$, respectively, and q_a/q_o is 11.96%. Based on the method provided by Wachtmeister *et al*. [21], in which the contribution of DNPAOs to the total phosphorus removal is calculated from the ratio q_a/q_o. It is estimated that the proportion of DNPAOs is 11.96% of total PAOs in the seeding sludge, though no anoxic time is provided for the culture of seeding sludge. With the modified SFA^2/O system, the effect on the accumulation of PAOs and DNPAOs is expected to be different because of a changed biomass distribution in the system, and proved by the results on day 70. In Fig. 5 (b), the anoxic and aerobic phosphate uptake rates are 5.24 and 14.22 $mg\cdot g^{-1}\cdot h^{-1}$, respectively. The proportion of DNPAOs is increased from 11.96% to 36.85% of the total PAOs. Therefore, it is concluded that the DNPAOs are accumulated and enriched gradually in the modified step-feed A^2/O nutrient removal process, which presents a satisfactory performance for denitrifying phosphorus removal.



(a) Day 0



(b) Day 70

Figure 5 Phosphate uptake tests under aerobic and anoxic conditions with the activated sludge from the A^2/O step-feed system on day 0 and day 70
▲ aerobic; ▲ anoxic

3.3 Nitrogen removal: Nitrification and denitrification

Figure 6 shows the total N-removal of the system with different influent flow distribution ratios. The influent TN concentration is 56.37 to 74.87$mg\cdot L^{-1}$ and C/N is 3.23 to 5.17, which represents the characteristics of municipal wastewater in China. The average ammonia removal efficiency from run 1 to run 4 is 91.89%, 98.01%, 98.0% and 95.92%. The higher effluent ammonia of run1 may be due to the higher influent flow rate of Q_3 ($0.5Q_{total}$) with the optimal coefficient method developed by Wang *et al* [23]. Nitrification is affected by influent ammonia loading significantly when the optimal coefficient is applied to treat low C/N sewage, which leads to a relatively short hydraulic retention time in the third stage (3.2 h) and induces an incomplete ammonia removal. In Fig. 7 (a), the ammonia concentration in effluent of run 1 is 5.94 $mg\cdot L^{-1}$. In other three runs, higher oxygen concentration with the descending influent distribution in each stage results in lower effluent ammonia, usually below 1.0 $mg\cdot L^{-1}$. However, due to the constant nitrification capacity of each stage, incomplete nitrification occurs frequently in stage 1 or stage 2 when Q_1 is higher, such as the situations in runs 3 and 4, shown in Figs. 7 (c) and 7 (d). The change of ammonia concentration suggests the importance of the feeding ratios to the

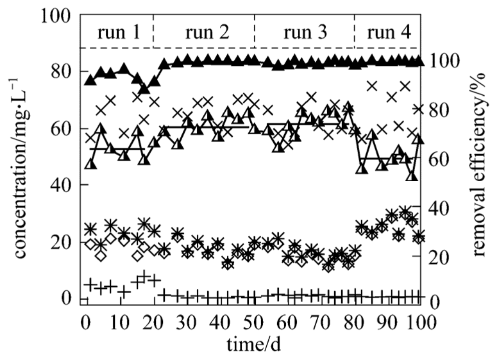


Figure 6 N removal with different influent flow distribution ratios
× influent TN; + effluent ammonia; ◇ effluent nitrate; ✳ effluent TN; ▲ ammonia removal efficiency; ▲ TN removal efficiency

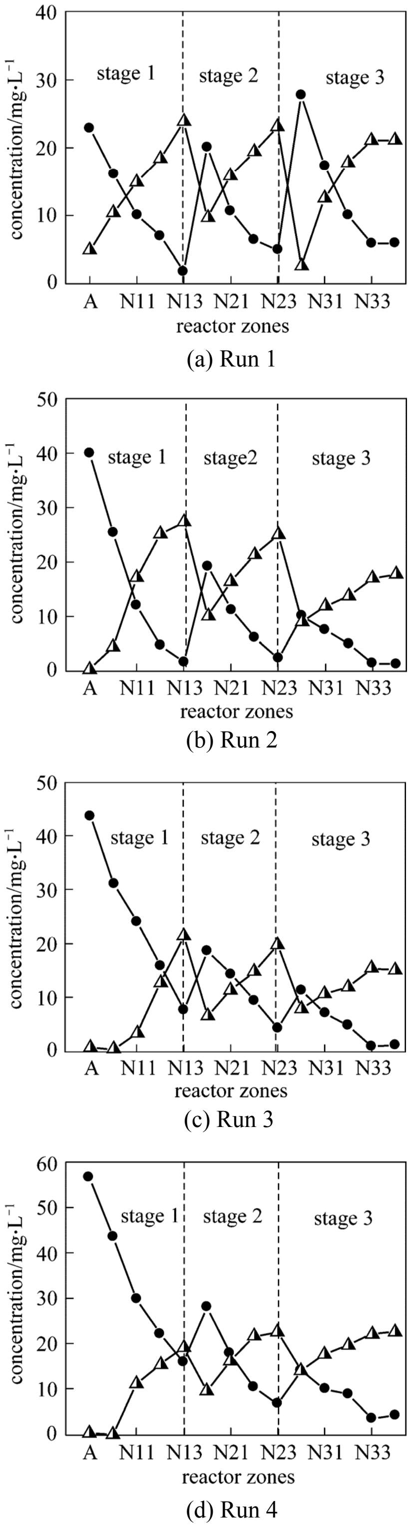


Figure 7 Concentration of ammonia and nitrate in different reactor in each run

● NH_4^+-N; ▲ NO_3^--N

nitrification process. The nitrification should be considered when different influent feeding ratios are applied especially in wastewater treatment systems with high ammonia loading and low C/N ratio.

Figure 6 also shows that TN removal efficiencies is 63.49%, 72.35%, 73.61% and 59.62% from run 1 to run 4. Run 3 is more effective for TN removal since more nitrate is removed in anoxic zone (D1), which serves as electron acceptor for denitrifying phosphorus removal. As a result, more carbon source is saved for denitrification in the subsequent stages. In addition, the low concentration nitrate transferred into the anaerobic zone reduces the competition of carbon source between ordinary heterotrophic denitrifying bacteria (OHOs) and phosphorus removing organisms, which enhances the phosphorus release significantly. More influent feeding to the anaerobic zone provides sufficient organic substrate for phosphorus release and more PHA is stored as intracellular compounds, which serves as the carbon source for phosphorus uptake in the anoxic or aerobic zone and improves the phosphorus removal. When Q_1 is increased to $0.6Q_{\text{total}}$ in run 4, however, the TN removal efficiency is the lowest, 59.62%. The higher COD concentration of anaerobic effluent and a little nitrate in anoxic zone, shown in Fig. 7 (a), suggests that the nitrate returned with activated sludge to the anoxic zone, as terminal electron acceptor, is not enough for denitrifying phosphorus uptake or heterotrophic denitrification. As a result, the carbon source is wasted and denitrification rate is decreased significantly.

It is concluded from above results that higher Q_1 feeding rates supply sufficient organic compounds for sufficient phosphorus release and improve phosphorus removal, but the carbon source may be wasted and N-removal efficiency may decrease to a large extent. To avoid this situation, another internal nitrate recycling flow is suggested from the end of system to D1 as an alternate choice. To ensure stable operation, keep the nitrate at a lower but non-zero level in anoxic zone *via* real-time control of returned activated sludge and internal recycling flow would to be a feasible strategy.

3.4 Removal of organic compounds

The COD concentrations of the influent, anaerobic effluent (A), anoxic effluents (D1), aerobic effluent (N13) and final effluent are shown in Fig. 8. The effluent COD concentrations are maintained although the influent flow distribution ratios are different and the COD removal efficiency is 90.26%, 87.80%, 86.59% and 90.05% from run 1 to run 4. The COD profiles of anaerobic zone shows that the average COD concentration in run 1 is the lowest and it is the highest in run 4, fed with $0.15Q$ and $0.60Q$, respectively. With the higher nitrate concentration and denitrification rate in the anaerobic zone, the COD concentrations in the anoxic zone are not different significantly in runs 1, 2 and 3. However, the anoxic effluent COD concentration in run 4 is relatively high, 66.68 mg·L^{-1}, which may be due to a lower Q_r, so that the electron acceptors are not sufficient for denitrification in this zone.

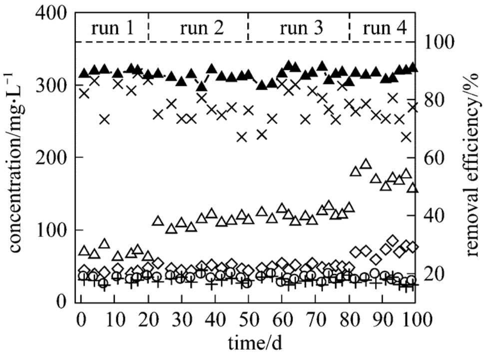


Figure 8 COD removal under different operation conditions
× influent; △ anaerobic (A) effluent; ◇ anoxic (D1) effluent; ○ aerobic (N13) effluent; + effluent; ▲ removal efficiency

Figure 9 exhibits the rate of COD consumption in each stage calculated based on the mass balance. The notations N*i* indicates the total aerobic zone of stage *i*. Due to the phosphorus release and denitrification, most of the available organic substrate is consumed in anaerobic zone or anoxic zone and the residual biodegradable COD is usually very low in aerobic zone. As a result, the COD consumption rate in anaerobic or anoxic zones is usually higher than that of aerobic zone. However, the variation of COD consumption rate in aerobic zone is higher than that in anoxic or anaerobic zone in run 1 and run 4. The higher COD consumption in aerobic zone and lower nitrate in anoxic zone verifies the fact that carbon source is wasted in the operation, resulting in a lower TN removal efficiency, 63.49% and 59.62%. The amount of the total COD consumption in anaerobic zone is similar in runs 3 and 4, though the feeding ratios are different. It indicates that the utilization of organic substrate in anaerobic zone in run 3 almost reaches the maximum capacity, so that more organic substrate available in anaerobic zone of run 4 does not enhance the phosphorus release further. Therefore, the feeding ratio should be controlled carefully to avoid the carbon source wasting. The total nutrient and organic substrate removal are summarized in Table 5.

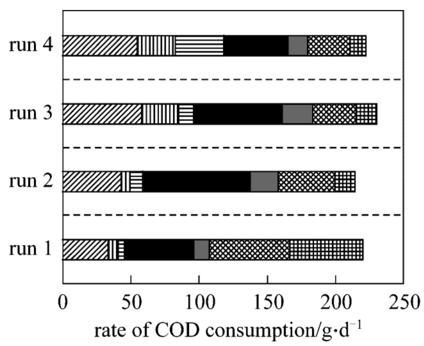


Figure 9 Rates of COD consumption under different operation conditions
A; D1; N1; D2; N2; D3; N3

4 CONCLUSIONS

It was confirmed that the modified step-feed A^2/O system was a technically feasible and economically favorable dynamic process for simultaneous nitrogen and phosphorus removal from municipal wastewater. Four different feeding patterns were applied. The feeding ratio of 0.45∶0.35∶0.2 in run 3 was the optimal for phosphorus and nitrogen removal due to an increased denitrifying phosphorus removal, in which the total nitrogen removal was not decreased, and the overall nitrate reduction by intracellular carbon source was higher. The higher anoxic phosphorus uptake attained in run 3 suggested that appropriate influent flow distribution would enhance the contribution of denitrifying phosphorus bacteria. The batch tests for sludge phosphorus uptake showed that the proportion of DNPAOs was increased in the modified SFA^2/O system, from 11.96% to 36.85% of total PAOs. It indicated that more DNPAOs would be accumulated when feasible control strategy was applied in this system. For practical applications, appropriate feeding ratio is essential for energy saving and sufficient nitrogen and phosphorus removal. The combination of different operational strategies, such as the control of nitrate recycling, returned activated sludge ratio and influent feeding ratios, is needed for stable nutrient removal, especially denitrifying phosphorus removal. Future studies on the real-time control should be carried out so that the modified process could be successfully applied to full-scale municipal wastewater treatment plant with fluctuating influent.

NOMENCLATURE

BOD	biochemical oxygen demand, mg·L^{-1}
COD	chemical oxygen demand, mg·L^{-1}
DO	dissolved oxygen, mg·L^{-1}
HRT	hydraulic retention time, h
MLSS	mixed liquid suspended solids, g·L^{-1}
Q_c	internal recycling, L·d^{-1}
Q_i	influent flow rate of stage i (i = 1,2,3) , L·d^{-1}
Q_r	returned activated sludge, L·d^{-1}
Q_{total}	total influent flow rate of system, L·d^{-1}
q_a	maximum anoxic P uptake rate, mg·g^{-1}·h^{-1}
q_o	maximum aerobic P uptake rate, mg·g^{-1}·h^{-1}
SRT	solid retention time, d
SVI	sludge volume index, ml·g^{-1}
TN	total nitrogen, mg·L^{-1}
TP	total phosphate, mg·L^{-1}

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