Phosphorus removal under anoxic conditions in a continuous-flow A_2N two-sludge process

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Abstract The Anaerobic-Anoxic/Nitrification (A_2N) system is a continuous-flow, two-sludge process in which Poly-P bacteria are capable of taking up phosphate under anoxic conditions using nitrate as an electron acceptor. The process is very efficient because it maximizes the utilization of organic substrate for phosphorus and nitrogen removal. An experimental lab-scale A_2N system fed with domestic sewage was tested over a period of 260 days. The purpose of the experiment was to examine phosphorus removal capacity of a modified A_2N two-sludge system. Factors affecting phosphorus and nitrogen removal by the A_2N system were investigated. These factors were the influent COD/TN ratio, Sludge Retention Time (SRT), Bypass Sludge Flow rate (BSF) and Return Sludge Flow rate (RSF). Results indicated that optimum conditions for phosphorus and nitrogen removal were the influent COD/TN ratio around 6.49, the SRT of 14 days, and the BSF and RSF were fixed at about 26–33% of influent flow rate.
Keywords A_2N two-sludge system; COD/TN ratio; denitrifying phosphorus removal bacteria (DPB); phosphorus and nitrogen removal

Introduction

Denitrifying Phosphorus removal Bacteria (DPB) which are capable of using nitrate as an electron acceptor simultaneously removing phosphorus and nitrogen from wastewater have been introduced since the 1980s (Vlekke *et al*., 1988; Wanner *et al*., 1992; Kerrn-Jespersen and Henze, 1993; Kuba *et al*., 1993). For these bacteria, nitrate is utilized for the oxidation of stored PHB (Poly-β-Hydroxybutyrate) and is removed as nitrogen gas (N_2) from wastewater. Besides phosphorus, nitrogen that also induces the eutrophication of water is removed simultaneously by DPB. It was reported that DPB have not only a similar potential for phosphorus removal but also a similar biological metabolism of phosphorus and intracellular organic substances like PHB and glycogen to the conventional A/O phosphorus-removing organisms (Kuba *et al*., 1993). The main advantage of applying DPB is the possible saving of COD and energy (aeration) and less sludge production (Kuba *et al*., 1996b).

To effectively apply DPB for nitrogen and phosphorus removal, a new system was proposed in which an anaerobic phase is placed at the influent side, followed by anoxic and external nitrification phases. This system is referred to as the "A_2N" system hereafter (Hao *et al*., 2000). By using an A_2N two-sludge system process, the severe problem of the competition for COD between phosphorus and nitrogen removing organisms that occurs in the conventional united phosphorus and nitrogen removal process has been resolved perfectly (Kuba *et al*., 1996b; Bortone *et al*., 1999).

The purpose of this study is to investigate the denitrifying phosphorus removal capacity

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in A_2N two-sludge system with continuous flow. Moreover, the factors affecting simultaneous phosphorus and nitrogen removal using the two-sludge system were studied in order to determine favorable influent COD/TN ratio, SRT, BSF and RSF for optimal phosphorus and nitrogen removal.

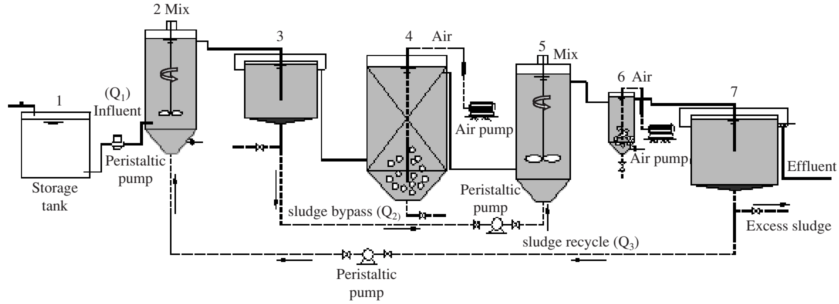
Materials and methods

A_2N two-sludge system with continuous flow configuration and layout

The lab-scale A_2N two-sludge system shown in Figure 1 was operated. Raw domestic sewage stored in a storage tank (1) is introduced into the anaerobic tank (2) where phosphate is released from Poly-P bacteria. Most of the readily biodegradable COD in raw wastewater has been taken up by the sludge in the anaerobic tank. Internal settler (3) separates the sludge from the ammonia-rich supernatant. The supernatant goes to the biofilm reactor (4) where the nitrification occurs. The substrate-storing sludge (Q_2) passes nitrification and is resuspended with the nitrified effluent in the anoxic tank (5). Here DPB can take up phosphate by using the nitrate as electron acceptors and the stored PHB as electron donors. A post-aeration tank (6) allows nitrogen gas stripping from the sludge and assists in take up of the residue phosphorus before final settling (7) when influent C/N ratio is too low.

The effective volumes of the anaerobic tank, fixed-biofilm nitrification tank, anoxic tank and post-aeration tank were 6 L, 13 L, 10 L and 2 L, respectively. The settle volume was 7.2 L for the internal settler and 4.9 L for the final settler. Influent wastewater, bypass sludge and return sludge were introduced to the reactors using three variable speed peristaltic pumps. The influent flow rate (Q_1) was maintained at 43.2–48 L/d while BSF (Q_2) and RSF (Q_3) were both set at 12.9–14.4 L/d (Q_1:Q_2:Q_3≈3.7:1:1). Overall hydraulic retention time (HRT) was 17 hours and the MLSS in the anaerobic tank, anoxic tank and post-aeration tank were maintained at 4,300–4,700 mg/L, resulting in DPB sludge age of approximately 14 days. The dissolved oxygen (DO) concentration was controlled at 3 mg/L in the biofilm nitrification reactor for expected nitrification and at 2.0 mg/L in the post-aerated reactor for taking up residual phosphate. Room temperature was controlled at 20–23 centigrade during the operational period, and the mixers were installed in the anaerobic and anoxic reactor to prevent the activated sludge from settling.

The bio-film contact medium is made with carbon fiber which is made in Japan (Figure 2). The diameter of a carbon fiber filament is 7 micrometres, thereby providing increased surface area for bacteria. The carbon fiber filaments are free from deterioration in wastewater and improve nitrification ability.



1) storage tank; 2) anaerobic tank; 3) internal settler; 4) fixed-biofilm nitrification; 5) anoxic tank; 6) post-aeration tank; 7) final settler

Figure 1 Configuration of the A_2N system

Sludge and wastewater

The A_2N two-sludge system was inoculated with sludge taken from the EBPR process in Beijing Gaobeidian Wastewater Treatment Plant. Raw wastewater for the laboratory system was taken from fresh domestic sewage that was characterized with a low C/TN ratio. The glucose was added to improve the influent C/TN ratio. The pH control instrument was set in the wastewater storage tank to keep inflow pH at 7.0 by adding sodium hydrogen carbonate ($NaHCO_3$) solution. The major characteristics of the influent are shown in Table 1. The operating stages are listed in Table 2. After the culture of the activated sludge, the A_2N two-sludge system was operated continuously over 260 days. Experimental tests were started after stable operation was attained.

Analytical methods

The dissolved oxygen (DO) and temperature were measured continuously using a WTW oxygen probe. Continuous monitoring of pH was carried out using two WTW inolab pH level 2. COD_{Cr}, TN, TP, NH_4^+-N, NO_3^--N and MLSS were measured according to standard methods (APHA, 1995).

Results and discussion

Phosphorus and nitrogen removal

Variation of total phosphorus concentration (TP) was depicted in Figure 3. Phosphorus release activity was shown to increase steadily with increasing influent COD/TN ratio. A majority of phosphorus was removed in the anoxic stage. The average phosphorus removal efficiencies of period I, II and III were 92.87%, 97.12% and 73.61%, respectively. The effluent TP in period I and II were usually below 0.5 mg/L. However, in period III, the average TP in the final effluent was as high as 1.95 mg/L.

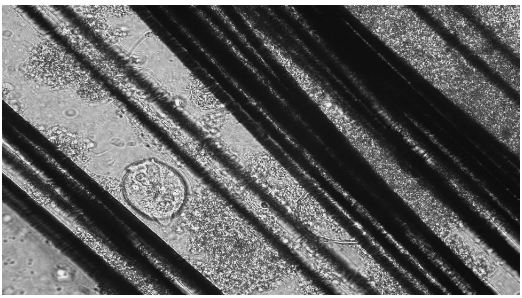


Figure 2 Immobilization of microorganism on the Carbon fiber (10 × 40)

Table 1 Characteristics of the influent wastewater

Term (Unit)	CODcr (mg/L)	BOD_5 (mg/L)	NH_4^+-N (mg/L)	NO_3^--N (mg/L)	TN (mg/L)	TP (mg/L)	pH	Alkalinity
Max/min	201–537	200–430	42–62	0.5–2.3	44–71	3.9–9.2	6.8–7.4	340–400
Average	349	230	51.2	1.08	56	6.07	7.21	360

Table 2 Testing period and influencing factors (mean value)

Period	Influent COD (mg/L)	COD loading (kg/m³•d)	COD/TN	TN/TP
Period I (1–60 d)	237	0.479	3.94	10.54
Period II (61–160 d)	371*	0.704*	6.49*	9.94
Period III (161–200 d)	504*	1.018*	9.64*	8.28

* Glucose added in the influent wastewater

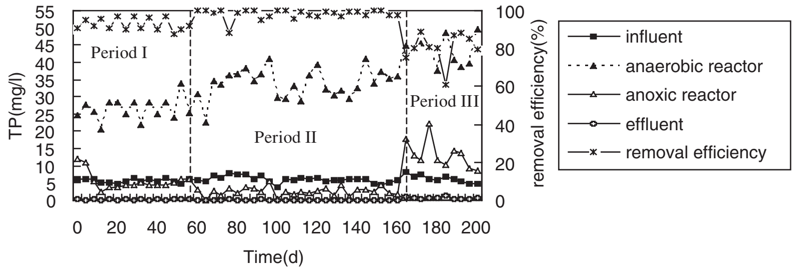


Figure 3 Variation of TP concentration in the A_2N system

Efficiency with respect to nitrogen is shown in Figure 4. The variation of nitrate nitrogen concentration (NO_3^--N) in an individual reactor is shown in Figure 5. It can be noticed in Figure 4 that as the COD loading rate was progressively increased from 0.479 to 1.018 kg/m^3·d, nitrogen removal was increased from 80.99 to 92.99% with TN in the effluent ranging from 3.67–11.47 mg/L. The nitrification biofilm reactor was the most reliable operation unit in the system. All ammonium nitrogen (NH_4^+-N) in the supernatant was oxidized to nitrate in the aerobic nitrification reactor, with nitrification rate up to 100%. Therefore nitrification was not the rate-limiting step in this particular arrangement. In the anoxic tank, the previously stored (PHB) and adsorbed substrate is used as the C-source by DPB for the denitrification and phosphorus uptake. Consequently considerable nitrate had been removed in the anoxic tank. As shown in Figures 4 and 5, the dosage of glucose in period II and III contributed to low concentration of nitrate and TN in the effluent.

NH_4^+-N in the DPB sludge stream was transferred into the anoxic tank. The NH_4^+-N residue in the DPB sludge stream decreases in the anoxic tank, mainly because of the dilution by the nitrified supernatant stream from the nitrification tank and partially because of the utilization for growth of DPB. The mean NH_4^+-N concentration in the effluent was less than 6 mg/L throughout all runs.

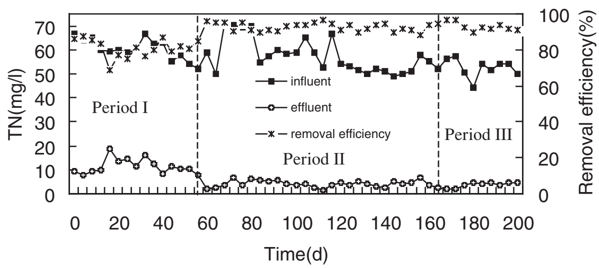


Figure 4 Variation of TN concentration in the A_2N system

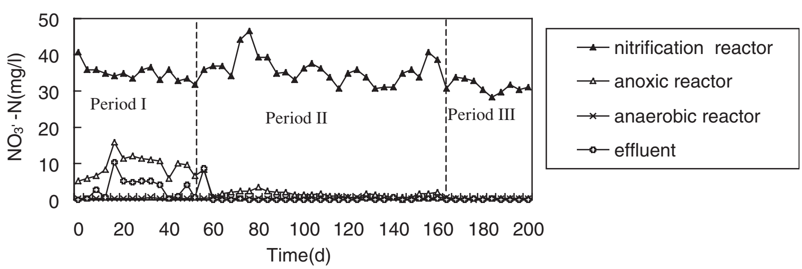


Figure 5 Variation of NO_3^--N concentration in the A_2N system

COD removal

COD performances in the influent and effluent are shown in Figure 6. COD removal was quite stable in spite of a great variation in the COD value of the influent water. The effluent COD ranged between 10 and 40 mg/L and the average COD removal efficiency was up to 93%.

Figure 7 shows that COD was primarily utilized by activated sludge in the anaerobic phases. Approximately 72.8% of COD was consumed in the anaerobic stage compared to the other stages. Results also demonstrate that good anaerobic COD removal in the anaerobic stage leads to the subsequent biological nitrogen and phosphorus removal. High COD removal in the anaerobic stage implies more PHB (electron donors) stored by PAOs and this facilitates nitrogen/phosphorus removal in the anoxic stage. Moreover, large amounts of COD consumed in the anaerobic stage drop the COD/TN ratio of supernatant flowing to the nitrification reactor, therefore, growth of nitrifying organisms in the biofilm system is promoted and nitrification is enhanced.

Effect of the influent COD/TN ratio

The correlations between influent COD/TN ratio and nitrogen removal, and phosphorus removal efficiency are shown in Figure 8 and Figure 9 respectively. It is noted from Figure 8, when the influent COD/TN ratio increased up to 8.75, that TN removal efficiency increases rapidly from 74.75 to 96.5%. With influent COD/TN greater than 8.6, TN removal appeared to decrease slowly. The inhibition of a high organic loading on nitrification has been reported in the literature (Hanaki *et al*., 1990). In their results, an increase in influent COD concentration was shown to provoke the growth of heterotrophic biomass and resulted in inhibition of ammonia oxidizer. The biofilm nitrification process applied in the A_2N system weakens this inhibition to some degree, but long-term operation under high organic loading must inhibit growing of the nitrifying organisms ultimately. This is why TN removal was decreased slightly when influent COD/TN was greater than 8.6 in period III.

When the mean influent COD/TN ratio was around 3.94 (in period I), the mean nitrogen removal efficiency was 80.99%. Obviously, nitrate was not removed completely in the anoxic tank and can be detected in the final effluent because of shortage of C-source as an

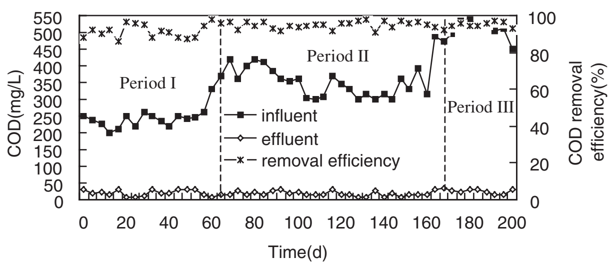


Figure 6 Variation of COD concentration in the A_2N system

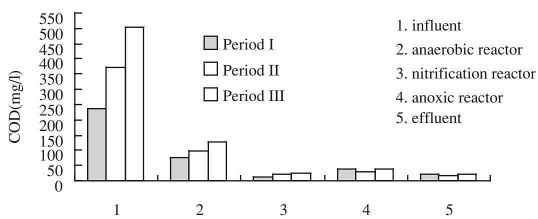


Figure 7 Variation of COD concentration in each stage of A_2N system

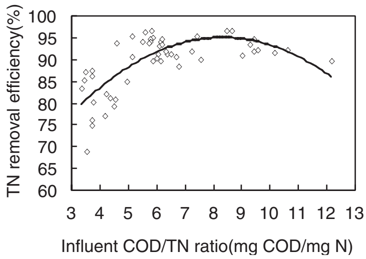


Figure 8 Effect of influent COD/TN ratio on TN removal efficiency

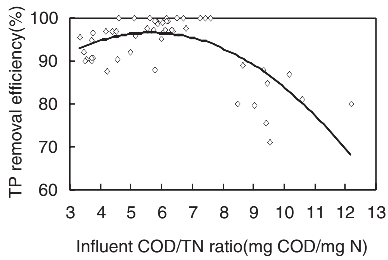


Figure 9 Effect of influent COD/TN ratio on TP removal efficiency

electron donor (Figure 5). It might be explained that the low concentration of COD into the anaerobic reactor reduced the scale of PHB accumulated by DPB, thus excess nitrate was supplied from the nitrification reactor.

As a satisfactory result was not obtained during period I in terms of nitrogen removal, the wastewater was supplemented with glucose in the periods II and III, and the influent COD/TN ratio of these two periods increased to 6.49 and 9.64 respectively. It appears that more nitrates had been removed in these two periods and both of the average nitrogen removal efficiencies increased up to 92%. As a consequence, the higher influent COD/TN ratio allows achievement of a very low concentration of nitrate and the TN removal efficiency was also improved. But when the influent COD/TN ratio continued to increase, the TN removal efficiency dropped just as the front had been discussed.

As shown in Figure 9, TP removal also appeared to be affected significantly by the influent COD/TN ratio. In periods I and II with the influent COD/TN ratio of 3.94 and 6.49 respectively, an excellent phosphorus removal efficiency could be obtained. Apparently the phosphorus removal efficiency was not influenced negatively by the low influent COD/TN ratio. In the A_2N system, the DPB sludge storing maximal PHB content in the anaerobic tank allowed for denitrification and phosphorus uptake taking place simultaneously under anoxic conditions. This facilitates the prime use of PHB-COD for denitrifying dephosphorus and minimizes aerobic PHB oxidation. This is the main reason that the A_2N two-sludge system can obtain high phosphorus removal efficiency even with low COD/TN as 3.94. While the conventional processes (like UCT, A^2/O and A/O process) seldom possess biological phosphorus removal activity unless they have influent COD/TN ratio greater than 7 (Shen and Wang, 1999). In these conventional processes, the available amount of COD in the wastewater is a crucial limiting factor for both EBPR and denitrification. Thereby saving significant amounts of COD can be considered as a specific advantage of the A_2N system over conventional biological phosphorus removal systems.

On the other hand, with influent COD/TN ratio increasing to 9.64 (period III), TP removal decreased rapidly. It can be noticed that in spite of the higher COD availability, anoxic TP uptake was incomplete (Figure 3). And the phosphorus removal efficiency declined to 72.61%. This result suggests once the influent COD/TN ratio increased beyond a certain level (just as the value of period III), declining of phosphorus removal may occur in the anoxic reactor and no longer support the low effluent TP concentrations. It is worth pointing out that the reason for this decline could be due to the limiting of the nitrates count. Further, two pieces of evidence attributing to the limitation of nitrate need to be put forward: (i) The electron acceptor (nitrate) amount surely has been a limitation factor. The released phosphorus amount is so high because of high COD supplied in the anaerobic stage that resulted in the released phosphorus being not taken up fully in the anoxic stage for lack of electron acceptors. (ii) Probably simultaneous presence of COD and NO_3^- in the anoxic reactor inhibited the denitrification phosphorus removal. The influent COD concentration of period III was greater than 500 mg/L and this possibly leads to the organic substance not being fully utilized to produce PHB by PAOs in the anaerobic phase. Residual organic substance adsorbed by sludge flocs introduced to the anoxic reactor seemed to hinder phosphorus uptake at the very beginning because of the competition for nitrate by denitrification, which in turn led to a limitation of nitrate for phosphorus uptake. Therefore high TP concentration in anoxic stage could be observed.

The above results also illustrated that the post-aerobic tank is strictly needed in the A_2N configuration especially with high influent COD/TN ratio. Because phosphorus is fully anoxically removed only when nitrates are not limiting, in the case of full depletion of nitrates in the anoxic tank, phosphorus uptake can continue under aerobic conditions in the post-aerobic compartment. Just as Figure 3 shows, a considerable amount of phosphorus was removed by DPB with oxygen instead of nitrate as an electron acceptor in period III. Moreover it has been proved that oxygen has no direct detrimental effect on denitrifying dephosphatation activities by DPB (Kuba *et al*., 1996a).

Effect of MLSS and SRT

The MLSS concentration of the anaerobic, anoxic and aerobic reactor ranged between 4,300–4,700 mg/L. Results demonstrated when the ratio of the MLSS in these three reactors was controlled near to 1:1:1, the nitrogen and phosphorus removal can obtain the optimal state simultaneously.

At days 0–71, the SRT of DPB sludge is 14 days. From day 72, SRT was decreased from 14 to 7 days. It was expected that the lower SRT led to improve the phosphorus removal efficiency. However the phosphorus removal efficiency decreased swiftly from 98.18 to 78.5% and the MLSS concentration in each reactor declined to 2,700–3,200 mg/L subsequently. From day 82, the SRT increased back to 14 days in order to restore the phosphorus removal efficiency, and it was found that the phosphorus removal efficiency increased gradually. Comparison of these two SRTs impacting on the phosphorus removal shows that SRT of 14 days is more beneficial for phosphorus removal than that of 7 days. The reason for this is that decrease in SRT causes a washout of PAOs from the reactor and consequently decreases the phosphorus removal. In the activated sludge model No. 2D (Henze *et al*., 2000), it is estimated that the biomass growth and the Poly-P regeneration under anoxic conditions is reduced to 60% compared to aerobic conditions. So, a longer SRT for DPB sludge is needed to maintain a low phosphorus effluent from the A_2N process compared to the conventional EBPR process. Moreover, the report dictates that the small SRT in the EBPR process allows for quick changes in the biomass, which can result in quick changes in the characteristics, such as differences in phosphorus release rate and capacity (Tykesson *et al*., 2001). Therefore, the designed SRT of 14 days was recommended in the A_2N system for the domestic wastewater treatment.

The bypass sludge flow rate and recycle sludgy flow rate

Special attention must be paid to the control of BSF because this determines the amount of ammonia distributing to the anoxic reactor directly. Experimental results demonstrate that the BSF should be controlled at a low level so long as enough DPB sludge has been maintained in anoxic reactor. In this study, the BSF was controlled around 26–33% of influent flow rate. With this controlled ratio, a large amount of ammonia entering the anoxic tank has been avoided, simultaneously, enough sludge can be maintained in the anoxic reactor to guarantee the excellent TN effluent quality. As to the RSF rate, it is recommended that this should be the same as or a little larger than the BSF aiming to avoid the sludge accumulating in the internal or the final settler, otherwise the actual amount of PAOs available for the phosphorus removal will be reduced.

By way of contrast, in the conventional BNR system, a large recirculation no less than 200% is absolutely necessary to improve the nitrogen removal efficiency. However, the A_2N system in which DPB and nitrifiers are separated operating as a post-denitrification process need not to recirculate between the aerobic and anoxic stage contrast to pre-denitrification in conventional single-sludge system and can also obtain the low effluent nitrate concentration.

Conclusion

Based on the results obtained from the operation of the A_2N system, we conclude:

1. Optimizing anoxic biological phosphorus removal in the A_2N system was dependent on some parameters: COD/TN ratio, sludge retention time (SRT), BSF and RSF. The influent COD/TN ratio around 6.49, the SRT of 14 days and the BSF and RSF fixed about 26–33% of influent flow rate were determined as optimal for the phosphorus and nitrogen removal in the A_2N two-sludge system.
2. The separation of nitrifiers and PAOs in two systems and introducing the DPB to the process are the two key factors which enable the A_2N system to treat the wastewater with the low influent COD/TN ratio. The COD/TN of the municipal wastewater was ranged between 5–8 in common, so the A_2N process has a good prospect for municipal wastewater treatment.
3. The external biofilm reactor stabilizes the nitrification population in the system and complete nitrification in the fixed-biofilm reactor was achieved. Further, the carrier of carbon fiber was observed to possess excellent characteristics for more microorganisms to immobilize on it.

Acknowledgements

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