Evaluation of sludge reduction and phosphorus recovery efficiencies in a new advanced wastewater treatment system using denitrifying polyphosphate accumulating organisms

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Abstract A new biological nutrient removal process, anaerobic–oxic–anoxic (A/O/A) system using denitrifying polyphosphate-accumulating organisms (DNPAOs), was proposed. To attain excess sludge reduction and phosphorus recovery, the A/O/A system equipped with ozonation tank and phosphorus adsorption column was operated for 92 days, and water quality of the effluent, sludge reduction efficiency, and phosphorus recovery efficiency were evaluated. As a result, TOC, T-N and T-P removal efficiency were 85%, 70% and 85%, respectively, throughout the operating period. These slightly lower removal efficiencies than conventional anaerobic–anoxic–oxic (A/A/O) processes were due to the unexpected microbial population in this system where DNPAOs were not the dominant group but normal polyphosphate-accumulating organisms (PAOs) that could not utilize nitrate and nitrite as electron acceptor became dominant. However, it was successfully demonstrated that 34–127% of sludge reduction and around 80% of phosphorus recovery were attained. In conclusion, the A/O/A system equipped with ozonation and phosphorus adsorption systems is useful as a new advanced wastewater treatment plant (WWTP) to resolve the problems of increasing excess sludge and depleted phosphorus.

Keywords Anaerobic–oxic–anoxic (A/O/A) system; denitrifying polyphosphate-accumulating organisms (DNPAOs); excess sludge reduction; ozonation; phosphorus recovery; zirconium ferrite adsorbent

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Introduction

In recent years, advanced biological nutrient removal processes such as the anaerobic–anoxic–oxic (A/A/O) system have been widely introduced in wastewater treatment plants (WWTPs). In the A/A/O system, nitrogen is removed by nitrifying bacteria in oxic conditions and denitrifying bacteria in anoxic conditions, and phosphorus is removed by polyphosphate-accumulating organisms (PAOs). However, in the A/A/O system, competition for organic carbon source uptake between PAOs and denitrifying bacteria in the anaerobic zone reduces water quality of the effluent.

In the anaerobic–oxic–anoxic (A/O/A) system, denitrifying PAOs (DNPAOs), which could perform denitrification and phosphorus removal simultaneously under anoxic conditions without any carbon substrate, could be enriched (Tsuneda *et al.*, 2003). In the anaerobic conditions, DNPAOs take up volatile fatty acids (VFAs) and release phosphate. In the subsequent oxic conditions, nitrifying bacteria oxidize ammonia to nitrate, and in the last anoxic conditions, DNPAOs accumulate polyphosphate (polyP) by using nitrate as an electron acceptor (Ahn *et al.*, 2002; Tsuneda *et al.*, 2003). Therefore, competition

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for organic carbon source uptake between PAOs (DNPAOs) and denitrifying bacteria never occurs in A/O/A systems. Moreover, in the A/O/A system, the reactor can be simplified because liquid circulation after nitrification is unnecessary.

Phosphorus in wastewater is not only thought to be removed, but also recovered and recycled, because the phosphorus resource tends to be depleted. To achieve enhanced phosphorus recovery in wastewater, it is essential to recover phosphorus from excess sludge and recycle it immediately. For this social background, development of phosphorus recovery technology is intensively studied (Takai *et al.*, 2001). By using zirconium-ferrite ($ZrFe_2(OH)_8$) adsorbent, higher efficiency of phosphorus removal and higher purity can be achieved.

Treatment and disposal of excess sludge produced in WWTPs became also one of the serious problems. Recently, a combined system of activated sludge process and ozonation for excess sludge reduction has been successfully developed (Yasui and Shibata, 1994; Kamiya and Hirotsuji, 1998). In excess sludge reduction by ozonation, a part of the sludge is ozonized to improve the biodegrability. Conceptually, this technology can develop the non-excess-sludge process.

In this study, a new advanced sewage treatment process was developed with combination of three sub-processes, which were: (1) A/O/A process for nitrogen and phosphorus removal, (2) sludge ozonation process for excess sludge reduction, and (3) phosphorus adsorption process for phosphorus recovery (Figure 1). Then water quality of the effluent, sludge reduction efficiency and phosphorus recovery efficiency were evaluated through 92 days operation with changing amounts of ozonized sludge. In addition, populations and activity of DNPAOs in the present system were discussed based on fluorescence *in situ* hybridization (FISH) analysis.

Materials and methods

Reactor operation

Two types of reactors with 36 L of effective volume: (i) A/O/A process equipped with ozonation and phosphorus adsorption process (Run 1: Ozonation system), and (ii) A/O/A process without physicochemical process (Run 2: Reference system) were operated continuously. Reactor operation condition is shown in Table 1. The whole operation period (92 days) was divided into three phases (Phase 1: 0–48 days, Phase 2: 49–71 days and Phase 3: 72–92 days), and the amount of ozonation sludge was changed in each phase. Ozonation condition in each phase is shown in Table 2. During the periods, the temperature was controlled to be 20 °C. In this study, domestic wastewater was used and its characteristics are shown in Table 3. In Phases 2 and 3, sodium acetate, urea and potassium dihydrogenphosphate were added into the domestic wastewater to control 90–120 mg L^{-1} of total organic carbon (TOC), 40–50 mg L^{-1} of total nitrogen (T-N) and 4–5 mg L^{-1} of total phosphate (T-P). Ozone concentration and dosage were set at 20 mg L^{-1} and around 300 mg-O_3 (g-MLSS)$^{-1}$ throughout the periods, respectively.

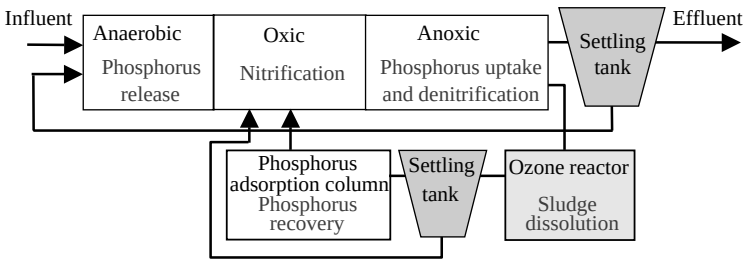

Figure 1 New wastewater treatment process combined with ozonation and phosphorus adsorption

Table 1 Reactor operation conditions

	Run 1	Run 2
Ozonation, Phosphorus adsorption	–	–
HRT (h)		
Anaerobic	4.3	
Oxic	4.3	
Anoxic	6.4	
Total	15	
MLSS (mg L^{-1})	2,800–3,000	
Sludge return (%)	60	

Table 2 Ozonation conditions in Run 1

	Phase 1	Phase 2	Phase 3
Ozone concentration (mg-O_3 L^{-1})	20	20	20
Ozone dosage (mg-O_3 (g-MLSS)$^{-1}$)	290	320	300
The sludge amount for ozonation (L day^{-1})	2.2	3.8	7.7

The amount of ozonized sludge was increased gradually (Phase 1: 2.2 L day^{-1}, Phase 2: 3.8 L day^{-1}, Phase 3: 7.7 L day^{-1}). Ozone concentration and dosage were controlled using Ozone Generator (Fuji Electric) and UV Ozone Monitor PG-620HA (E. J. Ozone Products).

Evaluation of sludge reduction efficiency

The sludge production quantity of each reactor and sludge reduction rate in Run 1 were calculated. Sludge production throughout the operation terms was calculated from (1) overflow of SS, (2) sludge drawing from settling tank, (3) variation of sludge quantity in the A/O/A system. Sludge reduction rate was defined as (sludge production quantity)/(removed TOC in influent water).

Evaluation of phosphorus recovery efficiency

During the operation periods, the amount of adsorbed phosphorus of Run 1 was calculated. Phosphorus recovery efficiency was defined as (the amount of adsorbed phosphorus)/(the amount of phosphorus in the influent).

Evaluation of treated water quality

To evaluate the effect of ozonation on treated water, water quality between Run 1 and Run 2 was compared. TOC, T-N, T-P and SS of treated water of both reactors were analyzed according to *Standard Methods* (APHA *et al.*, 1998).

Table 3 Properties of domestic wastewater

	Concentration (mg L^{-1})	
	Phase 1	Phases 2 and 3
TOC	40–60	90–120
T-N	20–35	40–50
NH_4-N	15–25	30–45
T-P	3.0–4.5	4.0–5.0
PO_4-P	2.0–3.0	2.5–3.5
SS	80–120	80–120

FISH analysis

For FISH analysis, grab sludge samples were collected and fixed with 3% paraformaldehyde for 2 h at 4 °C. Hybridization was performed according to the standard hybridization protocol described by Amann (1995). Oligonucleotide probes used in this study were FITC labeled EUB338 (Amann *et al.*, 1990) and Cy3-labelled PAOs mix probe (PAO462, PAO651 and PAO846) (Crocetti *et al.*, 2000). The washing buffer was removed by rinsing the slides with ddH_2O. Then the samples were mounted in VECTASHIELD Mounting Medium (Vector Laboratories, UK), and observed under a confocal laser scanning microscope (TCS4D; Leica Lasertechnik, Heidelberg, Germany).

Results and discussion

Evaluation of sludge reduction efficiency

Sludge yields and sludge reduction efficiencies in each phase are shown in Figure 2. Decrease in sludge yield of the ozone system was confirmed throughout the periods. Phase 1 exhibited 34% of the sludge reduction efficiency, Phase 2 52% and Phase 3 127%. Sludge reduction efficiency was over 100% at Phase 3 indicating that sludge production quantity was lower than sludge reduction quantity. Therefore, by changing the quantity of ozonized sludge, the quantity of reduced sludge could be controlled. In this study, to achieve 100% of sludge reduction efficiency, ozonation of 6 L sludge was found to be proper.

Evaluation of phosphorus recovery efficiency

The adsorption efficiency of dissolved phosphorus from ozonized sludge is shown in Figure 3. Throughout the period, 90–100% of dissolved PO_4-P originating from ozonized sludge was adsorbed by zirconium-ferrite adsorbent. However, the suspension after ozonation contained not only PO_4-P but also organic P (Org-P). As a result, around 70% of Org-P and around 80% of T-P recovery were adsorbed. From these results, by using zirconium-ferrite adsorbent, effective phosphorus recovery from excess sludge could be confirmed.

Evaluation of characteristics of treated water

Figure 4 shows the treated water quality of Run 1 and Run 2. In Phase 1, TOC, T-N and T-P were removed effectively in both systems because of lower loading of all compositions in domestic wastewater. The effluent water qualities of Run 1 and Run 2 were almost the same. This means that the quantity of the ozonized sludge was small and deterioration of effluent water quality by ozonation was not observed. However, in Phase

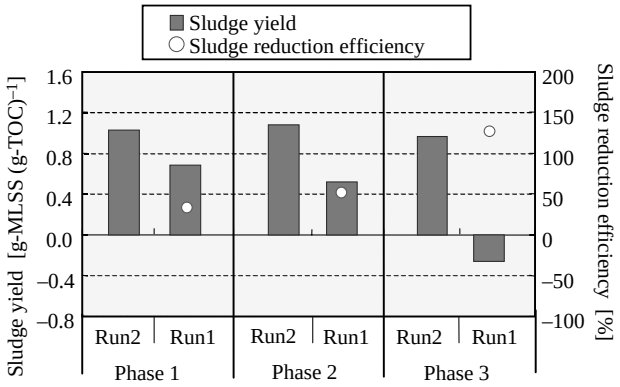

Figure 2 Sludge yield and sludge reduction efficiency in ozonation system

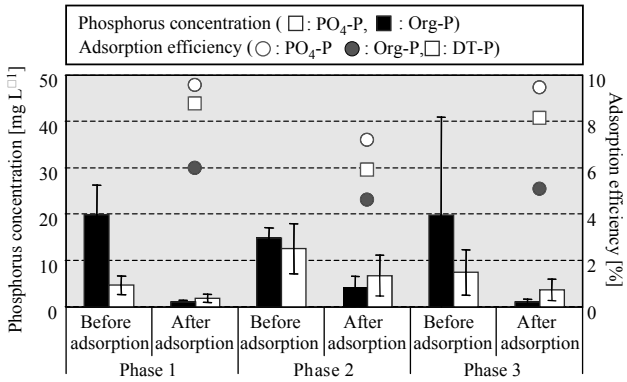

Figure 3 Phosphorus adsorption efficiency of zirconium-ferrite adsorbent in ozonation system

2 and Phase 3, deterioration of treated water quality was confirmed in Run 1. These results were attributed to the existence of Org-P and hard biodegradable organic carbon. As a result, TOC, T-N and T-P removal efficiencies were 85%, 70% and 85% in Run 1. To enhance biodegradability of organic carbon in ozonized sludge, improvement of ozone concentration and adjustment of ozone dosage for sludge were important.

Dominance of DNPAOs in A/O/A systems

FISH analysis demonstrates that *Rhodocyclus*-related PAOs (RPAOs), which were detected by the PAOs mix probe, occupy a considerable proportion of the A/O/A systems (Figure 5). Recently, Kong *et al.* (2004) reported that RPAOs were able to accumulate polyP when oxygen, nitrite, or nitrate was present as an electron acceptor by using microautoradiography and FISH (MAR-FISH). In this study, denitrification was also confirmed and it was supposed that nitrate was used as an electron acceptor in anoxic tanks by DNPAOs including RPAOs. Figure 6 shows the concentration changes of PO_4-P in each tank. In the reference system (Run 2), most of the phosphorus was removed in the oxic tank, whereas in the ozonation system (Run 1) phosphorus remained in the oxic tank. This was because return solution from ozone and adsorption processes contained a certain amount of organic carbon

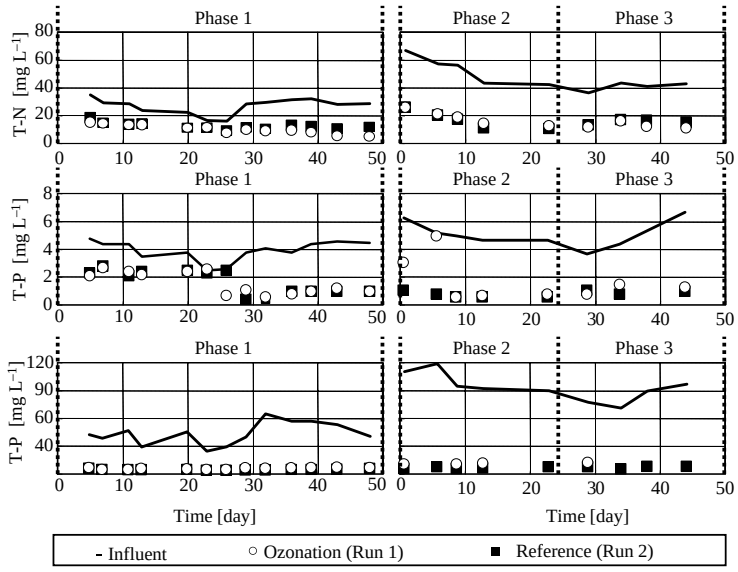

Figure 4 Effluent water quality in ozonation and reference systems

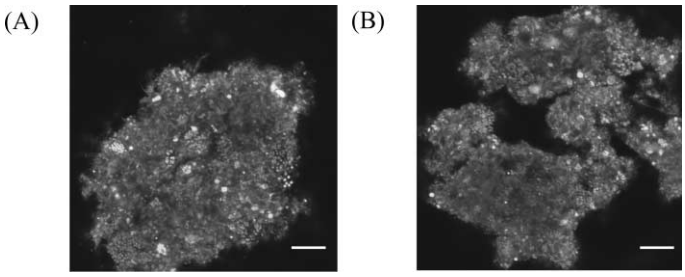

Figure 5 FISH images of RPAOs (yellow or red) and other bacteria (green) in the anoxic tank of Run 1 (A) and Run 2 (B) on day 59. Bar = 20 μm

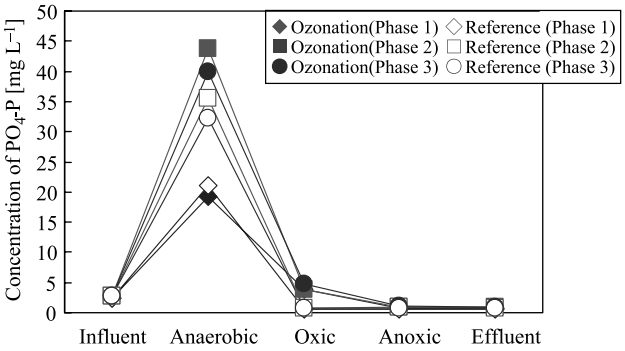

Figure 6 Concentration changes of PO_4-P in each tank

that inhibited phosphorus uptake in oxic conditions and then phosphorus uptake was confirmed in the anoxic tank. However, it was suggested that activity of PAOs was higher than that of DNPAOs in this study. Therefore, in a further study, consideration of the methods to increase the activity of DNPAOs in WWTPs is still needed.

Conclusion

In order to reduce excess sludge and recover phosphorus from the sludge, the A/O/A system equipped with ozonation and phosphorus adsorption systems was operated with changing of the sludge amount for ozonation. Conclusions are as follows.

(1) By controlling the sludge amount for ozonation, the sludge reduction rates could be regulated. In this study, 34–127% of sludge reduction was attained.
(2) PO_4-P and Org-P were dissolved by sludge ozonation and over 90% of PO_4-P and 70% of Org-P could be recovered by zirconium-ferrite adsorbent.
(3) In the A/O/A system equipped with ozonation tank and phosphorus adsorption column, treated water quality deteriorated measurably. However, TOC, T-N and T-P removal efficiencies were 85%, 70% and 85%, respectively, throughout the operating period.
(4) Although improvement of the methods to increase the activity of DNPAOs in WWTP is still needed for stable operation, RPAOs occupy a considerable proportion of the A/O/A systems.

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