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Enhancement of denitrifying phosphorus removal and microbial community of long-term operation in an anaerobic anoxic oxic–biological contact oxidation system

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A two-sludge system consisting of anaerobic anoxic oxic–biological contact oxidation (A^2/O–BCO) was developed to treat domestic wastewater with a low carbon/nitrogen (COD/TN) ratio (around 3.21) by shortening sludge retention time (SRT) for phosphorus accumulating organisms (PAOs) in the A^2/O reactor and prolonging SRT for nitrifiers in the BCO reactor. Specifically, the BCO reactor was composed of three stages in series (N_1, N_2 and N_3), so that simultaneous nitrogen and phosphorus removals by denitrifying PAOs (DNPAOs) was achieved in the A^2/O reactor with NO_x^-–N as the electron acceptor from the BCO reactor. Long term operational tests (600 days) were conducted with various operational parameters [e.g., hydraulic retention time (*HRTs*), nitrate recycling ratio (*Rs*), volume ratio (*Vs*)] to examine the denitrifying phosphorus removal performance. The system exhibited the highest removal of TN and PO_4^{3-}–P at the *HRTs* of 8 h, *Rs* of 300% and *Vs* of 2:4:1. The optimal TN and PO_4^{3-}–P removals were 80.30% and 96.61% at low COD/TN of 3.21. The species diversity and microbial community examined by the Illumina MiSeq method demonstrated the fact of two-sludge system, and the improved community structure by long-term optimization was prominent comparing with the seed sludge. Additionally, *Accumulibacter* and *Dechloromonas* were the dominant functional PAOs with 25.74% in the A^2/O reactor, while nitrifiers (including *Nitrosomonas* and *Nitrospira*) were gradually enriched with 13.10%, 21.33%, and 31.10% in the three stages of the BCO reactor.

[**Key words:** Anaerobic anoxic oxic–biological contact oxidation; Denitrifying phosphorus removal; Optimization; Illumina MiSeq; Microbial diversity]

Developing cost-effective nitrogen and phosphorus removal processes is critical to alleviate eutrophication. In the last decade, denitrifying phosphorus removal has received a high attention due to low aeration requirement and sludge production (1). Enhanced biological phosphorus removal (EBPR) was widely used with phosphorus accumulating organisms (PAOs) or denitrifying PAOs (DNPAOs) (2). Comparing with the conventional biological nutrient removal (BNR) processes requiring high amount of carbon source as electron donor and high aeration (oxygen) as electron acceptor, denitrifying phosphorus removal can simultaneously remove nitrogen and phosphorus with nitrate (NO_3^-) or nitrite (NO_2^-) as electron acceptors, and thus efficiently utilizing the limited carbon sources contained in wastewater with low carbon/nitrogen (COD/TN) ratio (3).

A two-sludge system of anaerobic anoxic oxic–biological contact oxidation (A^2/O–BCO) was developed to combine the advantages of activated sludge and biofilm, and favor the development of specialized microbial communities (4). In contrast to the previous A^2/O–biological aerated filter (BAF) process (5), the BCO reactor was filled with suspended carriers, which prolonged the retention time of nitrifying bacteria with slow growth (6). The BCO reactor also solved the clogging problem of BAF and enhanced the system operational stability (7). In addition, the enrichment of DNPAOs in the A^2/O reactor substantially improved the efficiency.

The newly developed A^2/O–BCO system was successfully operated over one and a half years (600 days), and diverse parameters (e.g., hydraulic retention time (*HRTs*), nitrate recycling ratio (*Rs*), and volume ratio (*Vs*)) were studied for enhancing denitrifying phosphorus removal. Even though the system ran well, the nitrogen and phosphorus removal capacity deteriorated sometimes when the operating conditions changed (e.g., *HRTs*, *Rs* and *Vs*). It was reported that denitrifying phosphorus removal was not only determined by the reactor configuration but also the function of microbial community (8). So the metabolic pathway of nutrients removal might be potentially influenced by the structure and distribution of functional microbial communities (e.g., PAOs, DNPAOs, nitrifiers) (9). Recently, functional species (e.g., *Acinetobacter*) was isolated for EBPR enhancement (10), and many studies were conducted to understand the growth mechanism of DNPAOs (11). However, the fraction of DNPAOs was not high in traditional single-sludge EBPR systems (12), and the cultivation and enrichment of DNPAOs by the separation of sludge retention time (SRT) provided feasibility in A^2/O–BCO system.

Traditional metagenomic approach generally referred to amplification of target gene fragments using polymerase chain

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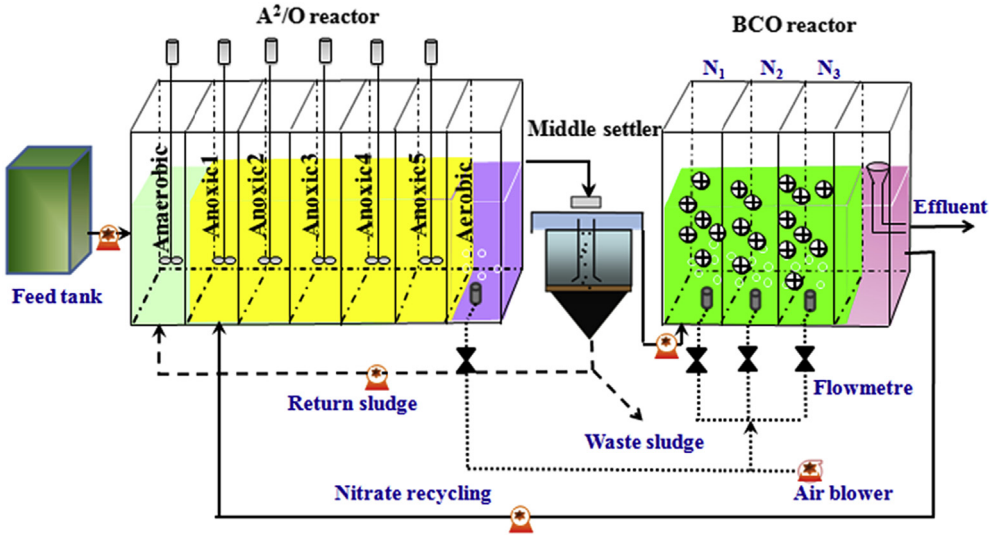

FIG. 1. Schematic diagram for A^2/O–BCO reactor.

reaction (PCR) or quantitative fluorescent in situ hybridization (qFISH), following by building a library for sequencing (13). However, the bioinformatics tools and databases cannot accurately classify bacteria to the species level (14). A low cost but high coverage technology of high-throughput sequencing (HTS) (15) has been widely applied to increase the sensitivity of mutation detection. Given the poor understanding of the microbial ecology in activated sludge and biofilm (16), it was urgent to master microbial information of the two-sludge denitrifying phosphorus removal process treating real domestic wastewater.

To strengthen the stable operation of A^2/O–BCO system, the effect of process parameters on denitrifying phosphorus removal combining with microbial metabolism was conducted, where microbial community structure of long-term operation was also analyzed to confirm the metabolic mechanism. The objectives of this study were to: (i) explore the performance through the optimization of different operational parameters, (ii) analyze the achievement and enhancement of denitrifying phosphorus removal, and (iii) evaluate the microbial characteristics of the two-sludge system based on HTS technology. An attempt was made to seek the interpretation between macro performance and micro behavior.

MATERIALS AND METHODS

Experimental equipment The A^2/O–BCO system was composed of an A^2/O reactor, a middle settler, and a BCO reactor (Fig. 1). The A^2/O reactor (working volume: 42 L) was evenly divided into seven chambers with anaerobic zones, anoxic zones and aerobic zone. For the anaerobic and anoxic zones, the agitators worked to keep a complete mixing of sludge and water. For the aerobic zone, air was supplied by diffusers with the dissolved oxygen (DO) of 1.0–1.5 mg/L to expel nitrogen gas from effluent. The effluent of the aerobic zone then flew into the middle settler (working volume: 15 L), and the settled sludge was recycled to the anaerobic zone of the A^2/O reactor, while the supernatant flew to the BCO reactor (working volume: 18 L) to complete the NH_4^+–N oxidation.

The BCO reactor for nitrification was divided into three identical chambers (N_1, N_2, and N_3) packed with cylinder polypropylene carriers (diameter: 25 mm and height: 10 mm). Different from traditional plastic rings, activated materials (e.g., polyacrylamide, calcium stearate, rare-earth compound) were added to improve hydrophilicity and biological affinity. The optimized carriers with higher specific surface area (950–1000 m^2/m^3) and larger porosity (93–98%) can speed up the biofilm formation and strengthen nitrification effect (17). The density of the carriers was 960–1000 kg/m^3, which can suspend in the reactor. The occupied percentage of carriers was determined to be 40%. The total aeration rate of 0.18–0.20 m^3/h was provided by an air blower and the DO concentration in the BCO reactor was 3.50–4.50 mg/L. The nitrate produced in the BCO reactor was recycled to the anoxic zone of the A^2/O reactor and acted as the electron acceptor for denitrifying phosphorus removal. The flow rates of influent, sludge return, and nitrate recycling were controlled by peristaltic pumps (Shenchen Co., Ltd., China).

Wastewater and experimental approach Domestic wastewater was taken from a septic tank in Beijing University of Technology (China), and the wastewater characteristics are shown in Table S1. The COD/TN was 3.21, which was a typical wastewater with low COD/TN ratio. When the COD/TN ratio was below 3.0, a little amount of sodium acetate was added to minimize the effect of fluctuated wastewater quality. The seed sludge was obtained from a pilot-scale oxic/anoxic sequencing batch reactor (O/A–SBR) system treating domestic wastewater on campus. According to the standard method (18), less than 3% of the PAOs population was able to use NO_3^-–N as electron acceptor in the seed sludge (Table 1).

TABLE 1. Comparison of the stoichiometric coefficients under different operational conditions in this study and previous studies.

References	Anaerobic transformations			Anoxic transformations			Aerobic transformations	PAOs (%)	DNPAOs/PAOs (%)
	$PHA_{production}$/VFA (mmolC/mmolC)	$GLY_{degradation}$/VFA (mmolC/mmolC)	$P_{release}$[a] mgP/(gVSS h)	$PHA_{degradation}$ (mmolC/gVSS)	$GLY_{production}$ (mmolC/gVSS)	P_{uptake}[a] mgP/(gVSS·h)	P_{uptake}[a] mgP/(gVSS·h)		
This study (seed sludge)	/	/	/	/	/	0.19	6.48	1.25	2.93
This study, run 2 (70 d)	1.06	0.17	10.20	1.12	0.68	4.84	8.73	/	55.4
This study, run 7 (250 d)	1.23	0.24	14.12	1.65	0.76	5.97	9.43	28.62	63.3
This study, run 11 (410 d)	1.34	0.39	11.28	2.87	0.97	7.18	10.24	35.50	70.1
41	1.35	0.85	/	/	/	/	/	38	45.9
45[b]	0.94–1.23	0.08–0.15	/	/	0.24–0.84	12.71–14.88		/	/
42[b]	0.64–1.39	0.21–0.91	/	/	/	/	22.32	/	/
44[b]	0.8–1.4	0.6–0.7	6.20	/	/	7.0	/	/	/
25	/	/	/	/	/	23.4	31.8	/	74

$PHA_{production}$: PHA production rate, mmolC/gVSS; $GLY_{degradation}$: glycogen degradation rate, mmolC/gVSS; $PHA_{degradation}$: PHA degradation rate, mmolC/gVSS; $GLY_{production}$: glycogen production rate, mmolC/gVSS; $P_{release}$: specific phosphorus release rate, mgP/(gVSS·h); P_{uptake}: specific phosphorus uptake rate, mgP/(gVSS h); DNPAOs/PAOs (%) = anoxic P_{uptake}/aerobic P_{uptake} × 100%.

[a] Data of $P_{release}$ and P_{uptake} reported in this study were the maximum values based on batch tests.

[b] Operated with propionate or acetate as electron donors.