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Development and Application of a novel anaerobic/aerobic/anoxic process in municipal wastewater treatment utilizing intracellular carbon sources

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Abstract Municipal wastewater treatment plants (WWTPs) face a significant challenge in controlling treatment costs while meeting increasingly stringent wastewater discharge standards. To address this challenge, A novel anaerobic/anoxic/aerobic (AOA) process has been proposed. In this study, long-term nutrient removal and microbial community variations were investigated in the AOA systems of different scales, including three laboratory-scale systems, one pilot-scale and one full-scale systems. In the AOA system, nitrogen removal exceeded 50% was carried out in the post-anoxic zone via endogenous denitrification driven by intracellular carbon sources. The synergistic effect of denitrifying bacteria (DNB), anaerobic ammonium oxidation (anammox) bacteria, and fermentative bacteria (FB) enhanced nitrogen removal. Advanced nutrient removal was successfully achieved in the AOA process under different environments and influent quality. Under a low influent carbon-nitrogen ratio, the effluent total nitrogen (TN) concentration was 3.6-8.7 mg/L, with a TN removal efficiency of 71-95%. Total phosphorus (TP) concentration in effluent of 0.2-0.3 mg/L was also achieved. Moreover, compared to the widely used anaerobic/aerobic/anoxic (AAO) process, the AOA process reduced 60% of energy consumption by aeration and recirculation. In conclusion, the AOA process has the potential for practical implementation and provides an economical and efficient nutrient removal process for municipal wastewater treatment.

Keywords: Wastewater treatment, Advanced nutrient removal, Anaerobic/aerobic/anoxic, Energy Saving, Endogenous denitrification

1. Introduction

Stringent discharge quality standards of wastewater treatment plants (WWTPs) can help to prevent surface water bodies from eutrophication (Yu et al. 2019). Municipal wastewater, due to its low C/N ratio, poses challenges in achieving high nitrogen removal efficiency using limited carbon sources. Without external carbon source, organisms could use intracellular organics as electron donor in the denitrification for nitrogen removal (Gao et al. 2022). Development and utilization of intracellular carbon sources and the mechanism of microbial synergy are considered feasible for enhancing nitrogen removal in WWTPs. A novel anaerobic/aerobic/anoxic (AOA) process has been proposed (Zhang et al. 2018), and it is beneficial for endogenous denitrification via enhancing the storage and utilization of intracellular carbon sources.

In this study nitrogen removal mechanism, performances, and advantages of the AOA system was clarifies the in combination with our series and recent research results. In order to provide a feasible process for efficient and economic wastewater treatment.

2. Materials and Methods

2.1 Establishment of AOA system

Three laboratory-scale AOA systems were established in temperate regions (Beijing, china). Activated sludge was obtained from the GaoBeiDian WWTP (Beijing, China). Raw municipal wastewater was collected from the residential area of the Beijing University of Technology (Beijing, China). The temperature varies naturally with the seasons between 15-26 ℃, and dissolved oxygen (DO) was controlled at 0.5 - 2.0 mg/L. No excess sludge was discharged, amd the mixed liquid suspended solids (MLSS) concentration were 4000 ± 1000 mg/L.

The pilot-scale AOA system was located in tropical region (Hainan, China). The activated sludge was obtained from the BaiSaMen WWTP (Hainan, China). The actual municipal wastewater was collected from the effluent of the grit chamber of BaiSaMen WWTP (Hainan, China). The sludge retention time (SRT) was about 25-30 d, and MLSS were 3000 ± 1000 mg/L.

The full-scale AOA system was in subtropical region (Guangxi, China). Seed sludge was excess sludge from the WWTPs and raw wastewater as the actual municipal wastewater in Cenxi city (Guangxi, China). The SRT was about 30 d, and MLSS were 2000 ± 1000 mg/L.

The processing capacity and hydraulic retention time (HRT) of the AOA system is shown in Table 1and Schematic diagram is shown in Fig.1.

2.2 Methods for chemical analysis

Before analysis, all samples underwent filtration using filter paper with an aperture no larger than 15-25 μm. Concentrations of NH_4^+-N, NO_2^--N, and NO_3^--N COD and MLSS were determined according to standard methods (APHA, 1998). The 3420 analyzer (WTW Company, Germany) was used to monitor DO and temperature. Poly-β-hydroxyalkanoates (PHAs) was determined by a gas chromatograph (Agilent 7890A) (Oehmen et al. 2005). Glycogen (Gly) was analyzed by anthrone method (Zeng et al. 2003). EEM spectra measurement was according to the method described by Miao using fluorescence spectrometer (PerkinElmer LS55,U.K.). Illumina high-throughput sequencing was used according to previous reported (Gao et al. 2023).

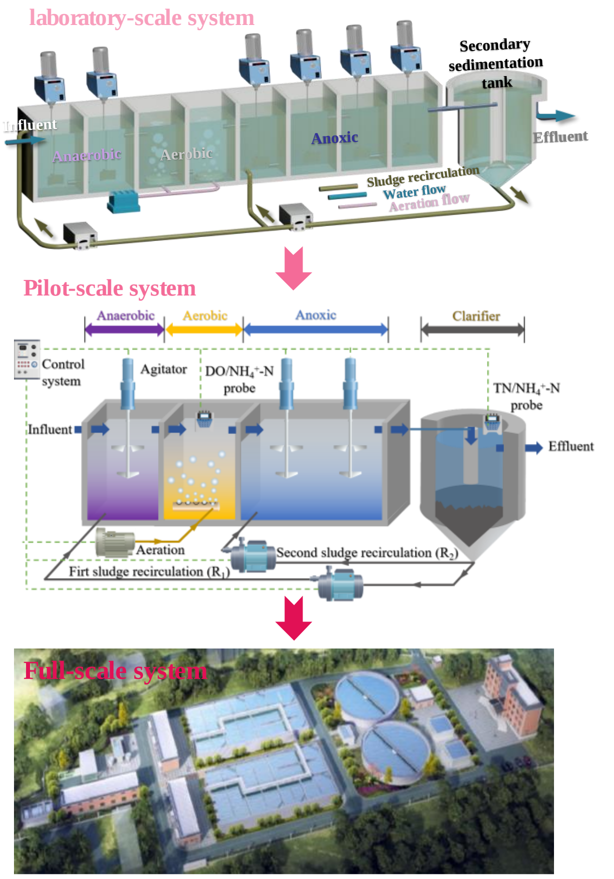

Figure 1 Schematic diagram of the AOA system

3. Results and discussions

3.1. Synergistic nitrogen removal mechanism of microorganism in the AOA process

The mechanism of nitrogen removal in a laboratory scale AOA system (Lab3-AOA) was explored. The variation of nutrient concentration in different zones is showed in Figure 1(A). Nitrogen removal was mainly carrried on the post-anoxic zone, accounting by 60.64%. Since the COD concentration remained unchanged, endogenous denitrification driven by internal carbon sources was suggested for nitrogen removal. PHA and glycogen decrease with the reduction of nitrate concentration, which was the possibile driving force of endogenous denitrification. The EEMs of the supernatant at the initial and the terminal of anoxic zone, and at the terminal of anoxic zone without inflow (no nitrate removal in the anoxic zone) were measured. Figure 1 (C) shows that the intensities of the two peaks are significantly enhanced at the terminal of anoxic zone. According to spectral information, aromatic protein like substances and by product like substances (e.g., tryptophan) (Li et al. 2020) might producted in the post-anoxic zone. They might come from the lysis and death of microorganisms, and driven for denitrification.

According to the results of high-throughput sequencing (Figure 3), Candidatus Competibacter were enriched, and it was able to transform carbon sources into pol-β-hydroxy alkanoates and glycogen for denitrification (McIlroy et al., 2014). It is worth noting that the decrease of ammonium was also observed in the poost-anoxic zone. Meanwhile, the abundance of anaerobic ammonium oxidation (anammox) bacteria increased from no detected to 0.9%. The anammox process might removed partial nitrogen in post-anoxic zone. Moreover, fermenting bacteria (FB) were detected, accounting for 9.71-38.50%. The large molecular organic compound producted by cell death and lysis, and decompose by FB to generate carbon source (such as Volatile fatty acids (VFAs)) for denitrifying bacteria (DNB). DNB reduce nitrate to nitrite and supply to anammox bacteria. The synergistic effect was enhanced the nitrogen removal under the condition of carbon limitation.

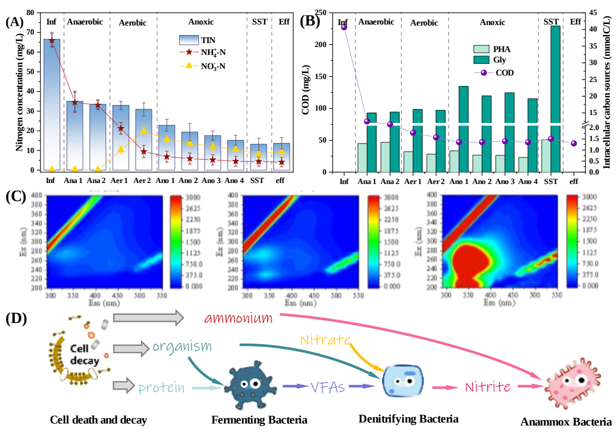

Figure 2 Mechanism of microorganism synergism in the anoxic zone (A: nitrogen removal performances in different zones; B: Variations of carbon sources in different zones; C: EEMs in initial and terminal of anoxic zone; D: Mechanism of microbial synergism in anoxic zone)

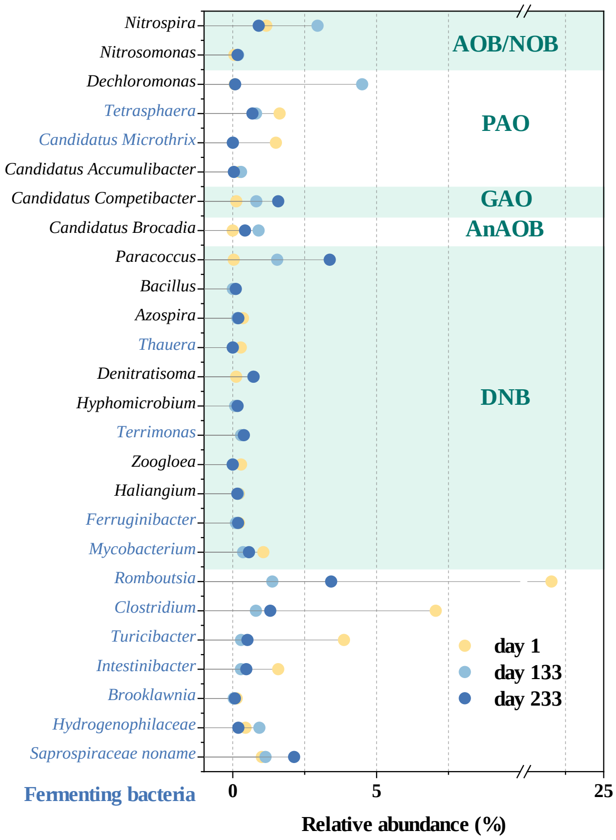

Figure 3 Relative abundance of functional bacteria at the genus level in the lab3-AOA system on days 1, 133 and 233

3.2. Long-term nutrient removal performances of the AOA process

The long-term nutrient removal efficiencies of the AOA systems are shown in Table 1. Three laboratory-scale systems were located in temperate regions with water temperatures ranging from 15 to 26 ℃. At stable operation periods, the influent C/N was about 3, and the TN in the influent was 50 - 70mg/L. The TN removal efficiency of 87 - 90% and the TN concentration in the effluent of 3.5-8.7 mg/L were achieved. Advanced nitrogen removal was successfully achieved in the AOA systems. Without excess sludge discharging, phosphorus release occurred in the secondary sedimentation tank, and poor TP removal efficiency was observed in laboratory-scale systems.

The pilot-scale AOA system was established in the tropical region. During nearly 400 days of operation, the water temperature was stable at 22 - 33 ℃, and the TN and TP removal efficiency was stable after one week of start-up. In low C/N (5.0) municipal wastewater treatment, the TN concentration in effluent of only 3.6 ± 1.6 mg/L was achieved, and the TP concentration in the effluent was only 0.24 ± 0.13 mg/L. The TP removal efficiency reached 93%. Simultaneous nitrogen and phosphorus removal nitrogen was realized in the AOA system for the first time.

The full-scale AOA system was in subtropical regions. The system entered a stable operation period after about one month of start-up. Overall one year of operation, with temperatures ranging from 18 to 28 ℃, the TN and TP concentration in the effluent was stable at 7.5 ± 2.5 and 0.32 ± 0.08 mg/L. The feasibility of advanced nitrogen and phosphorus removal had been proved for the first time on a full-scale scale AOA system.

Table 1 Nutrient removal preferences of the AOA systems

System	Lab1-AOA	Lab2-AOA	Lab3-AOA	Piolt-AOA	Full-AOA
Scale	126 L/d	110 L/d	90 L/d	100 m^3/d	30000 m^3/d
HRT (h)	16	13-16	16	10.8	10-12
Influent TN (mg/L)	50.4 ± 4.3	67.2 ± 3.9	67.1 ± 3.7	25.7 ± 4.15	25.9 ± 13.8
Effluent TN (mg/L)	5.0 ± 2.7	3.5 ± 1.7	8.7 ± 1.7	3.6 ± 1.6	7.5 ± 2.5
TN removal efficiency	90%	95%	87%	86%	71%
Influent TP (mg/L)	4.49 ± 0.19	5.56 ± 0.81	5.59 ± 0.91	3.26 ± 1.68	1.97 ± 0.62
Effluent TP (mg/L)	2.60 ± 1.02	3.39 ± 1.85	4.05 ± 2.00	0.24 ± 0.13	0.32 ± 0.08
TP removal efficiency	-	-	-	93%	84%

3.3. Energy saving advantages on the AOA process

Efficient nutrient removal in the AOA systems was successfully achieved. Meanwhile, outstanding advantages in energy conservation and consumption reduction in the AOA systems were also observed. Firstly, in the anaerobic zone of AOA systems, the carbon sources in the raw wastewater were removed by converting them into intracellular carbon sources, which reduced the carbon sources consumed by aeration. The carbon sources saved lead to more nutrient removal, low aeration energy and short HRT of aerobic zone. The proportion of aerobic zone in the AOA system was only 25-33%. Secondly, the AOA system as a post-denitrification process, without limitation by nitrified liquid recirculation ratio, could achieve complete nitrogen removal with sufficient carbon in theory. The energy consumption for recirculation was also saved. Therefore, compared with the widely used anaerobic/aerobic/anoxic (AAO) process (Jin et al. 2014), to achieve the same nitrogen removal efficiency, the AOA process reduced 60% of energy consumption by aeration and recirculation (Gao et al. 2022, Song et al. 2020). Finally, a lower excess sludge production had observed due to the utilization of intracellular carbon sources and the production of cell death and lysis. The cost of excess sludge treatment was saved.

4. Conclusions

This study provides an economical and efficient nutrient removal process for low C/N ratio municipal wastewater treatment, mainly including the following findings:

- ✧ The nitrogen removal of the AOA system was mainly in the post-anoxic zone driven by intracellular carbon sources. The synergistic effect of DNB, FB and anammox bacteria has enhanced nitrogen removal.

- ✧ Advanced nitrogen and phosphorus removal was achieved in AOA systems with TN and TP removal efficiency of 71-95% and 84-93%.
- ✧ The AOA system has the advantage of carbon saving and energy consumption reduction. The feasibility at full scale has been validated, which is conducive to promotion and application in municipal wastewater treatment under a low C/N ratio

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