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Improvement strategy on enhanced biological phosphorus removal for municipal wastewater treatment plants: Full-scale operating parameters, sludge activities, and microbial features

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ABSTRACT

The poor quality of effluent discharged by municipal wastewater treatment plants (WWTPs) is threatening the safety of water ecology. This study, which integrated a field survey, batch tests, and microbial community identification, was designed to improve the effectiveness of the enhanced biological phosphorus removal (EBPR) process for WWTPs. Over two-thirds of the investigated WWTPs could not achieve total P in effluent lower than 0.5 mg/L, mainly due to the high ratio of chemical oxygen demand to P (28.6–196.2) in the influent. The rates of anaerobic P release and aerobic P uptake for the activated sludge varied from 0.22 to 7.9 mg/g VSS/h and 0.43 to 8.11 mg/g VSS/h, respectively. The fraction of *Accumulibacter* (PAOs: polyphosphate accumulating organisms) was 4.8 ± 2.0% of the total biomass, while *Competibacter* (GAOs: glycogen-accumulating organisms) accounted for 4.8 ± 6.4%. The anaerobic P-release rate was found to be an effective indicator of EBPR. Four classifications of the principal components were identified to improve the EBPR effluent quality and sludge activity.

1. Introduction

Municipal wastewater, which may contain a considerable amount of nutrients and organic matter, can intensify water eutrophication if not adequately treated before discharging into surface waters. The current municipal wastewater treatment plants (WWTPs), particularly those located in rapidly growing regions, such as the coastal area of Southeast China, are facing increasingly rigorous standards for effluent discharge in the coming future, due to the very limited regional environmental capacity of the receiving water. Phosphorus (P) is a key nutrient that stimulates the growth of algae and other photosynthetic microorganisms such as toxic cyanobacteria. Reducing P input to nearby waters, along with other technical innovations, is an obligatory mission for both WWTP engineers and the neighboring communities.

Enhanced biological phosphorus removal (EBPR) has become an established process for many full-scale WWTPs that rely on an activated sludge system with polyphosphate accumulating organisms (PAOs), mainly *Accumulibacter* (Kong et al., 2005; Chua et al., 2006; Burow et al., 2007). Unlike most other microorganisms, PAOs can take up carbon sources such as volatile fatty acids (VFAs) under anaerobic conditions and store them intracellularly as carbon polymers, namely, poly-β-hydroxyalkanoates (PHAs) (Grady et al., 1999). Denitrifying polyphosphate accumulating organisms (DNPAOs), which can use nitrate/nitrite as an electron acceptor instead of oxygen to remove phosphate (Carvalho et al., 2007), have attracted attention because they can use the same carbon to simultaneously remove N and P and require 20–30% less microorganisms (Kishida et al., 2006). It has been reported that the fraction of DNPAOs to the total PAOs family in sludge varies from 0% to 80% in full-scale WWTPs (López-Vázquez et al., 2008; Brdjanovic et al., 2000). However, several reports have described the presence of glycogen-accumulating organisms (GAOs), mainly *Candidatus Competibacter*, at either lab-based or full-scale EBPR wastewater treatment plants (Saunders et al., 2003; Burow et al., 2007; Gu et al., 2008; López-Vázquez et al., 2009). The presence of GAOs is one of the main causes for EBPR deterioration because they do not contribute to P removal but compete with PAOs for substrate (mainly volatile fatty acids, VFAs) (Saunders et al., 2003; Kong et al., 2005). Therefore, it becomes a complicated issue to quantitatively address the P removal process associated with the relevant functional microbial performances in the BEPR system.

Recent studies in this field have taken great efforts to improve the EBPR effectiveness for full-scale WWTPs, from studying the microorganisms that are primarily responsible for the work, to the process to determine the microorganisms' biochemical

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pathways and develop mathematical models that facilitate better prediction of the process performance (Oehmen et al., 2007). Factors including pH (Zhang et al., 2007; López-Vázquez et al., 2008), temperature (López-Vázquez et al., 2008; Whang et al., 2007), wastewater source (Oehmen et al., 2005), and the ratio of P to acetate acid (HAc) in the influent (Chen et al., 2004; López-Vázquez et al., 2008) have been suggested as key elements influencing both the efficiency and effectiveness of EBPR. These are also important factors regulating the competition between PAOs and GAOs in both lab and full-scale studies. However, the decisive factors influencing the stability and reliability of EBPR that can reduce operating costs have yet to be determined.

The efficiency and effectiveness of EBPR for full-scale WWTPs are greatly influenced by a multi-factor process and the associated interactions among these factors in the bio-course. Essentially, an improvement strategy for WWTPs needs to integrate the technical process, operating parameters, sludge activities and microbial populations for EBPR. It is worth emphasizing that the improvement strategies in developed countries might not be suitable for some rapidly developing regions in developing countries, mainly due to the differences in influent quality, type of techniques used, and the specific operating parameters as well. This study combined a survey on the current performance of WWTPs located in the northern region of Zhejiang Province (China) with a series of laboratory-based batch tests of activated sludge and microbial community identification to characterize the performance and activity of EBPR in the investigated WWTPs. The regulations of operating parameters coupled with sludge features were further analyzed by the principal component analysis.

2. Methods

2.1. Regional information

Ten WWTPs were investigated between December 2008 and December 2009. The effluents of each plant were discharged into the following rivers: QiGe (QG) and XiaoShan (XS) into the Qiantang River, adjacent to the East China Sea; TongXiang (TX) to the Grand Canal; XingChang (XC) to the southwest of Taihu Lake; LinAi (LA), LiangZhu (LZ), and ShiShan (SS) to the East Tiaoxi River, which begins in the mountainous forest region to the southwest and merges into Taihu Lake. The rest of the plants, including JiangDong (JD), BeiLeng (BL), and LianHe (LH), discharge effluent directly into the East China Sea. Four plants in the study utilized the A^2O (Anaerobic–Anoxic–Oxic)-Phoredox process (QG, TX, XS, JD), three utilized the OD (oxidation ditch)-Carrousel process (XC, BL, LA) and the last three plants utilized the OD-Orbal (SS, LH) and OD-two stage series (LZ) process (Table 1).

2.2. Data collection and sampling

For individual field investigations, information related to plant process configurations, operating data, and influent compositions were obtained by reviewing records, interviewing plant operators, and visiting WWTP facilities. Influent and effluent samples were collected during the visits for the laboratory batch tests. Three liters of activated sludge were also collected from the end of the aerobic tank. After collection, all samples were kept on ice during transportation.

2.3. Batch tests

Batch tests were conducted within 24 h of sludge collection to evaluate EBPR activities at the WWTPs using two sequencing batch reactor (SBR) systems, one with a working volume of 2.2 L to carry out the corresponding anaerobic–anoxic batch tests, and the other with a working volume of 1.0 L to carry out anaerobic–aerobic batch tests. Both SBRs were constantly mixed at 90 rpm at a controlled temperature of 25 ± 1 °C and a pH of 7.0 ± 0.1. The sludge was diluted three times in a mineral medium: 0.6 g/L $MgSO_4{\cdot}7H_2O$, 0.0529 g/L $CaCl_2$, 0.1 g/L NH_4Cl, 0.1 g/L KCl, 2.0 mL/L trace solution (Kuba et al., 1997) before feeding the SBRs. N_2 gas was supplied at a flow rate of 136 L/m^3/min during the anaerobic and anoxic phases to avoid oxygen intrusion. During each test, mixed liquor of 12 mL was drawn and filtered using 0.45-μm-pore-size filter paper to analyze PO_4^{3-}–P and HAc. At the end of each test, another 100 mL of mixed liquor was sampled to determine the volatile suspended solids (VSS).

For the anaerobic test, 2.2 L of sludge was put into the SBR and was incubated anaerobically with excess HAc for 270 min. Samples were drawn and monitored at least 8 times during the whole batch test. The P and HAc concentrations were measured to determine (a) the maximum acetate uptake rate, (b) the maximum anaerobic P-release rate, and (c) the ratio of total released P to consumed HAc (anaerobic P/HAc ratio).

After 270 min, the anaerobic sludge was divided into two parts, one of which was retained in the SBR for the anoxic P uptake test. To supply nitrate as an electron acceptor for DNPAOs, a volume of 20 mL $NaNO_3$ solution (31 mg/L NO_3^-–N) was added into the anoxic SBR at the start of the anoxic stage, and the remaining 100 mL solution was continuously added by a pump over 120 min. The anoxic process lasted 270 min.

2.4. Fluorescence in situ hybridization (FISH) analysis

For quantification of PAOs and GAOs among the microbial communities, FISH analyses were performed following the methods de-

Table 1
The operational parameters of the investigated municipal wastewater treatment plants (WWTPs).

MWTP	P removal process	Pre-settling	Chemical dosing for P-precipitation	Anaerobic selector	Temperature (°C) and pH in anaerobic tank	Ratio of domestic wastewater	SRT (day)	HRT (h) (anaerobic/anoxic/aerobic)	MLSS (mg/L)	VSS (mg/L)
QG	A^2O-Phoredox	Yes	No	Continuous	18–20/7.0–7.3	0.75	12	12.36 (1/3.86/7.5)	3900	2300
XC	OD-Carrousel	Yes	No	Continuous	20–21/6.8–7.0	0.70	15	10.5 (1.44/2.56/6.5)	1280	840
SS	OD-Orbal	Yes	Yes	Continuous	26–27/6.2–6.8	0.50	15	12 (2/2/8)	6240	3250
LH	OD-Orbal	Yes	Yes	Continuous	30–32/7.3–7.6	0.50	3	6.2 (2/0/4.2)	3000	1600
TX	A^2O-Phoredox	Yes	No	Separate	25–26/7.3–8.1	0.55	21.7	8.6 (1/2.3/5.3)	3200	2000
BL	OD-Carrousel	Yes	No	Continuous	25–27/7.2–7.9	0.40	10	8.5 (0.5/3.5/4.5)	3500	1400
LZ	OD-two stage series	No	No	Continuous	20–22/6.9–7.6	0.70	20	3.0 (1.3/0/1.7)	3400	2000
LA	OD-Carrousel	Yes	No	Continuous	18–21/6.8–8.3	0.70	20	5.0 (2/2/1)	2000	1600
XS	A^2O-Phoredox	Yes	No	Separate	18–19/6.9–7.8	0.60	20	15.2 (1.5/2.7/11)	2500	1600
JD	A^2O-Phoredox	No	No	Separate	20–24/6.0–9.1	0.80	6	10.5 (2.5/2.0/6.0)	2300	800

A^2O refers to the Anaerobic–Anoxic–Oxic process and OD refers to the oxidation ditch process.