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Population dynamics of "*Candidatus* Accumulibacter phosphatis" under the modes of complete nitrification and partial nitrification (nitritation) in domestic wastewater treatment system

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ABSTRACT

In order to understand the enhanced biological phosphorus removal in a continuous-flow domestic wastewater treatment system, population dynamics of "*Candidatus* Accumulibacter phosphatis" using polyphosphate kinase (*ppk*1) gene as genetic marker was investigated under the modes of complete nitrification and partial nitrification (nitritation). Nitritation was achieved at hydraulic retention time (HRT) of 6.49 h and dissolved oxygen (DO) of 0.5 mg O_2/L. Denitrifying phosphate removal via nitrite pathway caused low PHAs consumption. Regardless of change of nitrifying modes, phosphorus removal always maintained at a high level of about 100%. Abundance of total Accumulibacter increased along with rising of carbon to nitrogen ratios (C/N). Under good performance of complete nitrification and nitritation, IIC and IID clade affiliated with type II (including clades II A-I) of Accumulibacter lineage was dominant (70%–80%), respectively. Clade IID exhibited high tolerance to starving, oxygen-limiting and high nitrite accumulation, and thus became dominant clade under insufficient carbon sources, low DO and nitritation mode. Clade IIC had a positive correlation with C/N ratios, and IIF had a positive correlation with temperature ($p < 0.05$). Shannon index under nitrification and nitritation mode was 3.54 and 2.38, respectively. That indicated the change of Accumulibacter diversities under different operational conditions.

1. Introduction

Excessive nitrogen and phosphorus through drainage into surface waters possibly causes eutrophication. Biological nitrogen removal (BNR) has been widely applied in wastewater treatment over the past 3 decades, since it is environmentally friendly and economically effective. The BNR includes two steps, i.e. nitrification and denitrification. In nitrification, ammonia is oxidized to nitrite by ammonia oxidizing bacteria (AOB), and then nitrite is further oxidized to nitrate by nitrite oxidizing bacteria (NOB). In the subsequent denitrification, nitrate is reduced to nitrite, and then to nitrogen gas by denitrifying bacteria [1]. While BNR system cannot remove phosphate from wastewater, enhanced biological phosphate removal (EBPR) processes have been confirmed to be economic and effective in wastewater treatment. The EBPR systems are functioned by polyphosphate accumulating organisms (PAOs). Under anaerobic condition, the PAOs discompose intracellular polyphosphate to orthophosphate, and release the orthophosphate into wastewater. Meanwhile they absorb volatile fatty acids (VFAs) from wastewater to synthesis poly-hydroxyalkanoates (PHAs) pools. Under aerobic condition, the PAOs degrade PHAs to generate reducing power and excessively absorb orthophosphate to form intracellular polyphosphate pools. Phosphate could be removed by discharging waste sludge [2]. Incorporating both nitrogen and phosphorus (P) removal is most commonly required in wastewater treatment plants (WWTPs), which is more complicated in microbial communities and operations than a sole EBPR or BNR system.

Recently, many studies focus on nitritation and denitritation, i.e. ammonia is oxidized to nitrite (nitritation, also named partial nitrification), and then directly reduced to nitrogen gas (denitritation) [1,3]. Compared with complete nitrification and denitrification, oxygen demand in nitritation and denitritation is reduced by 25%, and carbon source is decreased by 40% [4]. The factors favorable for nitritation, such as dissolved oxygen (DO), hydraulic retention time (HRT) and temperature had been investigated. Ruiz et al. reported

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that nitrite accumulation was observed when DO was lower than 1.1 mgO_2/L [5]. Zeng et al. found that a high ratio of internal cycle (nearly 540%) created a short aerobic HRT, leading to NOB washout due to the restriction of short HRT on metabolism and growth of NOB [6]. Tian et al. demonstrated the growth rate of AOB was higher than NOB when temperature was higher than 25 °C, and thus temperature above 25 °C was favorable for nitritation [7]. Another innovative process is anaerobic ammonium oxidation (anammox), which converts ammonium to nitrogen gas using nitrite as the terminal electron acceptor. Anammox is an anaerobic-autotrophic biochemical reaction, 60% less oxygen and no extra carbon source compared with the traditional nitrification/denitrification [7,8].

In a complex incorporating both nitrogen and P removal system, BNR and EBPR unavoidably interact, possibly causing the fluctuation of biological nutrients removal. Especially, along with nitritation-denitritation increasingly concerned and applied, the wastewater treatment system will undergo the change of nitrifying modes. Fluctuation of EBPR is possibly resulted from the change of nitrifying modes. The response of EBPR performance and PAOs population to the change of nitrifying modes is largely unclear, and thus we cannot gain a good insight into the operation of EBPR combined with nitriation.

"*Candidatus* Accumulibacter phosphatis" affiliated with *Rhodocyclus* was commonly found to be dominant PAOs in both wastewater treatment plants (WWTPs) and lab-scale reactors [9–11]. Based on polyphosphate kinase 1 (*ppk1*) gene from WWTPs, the Accumulibacter fell into two major types, i.e. Type I and Type II [12]. Previous researches reported Accumulibacter could be divided into fourteen different clades based on *ppk*1 gene, and demonstrated the variations of clade-level population dynamics of Accumulibacter under different environmental conditions [13,14]. The IIF clade could survive when temperature was higher than 30 °C, while other clades were washed out [15]. Flowers et al. reported IIA clade had a positive correlation with temperature; contrastively, IA clade had a negative correlation with ambient temperature [16]. But to our best knowledge, very few researches were carried out regarding population dynamics of Accumulibacter under nitrification and nitritation modes.

This study aims to (1) investigate the clade-level population dynamics of Accumulibater in a continuous-flow reactor under complete nitrification and nitritation modes, and (2) gain a good insight into the response of Accumulibacter clades to different operational conditions.

2. Materials and methods

2.1. Experimental set-up and operation

A Johannesburg (JHB) reactor with a working volume of 70 L and a secondary settler of 24 L was used in this study. The configuration of JHB reactor was previously described [17]. The flow rates of the feedings, nitrite/nitrate recirculation and returned sludge were controlled by peristaltic pumps. Aerobic zone were equipped with DO and pH probes (Fig. 1).

2.2. Seed sludge and wastewater

Seed sludge was obtained from WWTP with anoxic-aerobic (A/O) process in Beijing. Raw wastewater was taken from campus sewer line and pumped into a storing tank to sediment. The characteristics of raw wastewater were shown in Table 1. The average ratio of influent COD to nitrogen (C/N) was 1.69, indicating the carbon resources in the raw wastewater were extremely limiting.

Table 1
Characteristics of raw wastewater.

Contents	Range	Average
NH_4^+-N (mg/L)	34.4–93.5	61.2
NO_2^--N (mg/L)	0–0.91	0.15
NO_3^--N (mg/L)	0.36–2.64	0.98
SOP (mg/L)	2.11–9.38	5.85
COD (mg/L)	83.6–159	111.8
C/N	0.98–2.35	1.69

2.3. Operational procedures

Operational data of 354 days from JHB reactor were included in this study. Table 2 shows the operational conditions over experimental period. From phase IV, external carbon resources were added to increase the ratio of carbon to nitrogen (C/N), and to further improve nitrogen and phosphate removal efficiencies.

2.4. Analytical methods

Chemical oxygen demand (COD), ammonia (NH_4^+-N), nitrite (NO_2^--N), nitrate (NO_3^--N), soluble orthophosphate (SOP), mixed liquor suspended (MLSS) were measured according to APHA Standard methods [18]. Volatile fatty acids (VFAs) were measured using Agilent 6890 N gas chromatography (GC) with an Agilent DB-WAXETR column (30 m × 1.0 μm × 0.53 mm) equipped. PHAs including poly-β-hydroxybutyrate (PHB), poly-β-ydroxyvalerate (PHV) and poly-β-hydroxy-2-methylvalerate (PH2MV) were performed using Agilent 6890N GC with an Agilent DB-1 column (30 m × 0.32 μm × 0.25 mm), and each sample was in triplicates [19]. DO and pH were measured via DO/pH meters (WTW Multi 3420, Germany). Total nitrogen (TN) was measured with a TN analyzer (Jena Multi N/C3000, Germany). Nitrite accumulating rate (NAR) was calculated as follows:

$$\text{nitrite accumulation rate(NAR)} = \frac{c(NO_2^-\text{-N})}{c(NO_2^-\text{-N}) + c(NO_3^-\text{-N})} \quad (1)$$

where $c(NO_2^-\text{-N})$ and $c(NO_3^-\text{-N})$ is the concentration of NO_2^--N and NO_3^--N in effluent of the last aerobic zone, respectively.

P-value represents the correlation of several parameters, and is calculated using IBM SPSS Statistics v20.

2.5. Quantitative polymerase chain reaction (QPCR)

Operational data of 354 days from JHB reactor were divided into 12 phases based on reactor performance and operational parameters (Table 2). Due to population dynamics and stable performance needing long enough running period, sludge samples were taken in the later period of each phase to represent microorganisms composition under a certain condition, i.e. on day 34 of phase II, day 53 of phase III, day 80 of phase III, day 108 of phase IV, day 126 of phase V, day 160 of phase VI, day 22 of phase VII, day 60 of phase VIII, day 81 of phase IX, day 106 of phase X, day 145 of phase XI and day 170 of phase XII. Genomic DNA of sludge sample was extracted using Fast DNA SPIN kits for soil (Bio 101, Vistsa, CA, USA). The oligonucleotide primers, QPCR mixture and procedures were previously described [20].

2.6. Construction of ppk1 gene clone library and phylogenetic analysis

Genomic DNA was extracted on day 160 of phase VI and day 176 of phase XII, representing nitrification and nitritation phase, respectively. Clone library, operational taxonomic units (OTUs) and neighbor-joining (NJ) phylogenetic tree were carried out as described in the literatures [20–22].