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Sludge bulking impact on relevant bacterial populations in a full-scale municipal wastewater treatment plant

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ABSTRACT

We have investigated the changes of microbial community structures and the concomitant performance in two biological wastewater treatment systems (conventional and inverted A^2/O processes) over a whole cycle of sludge bulking. A low level of filament abundance was observed during non-bulking period, with types 0092 and 0041 as the dominant filamentous bacteria. With the increase of the sludge volume index values from 76 (73) to 275 (300) mg/L, the filament abundance estimated by microscopic examination increased from 1 (few) to 5 (abundant), with *Microthrix parvicella* becoming the dominant filament bacteria. Sludge bulking resulted in a significant shift in bacterial compositions from *Proteobacteria* to *Actinobacteria* dominance, characterized by the significant presence of filamentous *M. parvicella* (from not detected to higher than 60% of clones) and decrease of the dominant Betaproteobacterial population (from higher than 40% to less than 1%). Important relevant bacterial populations including polyphosphate-accumulating organism (PAO, *Candidatus* Accumulibacter phosphatis), ammonia-oxidizing bacteria (AOB, *Nitrosomonas*), nitrite-oxidizing bacteria (NOB, *Nitrospira*) and denitrifying bacteria (*Thauera*) were absent under the serious bulking condition. Accumulation of nitrite and ammonia was observed during serious bulking, while the phosphorus removal performance was not decreased because *M. parvicella* could behave as a PAO.

1. Introduction

The phylum *Proteobacteria* has been found to be the dominant bacterial group (35–65%) in conventional municipal wastewater treatment plants (WWTPs) [1], suggesting the importance of this group for pollutant removal. Among the *Proteobacteria*, *Betaproteobacteria* is the most abundant class, playing a key role in nitrification, phosphorus removal and the removal of many organic pollutants [2]. The betaproteobacterial *Candidatus* Accumulibacter phosphatis, the representative polyphosphate-accumulating organism (PAO) in full-scale systems, was found to be present in a range from 4 to 22% [3]. The genus *Nitrosomonas* in *Betaproteobacteria* has been found to be mainly responsible for nitrification in municipal WWTPs [4]. Denitrifying bacteria like *Thauera* spp. were normally detected in WWTPs [5]. In a word, many *Betaproteobacteria* members play a key role in the functioning of municipal WWTPs while few filamentous bacteria, such as *Sphaerotilus natans* and Type 1701, belong to this class.

Sludge bulking, with the overgrowth of filamentous bacteria as the main reason, is a frequently encountered problem in WWTPs, particularly for those with the function of enhanced nutrient removal [6]. Although the distribution of filamentous microorganisms varies in different geographical areas, *Microthrix parvicella* and Types 0092 and 0041/0675 have been considered as the major morphotypes responsible for the bulking events observed in municipal WWTPs [7]. The excessive growth of filamentous bacteria could result in the deflocculation of activated sludge and the decrease of non-filamentous bacteria, making these non-filamentous bacteria susceptible to being washed out from the WWTPs. A previous investigation on dynamics of bacteria communities of a sewage treatment plant using PCR-DGGE showed that *Betaproteobacteria* could not be detected when serious sludge bulking occurred [8]. Our previous study has found that *M. parvicella* possibly played a role in phosphorus removal when *Candidatus* Accumulibacter phosphatis was washed out due to sludge bulking [9]. However, knowledge of

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the sludge bulking impact on the main functional bacteria has been very limited. Since sludge bulking can normally continue for several months, deterioration of the performance of WWTPs due to the washout of the non-filamentous relevant bacteria could become of great concern. Our preceding study [9] has revealed that the phosphorus removal performance would not be adversely affected by sludge bulking because *M. parvicella* could act as a PAO when *Candidatus* Accumulibacter phosphatis is not available. However, the nitrogen removal performance may be affected because the roles of AOB and NOB could not be easily replaced by the filamentous bacteria. In this study, we tracked the filament levels as well as the performance of two parallel full-scale municipal wastewater treatment systems over a period covering a whole cycle of a sludge bulking event. In order to reveal the potential impacts of sludge bulking on microbial community structures, four biomass samples were taken from each system to construct four clone libraries representing bacterial compositions at the non-bulking, initial bulking, serious bulking and recovery phases, respectively. The results of this study provide valuable information for better management of WWTPs.

2. Materials and methods

2.1. Description of the investigated WWTP

The investigated municipal wastewater treatment plant in northern China consists of a conventional A^2/O system (anaerobic/anoxic/aerobic) and an inverted A^2/O system (anoxic/anaerobic/aerobic) (Fig. 1), each of which has a treatment design capacity of 200,000 m^3/day. Since the two systems were fed with the same sewage, one system could be used as the reference for another system. The sludge recycling ratio was approximately 100% for both the systems, while the mixed liquor recirculation ratio was 250% for the conventional system and none for the inverted one. In the inverted system, the influent was distributed into the anoxic and anaerobic tanks, respectively, at a ratio of 7:3. In the past 4 years, each system had received mean wastewater flows of 225,000 m^3/day and had exhibited sludge bulking from late December to early May, with the inverted A^2/O system exhibiting more serious bulking [8]. The operating conditions of the two systems, including sludge retention times (SRT, 12.7 ± 6.6 and 10.9 ± 5.8 days for the conventional and inverted A^2/O systems, respectively), mixed liquor suspended solids (MLSS, 3009–5021 and 3010–4810 mg/L, respectively) and food/microorganism (F/M, 0.09 ± 0.03 and 0.11 ± 0.04 kg BOD/kg MLSS/day, respectively) were kindly provided by the plant operators. The determination of biochemical oxygen demand (BOD), chemical oxygen demand (COD), total phosphorus and soluble orthophosphate concentrations was done according to the Standard Methods of Water and Wastewater Monitoring [10]. Two week average values for each sampling time were used for evaluating the performance of the two systems under different sludge conditions, and are shown in Table 1.

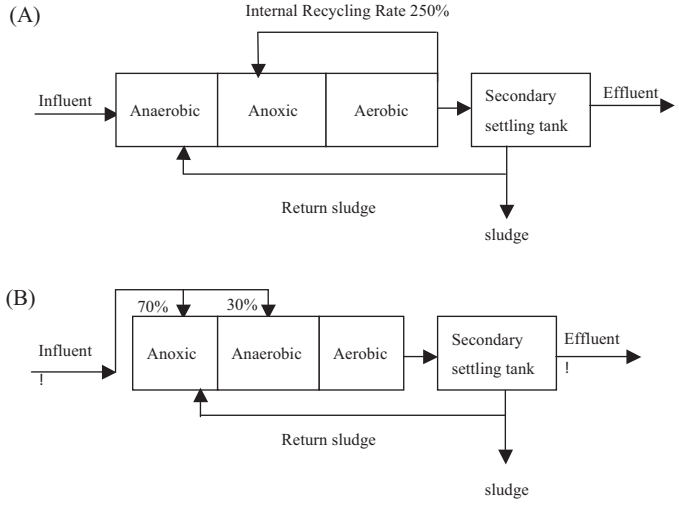

Fig. 1. Flow chart of the anaerobic/anoxic/aerobic process (conventional A^2/O system) and aerobic/anaerobic/aerobic process (inverted A^2/O system).

2.2. Activated sludge samples and DNA extraction

In total, four activated sludge samples were taken from the end of each system's aerobic unit: one in September when the systems were in normal state (SVI = 76 and 73 mL/g for the conventional and inverted A^2/O systems, respectively); one in January when significant sludge bulking occurred (SVI = 231 and 246 mL/g, respectively); one in March when serious sludge bulking occurred (SVI = 275 and 300 mL/g, respectively); one in July when sludge bulking began to disappear (SVI = 150 and 131 mL/g, respectively). The samples were centrifuged at 10,000 rpm for 10 min at 4 °C, and 0.25 g (wet weight) of activated sludge was used for DNA extraction using a FastDNA SPIN kit for soil (MP Biomedicals, USA) in accordance with the instructions provided by the manufacturer. The final volumes of the DNA solutions were 50 μL, which were then quantified with a Nanodrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE). Triplet genomic DNA was extracted from these samples and then pooled.

2.3. Cloning and sequencing of 16S rRNA gene

The 16S rRNA genes were amplified using bacterial universal primer 27f (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492r (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) [11] for bacteria. The conditions for PCR amplification were as follows: initial 95 °C for 10 min, followed by 35 cycles of 95 °C for 60 s, 55 °C for 60 s, and 72 °C for 90 s, with a final extension step of 10 min at 72 °C. The PCR products were confirmed by electrophoresis in 1.0% (w/v) agarose gel. Three separate reactions were conducted for each sample to minimize PCR bias in subsequent cloning steps, and all PCR products were purified using TIANpure Mini Plasmid kit (Tiangen, China). The 16S rRNA amplicons were cloned into TOPO TA cloning vector pCR2.1, with TOP10 *Escherichia coli* transformants further selected according to the manufacturer's instruction (Invitrogen, China). The transformants were selected by blue-white selection on Luria-Bertani (LB) agar plates containing ampicillin (100 μg/mL). The cloned inserts were amplified from lysed colonies by PCR with plasmid-vector-specific primers M13F (5′-GTA AAA CGA CGG CCA G-3′) and M13R (5′-CAG GAA ACA GCT ATG AC-3′). Positive clones were sequenced with an ABI 3730 automated sequencer (Invitrogen, Shanghai, China).

2.4. Phylogenetic analysis

After editing and trimming manually using BioEdit, the bacterial 16S rRNA gene sequences were searched against the GenBank database using BLASTN [12]. The most similar reference sequences were downloaded and aligned with the sequences of the present study using CLUSTALX [13]. Four 16S rRNA gene libraries were constructed for each system: AN, AB, ASB and AR for A^2/O samples taken in non-bulking, significant bulking, serious bulking and recovery states, respectively, and IN, IB, ISB and IR for corresponding inverted A^2/O ones. The operational taxonomic unit number was determined using the software MOTHUR by defining the sequences sharing 97% or greater similarity as one OTU [14]. The sequences obtained in the present study were deposited in the NCBI GenBank under accession numbers KJ807835–KJ808573. OTU