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Stable limited filamentous bulking through keeping the competition between floc-formers and filaments in balance

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ABSTRACT

Limited filamentous bulking (LFB) was proposed to save aeration energy consumption and enhance the capacity of filaments to degrade substrates with low concentrations in activated sludge systems. Operational parameters favorable for maintaining the LFB state were investigated in an anoxic–oxic reactor treating domestic wastewater. The experiments showed that the LFB state would deteriorate with sharply decreasing temperature, reducing substrate gradients or removing anoxic zones. The balance between filaments and floc-formers could be achieved by controlling dissolved oxygen and sludge loading rates to be in optimal ranges. Eikelboom Type 0041 and *Candidatus Microthrix parvicella* were the filamentous bacteria responsible for the LFB state. However, the excess growth of Eikelboom Type 021N and *Sphaerotilus natans* were observed when serious bulking occurred under low substrate gradients. It was demonstrated that stable maintenance of LFB for energy saving was feasible by process control and optimization.

1. Introduction

Although the activated sludge process has been the most commonly used technology for treating both domestic and industrial wastewaters, its stable operation is still plagued by sludge bulking or foaming (Martins et al., 2011; Petrovski et al., 2011). Filamentous bulking sludge, a term used to describe excessive proliferation of filaments, can lead to the deterioration of effluent quality and sludge washout with the final treated effluent, even the collapse of the overall system (Martins et al., 2004; Lou and de los Reyes, 2008). Substantial studies have attempted to develop suitable methods for preventing or controlling filamentous bulking both from engineering and microbial views (Eikelboom, 2000; Jenkins et al., 2004; Martins et al., 2004). Non-specific methods, such as chlorination (Caravelli et al., 2004), ozonation (Caravelli et al., 2006) or adding weighting and flocculating agents (Juang, 2005; Agridiotis et al., 2007) are commonly applied when the factors leading to sludge bulking are unidentified. The use of polyallumi-num salts has recently been proposed to control the growth of the filament *Microthrix parvicella* by causing either a flocculating or specific toxic effect (Nielsen et al., 2005). However, non-specific methods might result in some negative effects on operational performance of wastewater treatment plants (WWTPs). For example, the addition of chlorine to activated sludge would decrease the activity of nitrification bacteria or lead to the deterioration of effluent quality due to the formation of halogenated organic compounds (Wimmer and Love, 2004). On the other hand, specific methods are often effective to control the proliferation of filamentous bacteria after finding relationships between the dominant filaments and the operational conditions (Martins et al., 2004). Although specific methods are preventive methods, the challenge is to find the appropriate operational conditions that would consistently favor the growth of floc-formers but selectively suppress the growth of filaments (Martins et al., 2004; Al-Mutairi, 2009).

The limitation or deficiency of dissolved oxygen (DO) is often responsible for the proliferation of filamentous bacteria in activated sludge processes (Martins et al., 2003; Huang and Ju, 2007). It is an efficient method to control sludge bulking due to low DO by increasing aeration intensities, but which result to waste aeration energy consumption. On the other hand, if solids-liquid separation and effluent quality in clarifiers are not deteriorated by modestly decreasing DO, aeration energy consumption would be saved in the wastewater treatment systems by using of filamentous bulking due to low DO. A method of energy saving achieved by limited filamentous bulking (LFB) had been proposed in the previous study (Guo et al., 2010). This method provided an alternative pathway for reducing aeration energy consumption without decreasing the effluent quality. Field observations indicated the LFB state was resulted from a decline in DO concentration (Guo et al., 2010). This decline did encourage filamentous

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bacteria growth, but did not lead to severe bulking. Compared to floc-formers, filaments with their higher surface-to-volume (A/V) would have enhanced ability to degrade lower residual substrate concentrations. Suspended solids (SS) removal efficiency would also be enhanced through the enmeshment mechanism associated with the filaments, which resulted in a treated effluent with better clarity. The previous verification in a lab-scale anoxic–oxic (A/O) reactor also clearly showed that the blanket problem was not encountered in secondary clarifiers, while chemical oxygen demand (COD) and total nitrogen (TN) removal efficiencies were improved under the LFB state. The effluent SS and turbidity were distinctly lower than those under no bulking. It was also demonstrated that the LFB state caused by low DO could be used to reduce aeration energy consumption (Guo et al., 2010). However, some significant bottlenecks are still needed to be solved or prevented. In particular, long-term stable maintenance of the LFB state is crucial and should be primarily solved before applying this technology for wastewater treatment. In addition, it is still unknown if some operational factors would cause the deterioration from the LFB state into serious filamentous bulking.

The objectives of this study were: (i) to find optimal DO concentrations and sludge loading rates (Ns) for keeping the LFB state; (ii) to investigate the effects of operational factors (such as temperature, substrate gradient and anoxic zone) on the LFB state, and (iii) to reveal the response of LFB under various disturbances. Moreover, the dominant filamentous bacteria were identified by microscopic observation, staining reactions and fluorescence *in situ* hybridization (FISH). It is expected to find favorable operational parameters and to establish control strategies for maintaining the stable LFB state.

2. Methods

2.1. Experimental setup

Due to the wide application of A/O process (i.e. pre-denitrification) in China, a lab-scale A/O reactor made of plexiglass with a working volume of 66 L and an upright clarifier with a working volume of 20.5 L were used (Fig. S1, Supporting Information). To attain plug-flow effect, the A/O reactor was separated into two corridors and multiple compartments. Different substrate gradients could be obtained by changing the number of compartments, which was adjusted by a number of removable plastic flashboards. Two mechanical mixers were installed in the anoxic zone. Compressed air was provided to each aerobic zone through an air diffuser stone. Five air flow meters were used for adjusting the airflow rate in each aerobic zone. Two DO probes (WTW oxi330, Germany) were used, placed in the first and the last aerobic zones, respectively. At each stage, the DO levels in the first and last aerobic zones were controlled at the pre-selected set-point by adjusting the air-flow rate. The flow rates of influent, internal cycle and return sludge were controlled by three peristaltic pumps. The internal and external recycle ratios were set at 1.5 and 1.0, respectively. The seeding sludge was obtained from a secondary clarifier of Jiuxianqiao WWTP (Beijing, China). The sludge retention time (SRT) changed with sludge loading rates Ns, varying between 15 and 30 days. Mixed liquor suspended solids (MLSS) concentrations were in the range of 2500–3500 mg/L. The detailed experimental conditions of the A/O are described in Table 1. The reactor was operated to find the relationship between DO and sludge settleability under different Ns during Phase II. Factors (temperature, substrate concentration gradient and the presence of anoxic zone) influencing the LFB state were investigated during Phases III, IV and VI. The robustness and response of LFB under different disturbances were investigated in Phase V. Three different conditions were simulated in Phase VI to further investigate the effects of substrate concentration gradient and the presence of anoxic zone. In order to rapid recover sludge settleability, the reactor was switched into batch operation when changing from Phase II into III (from day 202–210), as well as from Phase III into IV (from day 264–292). During batch operation, the clarifier was idle and the pumps for returning sludge and recycling nitrifying liquor were switch off. The reactor was operated as a sequencing batch reactor. Each cycle consisted of 3 min feeding, 240 min aerobic reaction, 50 min settling, 30 min decanting, and idling. DO concentration was kept above 2.0 mg/L in aerobic reaction phase. Under these conditions, sludge settleability could be recovered in short time.

2.2. Wastewater characteristics

The influent was collected once per day from an on-campus sewer line. It was firstly pumped into an intermediate storage tank (0.75 m^3) before being pumped into the A/O reactor. The wastewater characteristics are described as follows. COD: 160–320 mg/L; NH_4^+-N: 40–80 mg/L; alkalinity: 280–400 mg/L; NO_2^-—N: 0.04–0.26 mg/L; NO_3^-—N: 0.12–1.08 mg/L; pH: 7.0–7.8. The detailed information of wastewater characteristics can be found in the previous study (Guo et al., 2010).

2.3. Extracellular polymer substances (EPS) extraction

The EPS were extracted from the sludge sample (collected from the end of aeration zone) by mixing with a cation exchange resin (Dower 50∗8, 20–50 mesh in the sodium form). The suspension was stirred at high shear force (2000 rpm) for 4 h at 4 °C. The extracted EPS was harvested by centrifugation at 6000 rpm for 15 min and filtration through a 0.45 μm membrane. The extracted EPS was stored at −20 °C. The EPS was quantified in terms of protein, DNA and carbohydrate. Total extractable EPS was defined as the sum of protein, carbohydrate and DNA.

2.4. Analysis methods

Biochemical oxygen demand after 5 days (BOD_5), COD, MLSS, mixed liquor volatile suspended solids (MLVSS), SS and sludge volume index (SVI) were measured according to Standard Methods (APHA, 1998). NH_4^+-N concentration was determined by Metrohm 761 Ion Chromatogram (IC, Switzerland) with a Metrostep C2 150 cation column. NO_2^-—N, NO_3^-—N and PO_4^{3-}—P concentrations were analyzed by Metrohm 861 IC with a Metrostep A supp 4 anion column (Switzerland). Turbidity was detected by WTW Turb 555 (Germany). Sludge zone height ratio was recorded by the division of sludge zone height to the total height of the secondary clarifier.

The extracted EPS were analyzed for protein, carbohydrate and DNA, as described in Frolund et al. (1996). Carbohydrate was determined using the anthrone method with a glucose standard (Aldrich). Protein was measured with the Lowry procedure using BSA (bovine serum albumin) as standard. DNA was measured in the extracted EPS samples according to the method described in Frolund et al. (1996).

2.5. Microscopic examination and filamentous bacteria identification

Microscopic examination of the mixed liquors sampled from the outlet of the reactor was extensively performed (2–3 times per week) using an OLYMPUS-BX52 (Japan), according to the manual given by Eikelboom (2000). Filamentous index (FI), a method of subjective scoring of filamentous bacteria abundance suggested by Eikelboom (2000), was used to evaluate the abundance of filamentous bacteria present in the samples. The dominant