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Effects of feeding pattern and dissolved oxygen concentration on microbial morphology and community structure: The competition between floc-forming bacteria and filamentous bacteria

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ABSTRACT

Understanding of the competition between floc-formers and filaments is critical to prevent filamentous bulking in practice. This study aimed to investigate the effects of feeding pattern and dissolved oxygen (DO) concentration on their competition in four sequencing batch reactors (SBRs). Short feeding under anoxic condition (fill time < 10 min) resulted in a well-settling sludge (sludge volume index (SVI) < 100 mL/g), in spite of DO concentrations. Sludge settleability deteriorated (SVI > 200 mL/g) and filamentous bulking was observed when the substrate was added in a limiting rate by prolonging the anoxic fill time up to 90 min. In contrast, sludge settleability in fully aerobic systems was quite poor (SVI > 500 mL/g) in spite of the feeding length. Compared to the systems with an anoxic fill phase, more types and abundant filamentous bacteria were identified in fully aerobic systems. Microscopic observation, staining reactions and fluorescence *in situ* hybridization analysis indicated that the extensive filaments, including *Thiothrix nivea*, Type 021N, Type 1851 and *Microthrix parvicella*, proliferated in fully aerobic systems. The results of this study indicated that substrate gradients played an important role on the competition between filaments and floc-formers. It is recommend the adoption of plug-flow selector configurations, with anoxic conditions in order to maintain good and robust sludge settleability.

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1. Introduction

Although the activated sludge process is widely used biological wastewater treatment process, its stable operation is still plagued by poor sludge separation problems, such as sludge bulking, foaming, pin-point sludge and poor macrostructure [1]. Filamentous bulking sludge due to excessive growth 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 [2,3]. Substantial studies have attempted to identify the ecophysiology of filamentous bacteria [4] and further develop control filamentous bulking methods [5–7]. However, it is difficult to develop specific control strategies for efficient solving filamentous bulking in practice, since relationships between the most predominant filaments and their physiology and the operational conditions are not completely established [8]. Therefore, it is significant to investigate some critical operating conditions on the competition between filaments and floc-formers in order to develop specific control methods and clarify the mechanism of sludge bulking from both engineering and microbial views.

Until now, many operational factors influencing the competition between filaments and floc-formers have been investigated, including F/M ratio [9,10], substrate concentration gradient [8], dissolved oxygen (DO) concentration [11,12], temperature [13,14], and nutrient deficiency [15]. Although the occurrence filamentous bulking due to low DO or low F/M was commonly encountered in lab-scale reactors and full-scale wastewater treatment plants (WWTPs) [5], relatively little is known regard as which filamentous bacteria would dominant under these unbeneficial conditions for sludge settleability. In particular, DO deficiency is one of the most typical factors responsible for filamentous bulking in activated sludge process [12,14,16]. The majority of filamentous bacteria under low DO are often identified by traditional microscopic observations according to the manuals [5,6]. Compared to traditional identifying methods, molecular biological methods exhibit the advantage of accuracy to identify and monitor

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filamentous bacteria [4]. Especially, fluorescence *in situ* hybridization (FISH) is an efficient technique for directly identifying the dominant filamentous bacteria in sludge bulking systems [17]. According to microscopic observation in the previous studies, *Sphaerotilus natans* were widely identified as the dominant filamentous bacteria under low DO [5,6]. However, according to the FISH experiments, it was found that the proliferation of *Thiothrix* spp., Type 021N and Type 1851 were usually caused under low DO (<1.1 mg/L) condition [12]. In addition, it is seldom documented that the combined effects of low DO and feeding patterns on the proliferation of filamentous bacteria.

The objective of this current study was to investigate the competition between floc-forming bacteria and filamentous bacteria under different DO concentrations and feeding patterns. Since the proliferation of filamentous bacteria seems to be connected to the feeding pattern of the system, the first approach was to simulate the plug flow reactor (PFR) and completely mixed flow reactor (CMFR) behaviour in a lab-scale system. In addition, low (0.5 mg/L) and normal (2.0 mg/L) DO levels were controlled to investigate the effect of DO on the competition between floc-formers and filaments. Multiple assessments, including sludge volume index (SVI), microscopic observations, Gram and Neisser staining, and FISH were used to monitor sludge properties and to track the changes of microbial morphology and community structure. It is expected to provide information for understanding the behavior of filaments and floc-formers, further reveal the mechanisms of their competition and, consequently, the correct design of biological nutrient removal process.

2. Materials and methods

2.1. Experimental set-up and operation phases

Depending on the mode of operation, a SBR system can be compared with a PFR or a CMFR [8]. In this study, four lab-scale SBR systems with different feeding patterns stimulate the substrate gradients bacteria experience in full-scale WWTPs containing selector [8]. Using different feeding time allowed us to simulate a variable relative size of selector and different bulk liquid substrate concentrations [8]. The lab-scale reactors were equipped with a monitoring and control system, as schematically shown in Fig. 1. On-line data provided by pH, DO and temperature probes (WTW, Germany) were acquired processed, and stored by every 1 min by means of a programmable logic controller (PLC) (Siemens, SIMATIC S7-200) connected to an IBM-compatible PC. Program commands were transmitted to SBRs through the control system, which controlled the switch on/off of all electrical devices.

The working volume of SBR1 and SBR2 was 5 L, while SBR3 and SBR4 with 14 L of working volume, respectively. SBR1 and SBR2 were operated as anoxic–aerobic process. The 6 h cycle consisted of a 2 h anoxic period, a 3 h aerobic period and a 1 h settle/decant period. Synthetic wastewater (composition detailed below) was fed to each SBR during the beginning of the anoxic period. The synthetic wastewater of 2.5 L (SBRs 1 and 2) or 7 L (SBRs 3 and 4) was fed each cycle, which results in a volume exchange ratio (VER) of 0.5. After the settling period, 2.5 L or 7 L of supernatant was removed, resulting in a hydraulic retention time (HRT) of 12 h, while the sludge retention time (SRT) was maintained at approximately 10–15 days by wasting mixed liquor at the end of aerobic phase. The pH was recorded but not controlled, and fluctuated between 7.0 and 7.5. The reactors were controlled at 25 °C using aquarium heaters. In the aerobic period, DO concentrations in SBR1 and SBR2 were controlled at 0.5 mg/L and 2.0 mg/L, respectively. SBR3 and SBR4 was operated as fully aerobic process, with 6 h cycles only consisting of a 5 h aerobic period and a 1 h settle/decant period. Other operating conditions including HRT, SRT and VER were identical with SBR1 and SBR2. The respective differences in the experimental conditions for the SBRs are highlighted in Table 1.

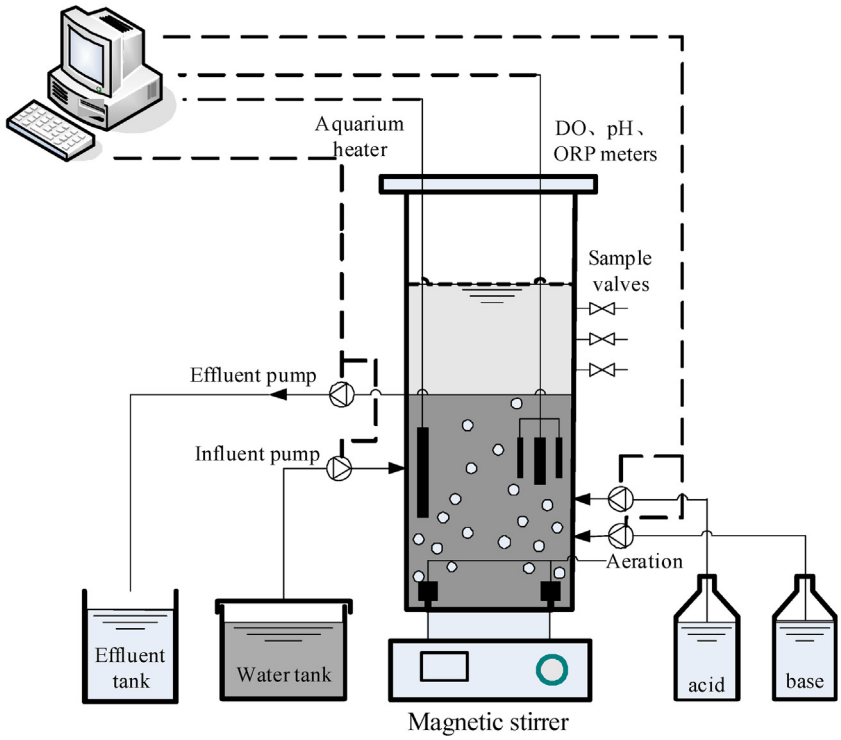

Fig. 1. Schematic diagram of the SBR used in experiments.

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