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Evaluations of biofilm thickness and dissolved oxygen on single stage anammox process in an up-flow biological aerated filter

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ABSTRACT

The overall influence of dissolved oxygen (DO) concentrations and biofilm thicknesses on single stage anammox process performance has been evaluated in this study. Results indicated the biofilm displayed a rapid initial increase and followed by a relatively slower formation rate during the operational period. The optimal DO concentration could be determined from a variety of biofilm thicknesses and as well the best biofilm thickness was required among different DO levels. In our lab-scale single stage anammox reactor with a constant hydraulic retention time of 1.0 h and influent ammonium of 400 mg L^{-1}, an optimal nitrogen removal capacity was acquired (TN removal loading of 2.18 kg-N m^{-3} d^{-1}) at the DO level of 0.6 mg L^{-1} and biofilm thickness of 700 μm. Species identification showed that *Nitrosomonas* related aerobic ammonium-oxidizing bacteria (AerAOB) and *Candidatus Brocadia fulgida*-like anaerobic ammonium-oxidizing bacteria (AnAOB) were the predominant functional bacteria mixed together with each other and exhibited no distinct niche. However, AerAOB exhibited higher biodiversity at the thinner biofilm while AnAOB showed a stable but lower biodiversity. Moreover, the population of AnAOB was smaller along with more scattered cells at the thinner biofilm while they trended to form specific irregular cauliflower-like zooglea as biofilm thickness increased.

1. Introduction

Biofilm wastewater treatment systems have been extensively used all over the world. As compared with activated sludge systems, they provide the basis for optimization of the volumetric conversion capacity, and assure long biomass residence time even at short hydraulic retention time (HRT) [1]. Consequently, they can offer favorable conditions for slow growing organisms such as nitrifying bacteria and anammox bacteria. As was well known, aerobic ammonium-oxidizing bacteria (AerAOB) and anaerobic ammonium-oxidizing bacteria (AnAOB) with long generation periods perform two sequential reactions simultaneously under oxygen-limited condition in single stage anammox process [2]. There is no doubt that biofilm-based processes are ideal candidates for AerAOB and AnAOB to co-exist in terms of aerobic region and anaerobic region along the biofilm depth.

Among the reported biofilm systems for single stage anammox process, up-flow biological aerated filter (UBAF) is one of the most common configurations since this type of reactor can transfer dissolved oxygen (DO) and substrate over the boundary layer into the biofilm in a more efficient way [3]. In UBAF-based single stage anammox process, oxygen partially penetrates the biofilm, leading to a generation of an oxygen concentration gradient to promote the balance between two types of functional bacteria. Despite the apparent advantages of UBAF, efficient nitrogen removal still demands rigorous microenvironment control because oxygen overloads and subsequent nitrite accumulations are harmful to AnAOB activity. As a result, more sophisticated monitoring requirements are urgently needed for single stage anammox process with respect to oxygen mass transfer efficiency and biofilm thickness [4]. Several researchers have also reported that DO and biofilm thickness are two key rates affecting the performance of the process [5–7]. Higher DO levels may contribute to a rapid buildup of nitrite oxidizing bacteria (NOB) to break the formed balance whereas too low DO concentration will limit ammonium oxidation by AerAOB. Consequently, a DO range of 0.2-1.0 mg·L^{-1} has been reported to be appropriate to achieve the optimal process performance [8,9]. Similarly, a very thin biofilm may not support the process because an anaerobic layer is hardly formed under aerobic conditions. Although a thicker biofilm usually correlates to the

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improvement of nitrogen removal efficiency, an optimal thickness of biofilm is always available under a given operation condition. An excessive thick biofilm seems to hardly promote process operation or even limit it for lack of substrates in the anaerobic layer [10].

Up to now, some research has been carried out to evaluate the effect of DO or biofilm thickness on single stage anammox process performance. Actually, an intimate correlation exists between the effects of biofilm thickness and DO on the process operation. That is to say, DO concentration governs the nitrogen conversion while biofilm thickness is a very important parameter determining the DO level at which the maximum nitrogen removal occurs. Lower DO level may be sufficient in a system with thin biofilm thickness while the relatively thick biofilm will restrict the diffusion of DO and further require higher DO levels [11]. Previous report demonstrated that different operation strategies on the process resulted in different optimum biofilm thickness [12]. Once the biofilm has reached the optimal thickness, nitrogen removal capacity stops improvement even the biofilm keeps developing. That is because the thick biofilm is not conducive to the formation of DO gradient, which will suppress the bioactivity of AerAOB and AnAOB. Unfortunately, previous work usually focused on the effect of either DO concentration or biofilm thickness on single stage anammox process, little literature addressed the overall influence caused by two factors. It is exciting that some efforts have been made to study the relationship among DO, biofilm and N removal capacity by building mathematical models [6,10–12], however, the relevant results were not further verified by laboratory experiments or full scale operations.

In this very study, the overall influence of DO concentration and biofilm thickness on single stage anammox process was evaluated based on a lab-scale UBAF-based reactor. During the biofilm development in the filter, five representative samples with the respective biofilm thickness of 50, 100, 300, 500 and 700 μm were selected and exposed to different DO levels to examine their nitrogen removal capacity. Moreover, in view of the microbial community structure, diversity and distribution will affect the ultimate performances of the process, the microbial features at different phases were also studied, which, were expected to reveal possible microbial mechanisms and further inspire us on the improvement or optimization of single stage anammox process implementation in the future.

2. Materials and methods

2.1. Experimental reactor and operational conditions

A lab-scale UBAF made of polymethyl methacrylate was used in this study. Volcanic rock (4–6 mm in diameter, 80% in porosity and 0.014-0.026 $m^2\,g^{-1}$ in surface area) was selected as carriers in this configuration since it showed distinct biomass retention capacity compared with other supporting materials such as sponge or modified polyethylene carriers [13].The inner diameter, total volume and working volume was 150 mm, 8.15 L and 1.80 L, respectively. Seeding sludge was obtained from another UBAF-based single stage anammox reactor that operated at high N concentration (400–480 mg L^{-1} of NH_4^+-N) and high temperature (30–35 °C). This reactor had been operated for ten months with TN removal efficiency and TN removal loading of 85% and 2.0 kg-N $m^{-3}\,d^{-1}$ respectively. The mixed liquid suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) of the seeding biomass was 1.25 and 1.13 g L^{-1} respectively, and the amount of sludge inoculated to the new reactor was 0.5 kg (wet weight). In this study, the reactor was fed with synthetic wastewater, containing $NaHCO_3$ (C source and buffer, approximate 1200 mg L^{-1} of inorganic C), $(NH_4)_2SO_4$ (N source, 400 mg L^{-1} of NH_4^+-N) and KH_2PO_4 (P source and buffer, 40 mg L^{-1} of PO_4^{3-}) together with a small amount of trace element solution. The liquid and air were pumped continuously from the bottom and output from the upper outlet with DO concentration and HRT of 0.5 mg L^{-1} and1.0 h respectively at ambient temperature (16–20 °C) throughout the whole operation.

To evaluate the nitrogen removal performance, concentrations of NH_4^+-N, NO_2^--N and NO_3^--N in influent and effluent were measured daily according to standard methods [14]. The pH value, temperature and DO were also detected by the online multi-function instruments (WTW noLabStirrOx).

2.2. Measurement of biofilm thickness

Biofilm thickness during the operation was measured using volume-area conversion method [15]. Considering constant water and air channel would form along the filter in UBAF-based reactor [16], it was necessary to rearrange the carriers at intervals to break the existing channels so as to ensure relatively uniform distribution of substrate and oxygen in the reactor. It meant the volcanic rocks at any height of the reactor were almost representative except those near the outlet that biomass lost due to hydraulic shearing or in the bottom that biomass might be effected by the supporting gravel. Therefore, the selection of volcanic rocks was done at the middle site of the filter every ten days to detect the total volume (V1). Afterwards, the carriers were submerged into 1% (w/V) NaOH solution under stirring for 30 min at 60 °C to detach the biofilm. Then the biofilm was further stripped from the rocks by ultrasonication for 20 min. The volume and mass of the rocks in the absence of biofilm were measured and recorded as V2 and M2 respectively. Surface area of volcanic rock ranged 0.014–0.026 m^2/g (averaged 0.020 m^2/g, S1). Biofilm thickness was calculated by the following equation:

$$\text{Biofilm thickness}\,(\mu\text{m}) = \left[(\text{V1}\,(\text{mL}) - \text{V2}\,(\text{mL}))\,/\,\left(\text{S1}\,\left(\text{m}^2/\text{g}\right)\right.\right.$$
$$\left.\left.\times\text{M2}\,(\text{g})\right)\right] \times 100$$

2.3. Effect of DO and biofilm thickness on N removal performance

Batch experiment was applied to evaluate the effect of DO and biofilm thickness on N removal performance: volcanic rocks with respective biofilm thickness of 50, 100, 300, 500 and 700 μm were selected from the reactor and then transferred to five beakers (the mass of each sample was around 20 g). To simulate the maximum conditions in the reactor, the influent liquid composition, HRT and temperature in the beakers were identical as in the original UBAF reactor except DO level. Various DO levels (0.2, 0.4, 0.6, 0.8 and 1.0 mg L^{-1}) were introduced in the five beakers by accurate control of aeration rate while HRT was controlled by two peristaltic pumps. Nitrogen removal capacity variation was investigated and the measurements were performed in triplicates.

2.4. Scanning electron microscope (SEM)

Surface morphology of the biofilm with different thicknesses was studied using an established SEM analysis. To be specific, some biofilm was cut off and fixed with 2.5% (V/V) glutaraldehyde for 1 h followed by dehydration in 50%, 70%, 90% and 95% (V/V) ethanol for 10 min per each step. Afterwards, the samples was steeped in hexamethyl disilazane (HMDS) twice for 10 min, air dried, and coated with gold. SEM images were then acquired on a SEM device (S-4300, Hitachi).

2.5. Fluorescence in situ hybridization (FISH)

The spatial distributions of AerAOB and AnAOB on biofilm with different thicknesses were examined by FISH. The exper-