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Physicochemical and microbial properties of settled and floating anammox granules in upflow reactor

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ABSTRACT

A lab-scale UASB reactor was used to assess the high-rate performance of anammox process after long-term operation by shortening HRT. The nitrogen removal rate (NRR) was developed to 15.51 kg N m^{-3} d^{-1} with a relative high nitrogen removal efficiency (NRE) of 90.5% under the short HRT of 1.0 h. However, continuous granules' flotation consequently initiated and was enhanced under the high nitrogen gas production condition, accompanying by the decrease in NRE to 69.8%. The physical, chemical and microbial characteristics of the settled and floated anammox granules were compared. Results indicated that the alternation of chemical properties (e.g. EPS over-secretion) of granules helped to prevent gas escaping out, thus decreasing sludge density. Therefore, granules' flotation becomes inevitable in high-rate anammox reactors. Microbial analysis showed that "*Brocadiacea*" dominated in settled and floated granules with abundance of 42% and 36%, respectively. Whereas, the abundance of "*Chloroflexi*" in floated sludge (20.69%) was much higher than that in settled sludge (12.66%).

1. Introduction

Anaerobic ammonium-oxidizing (anammox) reaction can directly convert NH_4^+ to N_2 gas using nitrite as the electron acceptor under anoxic conditions [1]. Since its discovery in mid-1990s, anammox has been regarded as a novel and promising biological process due to its higher nitrogen removal rate (NRR) (up to 77 kg N m^{-3} d^{-1} [2]), lower operational cost, less space requirement and reduction of greenhouse gas emissions, as compared with conventional nitrification-denitrification systems [3–7]. The anammox-based technologies have been successfully implemented for treatment of high-strength ammonium-rich wastewater during the past decades. Nowadays, more than 100 full-scale anammox plants have been installed worldwide. However, the widespread full-scale application of the anammox-based processes would involve some upcoming challenges. One of them is that the anammox bacteria, characterized by extremely slow growth rate and low cellular yield, have a high sensitivity to a variety of environmental factors, thus causing a difficulty in maintaining stability of anammox bioreactors [8,9]. So, it is important to enrich enough anammox bacteria in the reactor to acquire a stable reactor performance. Some operational strategies, e.g., gel immobilization and sludge granulation for rapid enrichment of anammox biomass have been developed accordingly [10–12], among which granulation emerges as a hot issue for anammox process.

Sludge granulation offers an effective strategy to improve performance of anammox reactors [2]. Granules possess compact density and better settling property, thus they can be retained efficiently in anammox reactors. Granules also offer a particle structure and a plenty of extracellular polymers (EPS) which would increase the resistance to adverse factors [13]. As reported, the metabolic selection pressure plays a vital role in the granulation of anaerobic biomass [14]. Such selection pressure like high loading rate with high shear stress (either gas-induced or liquid-induced) has been considered to be a crucial reason for granulation because of the stimulation of EPS secretion [2,15,16], which could accelerate sludge granulation [17,18].

Anammox is a gas-producing process that the anammox bacteria tend to locate in inner part of granules due to the strictly anaerobic condition. Thus, the nitrogen gas bubbles produced by anammox bacteria might not be squeezed out and then were trapped inside the granules [19]. Thus the density of granules

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would decrease and then sludge flotation and even washout might occur. Accordingly, reactor performance would eventually deteriorate during the continuous elevation of nitrogen loading rate (NLR). Moreover, the floated granules will assemble continuously in the settler, which would block up the effluent pipe, resulting in malfunction of reactor operation. Although, information about anammox granules' flotation has been reported in some references [19–21]. The detailed chemical and microbial characteristics of the granules before and after flotation are still limited. Furthermore, the description of granules flotation was not well-addressed from its initiation to the subsequent evolution in upflow reactors.

In this study, a granule-based UASB reactor was operated for a long term to achieve NLR higher than 15 kg N m^{-3} d^{-1} by either increasing substrate concentration or shortening HRT. The appearance and involvement of floated granules was carefully recorded and the correlation between nitrogen removal performance and flotation was established. Furthermore, the floated and settled granules were deliberately collected to determine the physical, chemical and microbial characteristics, in order to provide more detailed information about the possible mechanism of anammox granules' flotation. The obtained results would serve as a guidance for stable and high-rate operation of anammox reactors without sludge flotation.

2. Materials and methods

2.1. Anammox reactor

The experimental work was carried out in a plexiglas-made UASB reactor with an inner diameter of 50 mm. The working and total volume were 1.1 L and 1.8 L, respectively (Fig. 1). The temperature was controlled at 35 ± 1 °C by using a thermostatic water bath. The sludge retention time (SRT) of the reactor was maintained higher than 30 days in the present study since there was little sludge wasted from the reactors during the whole experimental period except for sampling.

2.2. Seed sludge

The reactor was inoculated with fresh anammox granular sludge obtained from a lab-scale anammox reactor operated over years. The anammox seed granules had a diameter range of 2–3 mm, with the specific anammox activity of 0.21 kg kg VSS^{-1} d^{-1}. The biomass inoculation volume was equal to the reactor working volume. The volatile suspended solids (VSS) of the sludge after inoculation was 21.4 g L^{-1}.

2.3. Synthetic wastewater

In this study, the anammox reactor was constantly fed with synthetic wastewater, which contained NaH_2PO_4 0.01 g L^{-1}, $CaCl_2{\cdot}2H_2O$ 0.0056 g L^{-1}, $MgSO_4{\cdot}7H_2O$ 0.3 g L^{-1}, $KHCO_3$ 1.25 g L^{-1} and 1 mL L^{-1} trace element solution I and II. Trace element solution I consisted of $FeSO_4$ 5 g L^{-1}, EDTA 5 g L^{-1}. Trace element solution II consisted of EDTA 15 g L^{-1}, $ZnSO_4{\cdot}7H_2O$ 0.430 g L^{-1}, $CuSO_4{\cdot}5H_2O$ 0.250 g L^{-1}, $NiCl_2{\cdot}6H_2O$ 0.190 g L^{-1}, $NaMoO_4{\cdot}2H_2O$ 0.22 g L^{-1}, and H_3BO_4 0.014 g L^{-1}. Ammonium and nitrite were supplemented to mineral medium as required in the form of $(NH_4)_2SO_4$ and $NaNO_2$. The pH of the influent was maintained at 6.8-7.2 by dosing hydrochloric acid solution throughout the whole operational period.

2.4. Experimental procedure

The UASB reactor was first operated for 224 days which can be divided into three phases. During Phase I (days 1–42), the reactor was initially inoculated with anammox sludge and then was started up by progressively increasing NLR via either increasing ammonium and nitrite concentration or shortening HRT. In Phase II (days 43–159), granules floatation initiated in the reactor and was further enhanced when the NLR of the reactor was increased to higher loading rates (up to 16.06 kg N m^{-3} d^{-1}). In Phase III (days 160–224), the rescuing measure of manual stirring with a stick was taken to overcome granules' floatation and then to recover the reactor performance. The influent substrate concentration was kept at an NO- 2-N/NH+ 4-N ratio of 1.1 during the whole study to protect the anammox sludge from nitrite inhibition [20].

The occurrence of flotation of anammox granules was recorded regarding the operation days and NLRs. The floated and settled granules were collected separately for subsequent detections of physical, chemical and microbial characteristics by using SEM, EDS, FTIR and high-throughput sequencing.

2.5. Analytical methods

The influent and effluent samples were collected on a daily basis and were analyzed immediately. The determination of ammonium, nitrite and nitrate concentrations, total suspended solids (TSS) and volatile suspended solids (VSS) concentrations were carried out according to the Standard Methods [21]. The pH of the wastewater samples was determined by the HANNA pH 211 acidometer with a selective electrode. Heme *c* content was determined according to Berry and Trumpower [22]. Digital macrophotography was performed by the Canon EOS 600D Digital Single Lens Reflex.

Extracellular polymeric substances (EPS) were extracted according to the method in Dai et al. [23]. Protein (PN) concentration was analyzed using a modified Lowry procedure [24], with bovine serum albumin as standard. Polysaccharide (PS) content was analyzed using phenol-sulfuric acid method, with glucose as standard [25].

The settling velocity and wet density of anammox granules was determined following the methods reported by Lu et al. [26]. The average diameters of the formed granules were estimated using the methodology proposed by Jeison and Chamy [27]. The Sludge volume index (SVI) assessment was carried out according to the procedure described by Chen et al. [19]. Specific anammox activity (SAA) assays were performed according to the methods described by Tang et al. [2]. Morphological characteristics of the biomass specimens were observed using SEM (JEOL, JSM-6360LV, Tokyo, Japan). The Energy dispersive spectrometry (EDS) analysis was carried out using SEM instrument equipped with a thermo EDS attachment. The organic functional groups of the anammox sludge were analyzed with an FTIR spectrometer (Nicolet iS10, Thermo Scientific, USA).

2.6. DNA extraction, PCR amplification and high-throughput sequencing

For the DNA extraction, sludge was collected from the reactor during stable operation (on day 180). After washed several times using phosphate buffer solution, the DNA was extracted from a 0.2 g sample of sludge with the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, Doraville, GA, USA), according to the instructions of the manufacturer. The quality of the DNA was assessed by 1% agarose gel electrophoresis, and the DNA concentrations were measured with a NanoDrop Spectrophotometer ND-1000 (Thermo Fisher Scientific, USA).

To determine the diversity and composition of the bacterial communities in the sludge sample, we used the protocol described in Caporaso et al. [28]. PCR amplifications were conducted in with the 515f/806r primer set that amplifies the V4 region of the 16S rDNA gene (average length of 254 bp). The PCR condition was as