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Achieving high performance completely autotrophic nitrogen removal in a continuous granular sludge reactor

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ABSTRACT

The start-up of completely autotrophic nitrogen removal over nitrite (CANON) process was investigated by seeding nitritation granular sludge in a continuous stirred tank reactor with typical geometry. Nitrogen loading rate (NLR) was increased from 1.5 to 3.3 kg N m^{-3} d^{-1}, by shortening hydraulic retention time from 2.0 to 0.9 h under oxygen-limiting conditions. An extremely high nitrogen removal rate (NRR) of 2.83 kg N m^{-3} d^{-1} was achieved, due to the high specific NRR of huge biomass in the reactor. With the improvement of granular structure compactness, it was believed that the EPS accumulation could be beneficial to enhance nitrogen removal performance of granules. In addition, high-throughput pyrosequencing analysis revealed that a co-culture of aerobic and anaerobic ammonium oxidizing bacteria, affiliated to genera *Nitrosomonas* and *Candidatus Kuenenia* respectively, had been established in mature CANON granules, while the growth of nitrite oxidizing bacteria (*Nitrospira* spp.) was effectively suppressed through substrate competitions between three groups of bacteria. Therefore, a single continuous reactor with granules is applicable to achieve high performance CANON process for treating ammonium-rich wastewater.

1. Introduction

For the pursuit of energy neutral (or even energy positive) processes in wastewater treatment plants, the CANON system (completely autotrophic nitrogen removal over nitrite) for nitrogen removal from ammonium-rich influent with low C/N ratio has been developed in recent decades [1–3]. It relies on the stable cooperation between aerobic and anaerobic ammonium-oxidizing bacteria (AerAOB and AnAOB), with the effective suppression of nitrite oxidizing bacteria (NOB) growth. Due to coupling nitritation (Eq. (1)) and anammox (Eq. (2)) reactions in a single, oxygen-limiting reactor, CANON (Eq. (3)) could theoretically save about 60% oxygen consumption, reduce 90% biomass production, avoid any organic carbon addition, and significantly reduce construction investment, compared to conventional nitrification-denitrification systems [4,5].

Nitritation reaction by AerAOB:

$$NH_4{}^+ + 1.38O_2 + 0.09HCO_3{}^- \rightarrow 0.98NO_2{}^- + 0.018C_5H_7O_2N + 1.04H_2O + 1.89H^+ \quad (1)$$

Anammox reaction by AnAOB:

$$NH_4{}^+ + 1.32NO_2{}^- + 0.066HCO_3{}^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3{}^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (2)$$

Combined reaction of Eq. (1) and Eq. (2):

$$NH_4{}^+ + 0.79O_2 + 0.079HCO_3{}^- + 0.055\,H^+ \rightarrow 0.434\,N_2 + 0.11NO_3{}^- + 0.010C_5H_7O_2N + 0.028CH_2O_{0.5}N_{0.15} + 1.46\,H_2O + 1.086H^+ \quad (3)$$

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Although simultaneous nitritation-anammox processes based on the suspended sludge system could be achieved under real-time control [6,7], the granules (a particular biofilm without additional biocarriers) take an obvious advantages in maintaining a steady CANON, in which functional microbes with different growth kinetics can be sustained effectively, due to excellent settling ability, the undefined solid retention time (SRT), and distinct substrate gradients [8,9]. In addition, there were three main technical routes reported for the initiation of autotrophic nitrogen removal in a single reactor: (1) to operate an anammox reactor with or without AerAOB inoculation and then create a nitritation zone by accurate aeration control [10–12], (2) to operate a nitrifying reactor under oxygen-limiting conditions to obtain the desired ammonium to nitrite molar ratio in the system and then inoculate AnAOB biomass [13–15], and (3) to cultivate AnAOB based on a steady nitritation process [16,17]. Because of a extremely low growth rate of AnAOB (estimated doubling time of 7–11 days), the third route is the most difficult and challenging in terms of reactor start-up, and could be achieved through adoption of a granule system.

Thus far, many laboratory studies have investigated the use of sequencing batch reactor (SBR) configurations for implementation of the CANON process with various sludge types, including flocs, biofilms, and granules, which enable the flexible application of different control strategies, such as intermittent aeration and feeding [11,18,19]. However, for large-scale wastewater treatment, a continuous-flow mode is preferable, due to the simpler operation control and more effective use of the reactor volume.

Therefore, this study aimed to demonstrate the feasibility of employing a continuous stirred tank reactor (CSTR) with nitritation granular sludge (NGS) for the start-up of a high performance CANON process. The stepwise increase in nitrogen loading rate (NLR) under oxygen-limiting conditions was chosen as the main control strategy, by shortening hydraulic retention time (HRT) of the reactor at a fixed influent NH_4^+-N concentration. During the 145 days operation period, variations in nitrogen removal performance and sludge morphology in the CSTR were investigated. The evolution of microbial community structure in the granules was also analyzed using a high-throughput pyrosequencing technique, to identify the mechanism behind the autotrophic nitrogen removal in a single reactor.

2. Materials and methods

2.1. Reactor setup and operation

A lab-scale CSTR consisting of an aerobic volume of 1.5 L (length × width × height: 80 × 80 × 235 mm), a rectangular internal recycling channel, and a settling zone of 0.6 L was set up with NGS, in which approximate completely mixing of liquid was achieved by introducing fine air bubbles through a gas diffuser at the base, as shown in Fig. A.1a. The airflow rate was set at 0.9 ± 0.1 L min^{-1} for the oxygen-limiting conditions, which controlled dissolved oxygen (DO) concentration in the range of 0.8–1.5 mg L^{-1} throughout the 145 days operation period. Synthetic inorganic wastewater was fed in from the top of the reactor. The temperature was maintained at $28 \pm 1\,^{\circ}$C using a water bath. In order to retain the slow-growth biomass in CSTR as much as possible, the organized sludge discharge was not implemented during the operation. Considering the washout of suspended solid (SS) in the effluent, the hydraulic selection pressure controlled by HRT would result in a long SRT varying from 33 to 56 days in the different phases.

The volumetric NLR as NH_4^+-N was initially set at 1.5 kg N m^{-3} d^{-1}, and finally reached 3.3 kg N m^{-3} d^{-1} by stepwise decreases in reactor HRT from 2.0 h to 0.9 h, as shown in Table 1.

2.2. Seed sludge and wastewater composition

Seed granular sludge was cultivated in a lab-scale SBR with a 4.0 L working volume, which had been operated at an NLR of 1.0–1.5 kg N m^{-3} d^{-1} with a nitrite accumulation percentage (NAP) higher than 90% for more than 160 days. NGS was brownish-red and had a compact and round-shaped structure, a mean size of 0.9 mm, and a sludge volume index after 5 min of sedimentation (SVI_5) of 20 mL g^{-1}. The initial concentration of mixed liquor volatile suspended solid (MLVSS) in the CSTR was set at 7600 mg L^{-1}, and the ratio of MLVSS to mixed liquor suspended solid (MLSS) was approximately 0.84.

Synthetic wastewater with the following composition was used: 124.2 ± 5.1 mg L^{-1} NH_4^+-N (as ammonium chloride), 750 mg L^{-1} $NaHCO_3$, 44 mg L^{-1} KH_2PO_4, 20 mg L^{-1} $MgSO_4{\cdot}7H_2O$, 0.15 mg L^{-1} $FeCl_3{\cdot}6H_2O$ and 1.0 mL of trace solution per liter [20]. Based on experimental results, a relative low NH_4^+-N concentration in the influent could bring an adequate hydraulic selection pressure at the same NLR, which was beneficial to maintain the stability of granular sludge by washing out loose flocs from the reactor. And the influent pH value was adjusted to 7.8–8.1 by adding 20 mg L^{-1} NaOH solution, since the pH optima for CANON process appear to be slightly on the alkaline side [2,5].

2.3. Analytical methods

The concentrations of NH_4^+-N, NO_2^--N, NO_3^--N, total nitrogen (TN), MLSS, MLVSS, and SS were measured using the procedures described in Standard Methods [21]. The pH value and DO concentration were monitored with PB-10 (Sartorius, Germany) and H1946N portable meters (WTW, Germany), respectively. Size distribution of the granules was measured regularly using different sieves with opening sizes of 0.30, 0.50, 0.80, 1.25, and 1.60 mm, and the mean diameter was calculated on the sum of products of dry weight percentages at intervals. The morphology of granular sludge was observed using both a CX41 optical microscope (Olympus, Japan) and a Quanta 250 scanning electron microscope (SEM; FEI, U.S.). Samples were prepared for SEM evaluation according to the pretreatment procedures described by Wu et al. [22]. Extracellular polymeric substances (EPS) were extracted using the formaldehyde-NaOH method, and protein (PN) and polysaccharide (PS) content were quantified using the Lowry and phenol-sulfuric acid methods, respectively [23].

2.4. Calculations

The NAP, TN removal efficiency, volumetric nitrogen removal rate (NRR) of the reactor, and specific nitrogen removal rate (sNRR) of biomass were calculated according to the following equations, respectively.

$$\mathrm{NAP}(\%) = NO_2^-\text{-}N_{eff}/(NO_2^-\text{-}N_{eff} + NO_3^-\text{-}N_{eff}) \times 100; \quad (4)$$

$$\mathrm{TNremoval}(\%) = (1 - TN_{eff}/TN_{inf}) \times 100; \quad (5)$$

$$\mathrm{NRR}(\mathrm{kgN\,m^{-3}\,d^{-1}}) = \mathrm{NLR} \times \mathrm{TNremoval}; \quad (6)$$

$$\mathrm{sNRR}(\mathrm{gN\,g^{-1}VSS\,d^{-1}}) = \mathrm{NRR}/\mathrm{MLVSS}; \quad (7)$$

where TN_{inf} is the ammonium concentration in the influent; and TN_{eff}, NO_2^--N_{eff} and NO_3^--N_{eff} are the ammonium, nitrite and nitrate concentrations in the effluent respectively.

2.5. DNA extraction, PCR and pyrosequencing

The sludge samples in CSTR were harvested on day 1 (sample S1), 50 (S2), 90 (S3) and 143 (S4), respectively. Microbial community structure was analyzed by high-throughout pyrosequencing