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Performance of Nitrogen Removal in Ceramic Anammox Reactor with Two-Inflow

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Abstract. Anaerobic ammonium oxidation (anammox) converted ammonium into nitrogen gas using nitrite as an electron donor in anaerobic conditions by anammox bacteria. Granular anammox was used commonly to remove ammonium from wastewater. It easy to be broken and tend to settle in the bottom of the reactor in the application. A pored ceramic carrier was proposed to cultivate anammox bacteria with two-inflow substrate feeding. The carrier with a diameter of 1.0 mm was set up in the center of the 1.0 L conical filtering flask reactor. The substrate with 70 mg-N/L of ammonium-supplemented substate was introduced into the reactor through the center of the ceramic carrier from the bottom and 70 mg-N/L nitrite-supplemented substrates into the outside of ceramic carrier with HRT 3 hours. The influent and effluent concentration measured once a week using ion chromatography. The experiment was conducted in the 35°C controlled temperature room. Anammox bacteria grew by filled and covered the ceramic carrier's pore, and a flock of anammox settled in the reactor. The reactor's performance achieved a maximum of 76.496 % NRE and 0.901 kg-N/m^3· d NRR, respectively.

Introduction

Anaerobic ammonium oxidation (anammox) process converted ammonium into nitrogen gas using nitrite as electron acceptor autotrophically under anoxic conditions [1]. The stoichiometry of anammox process is defined by Lotti et al. (2014),as shown in equation 1.

$$NH_4^+ + 1.146NO_2^- + 0.071HCO_3^- + 0.057H^+ \rightarrow 0.986N_2 + 0.161NO_3^- + 0.071CH_2O_{0.5}N_{0.15} + 2.002H_2O \quad (1)$$

Comparing with conventional traditional nitrification-denitrification technology for biological nitrogen removal, Anammox has economic advantages with lower operational costs, a smaller footprint, and used to remove nitrogen from wastewater. The first experience, high NRR reached 9.5 kg-N/m^3·dat the first full-scale Anammox process in the Dokhaven municipal wastewater treatment plant in Rotterdam, Netherlands [3]. It proved that anammox is a powerful method for nitrogen removal. As new technology in wastewater treatment, more than 100 reactors are currently applied to treat ammonium-rich wastewater, such as landfill leachate, sludge-digestion liquid, coke-oven wastewater, and swine wastewater, monosodium glutamate wastewater, pharmaceutical wastewater, and mainstream wastewater [4].

In the reactor, the anammox process occurs in the form of suspended sludge, granular, and biofilm. Granular is commonly used in the application. However, it could break in the reactor and tend to settle in the bottom part of the reactor, where the performance decreased to the reactor's high. Other carriers used to retain and to distribute biomass, e.g., sponge [5], gel [6], membrane [7], string wound filter [8], palm fiber[9].

Pored-ceramic was studied as biological supporting media to cultivate anammox bacteria with a two-inflow supplied substrate. For the first time, a two-inflow system was introduced by Zulkarnaini et al. (2018) to cultivate anammox bacteria in a string wound filter for start-up one-stage nitritationanammox process. However, the thick anammox biofilm grows only on the surface and decreases substrate penetration to the biofilm's inner part. The performance of the anammox process was evaluated during the whole operation of the reactor.

2. Materials and methods

2.1. Reactor configuration

Ceramic anammox reactor (CAR) was conducted on a laboratory scale with a 1.0 L conical filtering flask. A pored-ceramic (2 cm x 2 cm x 5 cm, pore size 1 mm) was used as microbial supporting media to grow anammox biofilm, with a total volume of 5 ml^3. The ceramic carrier is put in the center of the reactor. The substrates were delivered to the reactor by two-inflow lines, ammonium into the center of the ceramic carrier from the bottom and nitrite into the outside using a peristaltic pump (EYELA, Japan). The reactor was set-up in the 35 ^oC temperature-controlled room. CAR was operated until the carrier was covered with anammox bacteria. Anammox bacteria Genus *Candidatus*Brocadiasinica was inoculated in CAR for start-up.Table 1 showed the operation condition of the CAR.

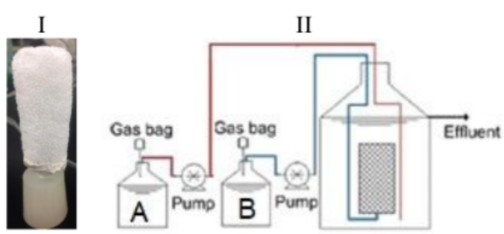

Figure 1. Ceramic carrier (I), Reactor configurations (II), tank A containing NO_2^--N and, tank B containing NH_4^+-N.

2.2. Substrates composition

The substrate solution's composition was (per L of tap water) KH_2PO_4, 27.2 mg; $MgSO_4.7H_2O$, 300 mg; $CaCl_2.2H_2O$, 180 mg; $CaCl_2$, 136 mg; $KHCO_3$, 500 mg; and 1 mL trace element solutions I and II[10]. The tank of the substrate is connected with an N_2-containing gas bag to maintain an anoxic condition. The solution was flushed with N_2 gas for 30 minutes before supplemented with substrate's compositions during preparation media. The substrate was supplied with 70 mg-N/L for both ammonium and nitrite with a Hydraulic retention Time (HRT) of 3 h.

2.3. Analytical methods

Samples for analysis were collected from influent and effluent lines once a week. The samples were filtered before the analysis with a 0.2-μm-pore-size membrane filter (Merc Millipore Ltd., Ireland). The concentration of ammonium, nitrite, and nitrate was analyzed using ion chromatography

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(Shimadzu,IC-A3, IC-C3 Type Column, Japan). Parameter pH was measured using a pH meter (F-71, Japan).

The performance of anammox was defined as nitrogen removal rate (NRR, kg-N/m^3·d), nitrogen removal efficiency (NRE, %), and ammonium conversion efficiency (ACE, %) were calculated according to the equations describedby Zulkarnaini et al. (2020)[12]:

Table 1. Operation Condition of CAR.

Process	Time (d)	NH_4^+-N (mg/L)	NO_2^--N (mg/L)	HRT (h)
Anammox	1-67	70	70	3

3. Results and discussion

The reactor was operated for 67 days for anammox process with two-inflow lines with separated supplied ammonium and nitrite. Anammox bacteria were grown in the carrier. Two-inflow supplied ammonium and nitrite caused the movement of enriched anammox biomass (*Candidatus* Brocadia sinica) to the carrier due to the physiological characteristics of anammox bacteria, which was inhibited by excess nitrite while ammonium is not an inhibitor for anammox bacteria. Figure 2 shown the correlation between ammonium conversion, nitrite conversion, and nitrate production during operation CAR. It was evident that the stoichiometric ratio of the anammox process close to the ratios obtained in this study with two-inflow CAR operation. This experiment indicated that anammox bacteria active and growing in the ceramic carrier. The lower conversion ratio of NO_3^--N/NH_4^+-N may be due to the denitrification process in the reactor where denitrification bacteria used decay of anammox biomass as a carbon source [13].

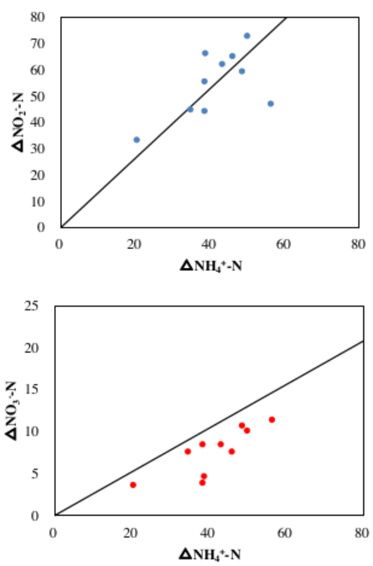

Figure 2. Correlation between ammonium conversion, nitrite conversion, and nitrate production.

Figure 3 shown the performance CAR. During the first week of operation, a small amount of ammonium and nitrite was removed from the reactor. This condition could be an adaptation of inoculant with operation condition where nitrite contacted directly. Nitrite could be an inhibitor for anammox process and decreased the cell metabolism of anammox bacteria. Then ammonium and nitrite converted to nitrogen gas where ACE achieved 85% and almost all supplied nitrite consumed for anammox process achieved 98%. Carmine biomass growth and covered ceramic carrier indicated anammox bacteria moved to and growth in the ceramic carrier. After a 1-month operation, nitrite concentration increased in the effluent while ammonium concentration stable.

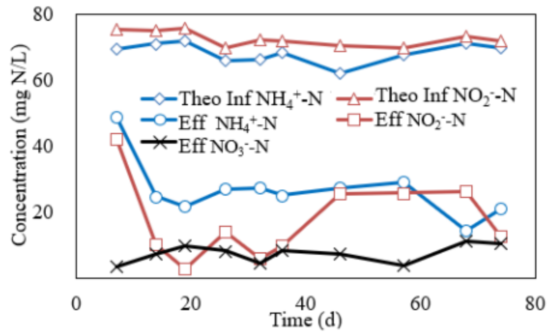

Figure 3. Nitrogen profile on CAR operation.

This problem occurred because a pump failure led to an unbalance of the flow rate two-line feeding substrate. The supplied nitrite was higher than ammonium slightly. This technical problem caused reactor exposure to nitrite concentration for a long time. Strous et al. 1999 reported at a level nitrite concentration of 5 mmol NO_2^-/L anammox activity completely lost in the reactor for more than 12 hours of exposure. This situation continued until the peristaltic pump changed with a stable flow rate. As a result, nitrite concentration decreased in the effluent, and anammox process could be recovered.

Figure 4 showed the performance of CAR. The reactor supplied with 1.120 kg-N/m^3·d during the whole operation of the reactor. Anammox process occurred at the first-week operation where NRE, NRR reached 34.590%, 0.400 kg-N/m^3·d, respectively. The maximum nitrogen removal was achieved at 19 days of operation with 76,496 % NRE and 0.901 kg-N/m^3·d NRR. The nitrogen removal tended to decrease and reached the lowest NRE and NRR of 54.124% and 0.574 kg-N/m^3·d, respectively. The nitrogen removal could be recovered after set-up the new peristaltic pump, and the performance showed NRE and NRR reached 68.667% and 0.779 kg-N/m^3·d, respectively.

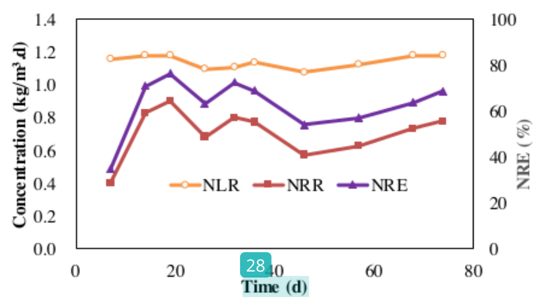

Figure 4. Performance of nitrogen removal in the CAR.

At the end of the operation, the ceramic carrier was taken out from the reactor to observeanammox bacteria's growth. Figure 5 showed the carrier covered by anammox bacteria and filled the ceramic carrier's pore until the inner part of the carrier. The anammox bacteria belong to

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*Candidatus*Brocadiasinica that was used as inoculum was sensitive to nitrite concentration. Two-inflow in CAR provided a better environment for anammox bacteria living in the ceramic carrier and pushed-up anammox bacteria to move and grow from seed to the carrier.

Figure 6 showed the proposed process of anammox bacteria moving in the reactor.Anammox bacteria prefer to grow in the filter supplied with ammonium than outside due to exposure to nitrite. It was reported that *Candidatus*Brocadia had lower tolerance on nitrite (70 mg/L) compared to other anammox bacteria such as *Candidatus* Kuenenia (70 mg/ L)[15]. This result better than others reported anammox biofilm using a filter where anammox biofilm grows only in the surface [8].

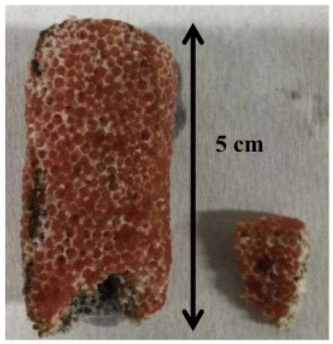

Figure 5. Anammox biofilm growth in the ceramic carrier.

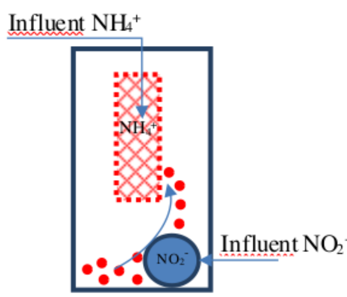

Figure 6. The Proposed moving process of anammox bacteria.

4. Conclusion

Fast start-up of anammox bacteria reached at 19 days operation. The reactor's performance reached maximum NRE, and NRR were 76,496 % and 0.901 kg-N/m^3·d, respectively. Anammox bacteria growth covered the pored-ceramic carrier and filled the pores. Two-inflow lines provide a suitable environment for anammox bacteria growth.

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