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Development of a simultaneous partial nitrification and anaerobic ammonia oxidation process in a single reactor

Sunja Cho [a,b], Naoki Fujii [b], Taeho Lee [a], Satoshi Okabe [b,*]

[a] Department of Civil and Environmental Engineering, Pusan National University, Busan 609-735, Korea
[b] Department of Urban and Environmental Engineering, Graduate School of Engineering, Hokkaido University, North-13, West-8, Sapporo 060-8628, Japan

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ABSTRACT

Up-flow oxygen-controlled biofilm reactors equipped with a non-woven fabric support were used as a single reactor system for autotrophic nitrogen removal based on a combined partial nitrification and anaerobic ammonium oxidation (anammox) reaction. The up-flow biofilm reactors were initiated as either a partial nitrifying reactor or an anammox reactor, respectively, and simultaneous partial nitrification and anammox was established by careful control of the aeration rate. The combined partial nitrification and anammox reaction was successfully developed in both biofilm reactors without additional biomass inoculation. The reactor initiated as the anammox reactor gave a slightly higher and more stable mean nitrogen removal rate of 0.35 (± 0.19) kg-N m^{-3} d^{-1} than the reactor initiated as the partial nitrifying reactor (0.23 (± 0.16) kg-N m^{-3} d^{-1}). FISH analysis revealed that the biofilm in the reactor started as the anammox reactor were composed of anammox bacteria located in inner anoxic layers that were surrounded by surface aerobic AOB layers, whereas AOB and anammox bacteria were mixed without a distinguishable niche in the biofilm in the reactor started as the partial nitrifying reactor. However, it was difficult to efficiently maintain the stable partial nitrification owing to inefficient aeration in the reactor, which is a key to development of the combined partial nitrification and anammox reaction in a single biofilm reactor.

1. Introduction

The anaerobic ammonium oxidation (anammox) process is a new, cost-effective and low energy consuming alternative to the conventional nitrogen removal processes (Hellinga et al., 1998). Anaerobic ammonium-oxidizing (anammox) bacteria belonging to the order *Planctomycetales* convert NH_4^+ directly to dinitrogen gas (N_2) using nitrite (NO_2^-) as the electron acceptor (van de Graaf et al., 1996; Schmid et al., 2001). To remove ammonia from wastewater using the anammox process, sufficient nitrite must be provided as an electron acceptor for anammox bacteria, since nitrite is rarely detected in typical wastewater (Jetten et al., 1997). Thus, ammonia must be partly converted to nitrite by aerobic autotrophic ammonia-oxidizing bacteria (AOB), and subsequently anammox bacteria would convert the remaining ammonia with the produced nitrite to N_2 gas. This sequential process has been extended by combining the partial nitrification with anammox reaction. The combination of partial nitrification and anammox process can be achieved in two different reactors as the SHARON – anammox process (Jetten et al., 1997; Hellinga et al., 1998) or in a single reactor such as OLAND (Oxygen-Limited Autotrophic Nitrification–Denitrification) (Kuai and Verstraete, 1998) or CANON (Completely Autotrophic Nitrogen removal Over Nitrite) process (Kuai and Verstraete, 1998; Third et al., 2001). If the entire nitrogen removal could be achieved in a single reactor with limited aeration (i.e., around 1.7 g O_2 per g-N), it would greatly reduce space and energy requirements as compared with the conventional nitrification–denitrification process and the SHARON–anammox process where nitrite is generated in a separate reactor. Thus, a single reactor system represents an economical and efficient option for wastewater treatment, especially for wastewater rich in ammonium but devoid of organic carbon.

Since the bacteria oxidizing ammonia to nitrite (i.e., AOB) require oxygen, while anammox bacteria are obligatory anaerobes and reversibly inhibited by low concentrations of oxygen, both reactions must occur under oxygen-limiting conditions (Kuai and Verstraete, 1998; Third et al., 2001). It is important to control DO concentration in the reactor not only due to the inhibition of anammox bacteria caused by DO concentrations but also in order to oxidize about a half of ammonia to nitrite (Kuai and Verstraete, 1998; Third et al., 2001). Thus, a single reactor process like the CANON requires efficient biomass retention and appropriate control of dissolved oxygen (DO) concentrations. It is assumed that AOB are

* Corresponding author. Address: Department of Urban and Environmental Engineering Graduate School of Engineering, Hokkaido University North-13, West-8, Kita-ku, Sapporo, Hokkaido 060-8628, Japan. Tel./fax: +81 (0)11 706 6266.
E-mail address: sokabe@eng.hokudai.ac.jp (S. Okabe).

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active in the outer oxic region of biofilms or aggregates, while anammox bacteria are present in the inner anoxic region (Egli et al., 2003; Nielsen et al., 2005; Kindaichi et al., 2007). The spatial distributions and their activities of AOB and anammox bacteria involved in the CANON aggregates have been investigated (Nielsen et al., 2005). Furthermore, the effects of original biomass and operational conditions on the aggregate sizes and microbial population have been reported (Vlaeminck et al., 2010; Vazquez-Padin et al., 2009). However, methods establishing cultures with a certain distribution of AOB and anammox bacteria have not been investigated yet. It is also important to control DO concentration in the reactor not only due to the inhibition of anammox bacteria caused by DO concentrations but also in order to oxidize about a half of ammonia to nitrite.

Partial nitrification and anammox in a single reactor has mainly been established by inoculating nitrifying sludge into anammox reactors (Third et al., 2001; Sliekers et al., 2003; Chen et al., 2009); however, inoculation of anammox biomass into partial nitrification reactors has been done recently (Gong et al., 2008; Vazquez-Padin et al., 2009). High nitrogen removal rates of up to 1.5 kg-N reactor-m^{-3} d^{-1} were achieved in a gas-lift reactor, which was developed by inoculating nitrifying biomass into an anammox reactor (Sliekers et al., 2003).

In this study, the combined partial nitrification and anammox reaction process was developed in single up-flow oxygen-limiting biofilm reactors, which were originally started as a partial nitrifying reactor and an anammox reactor, respectively. After achieving either stable partial nitrification or anammox, the aeration rate was carefully controlled to establish simultaneous partial nitrification and anammox reaction in each reactor. The feasibility and process performance (i.e., the entire nitrogen removal rate) were compared between two reactors. In addition, the microbial community structure and spatial organization of AOB and anammox bacteria in the biofilm of each reactor were analyzed by the denaturant gel gradient electrophoresis (DGGE) of PCR-amplified 16S rRNA gene and fluorescence in situ hybridization (FISH) techniques.

2. Methods

2.1. Reactors and startup

Two up-flow oxygen-controlled biofilm reactors equipped with a non-woven fabric support (Japan Vilene Co., Ltd, Japan) were developed as a single reactor for autotrophic nitrogen removal based on partial nitrification and anaerobic ammonium oxidation (anammox). The net working volume of each biofilm reactor was 1.25 L (440 mm in length and 60 mm in inner diameter). A peristaltic pump was used to introduce the medium from the bottom of the reactor. One reactor was started as a partial nitrifying reactor by inoculation with activated sludge from the Soseigawa municipal wastewater treatment plant (Sapporo, Japan) and feeding a synthetic medium containing only NH_4^+ (Tsushima et al., 2007b; van de Graaf et al., 1996). After attaining stable partial nitrification, the aeration rate (i.e., dissolved oxygen (DO) concentration) was lowered progressively until the anammox reaction occurred without inoculation of anammox biomass. This reactor was defined as "Reactor I". Another reactor was started as an anammox reactor by inoculation with anammox biomass originated from a previously developed reactor (Cho et al., 2010). After attaining a successful anammox reaction, a limited amount of air was gradually introduced into the reactor to establish simultaneous partial nitrification and anammox reaction without inoculation of the ammonia-oxidizing bacteria (AOB) biomass. The feeding synthetic medium was changed to contain only NH_4^+ (initial concentration; 150 mg-N L^{-1}) while other compositions of the medium were fixed. This reactor was defined as "Reactor II." The DO concentrations in both of the reactors were regulated by changing the aeration rate.

Both the reactors were operated at 37 °C, and the pH values ranged around 7.83 ± 0.20 and 7.60 ± 0.34 for the reactor I and reactor II, respectively. The reactors were operated in two different operational stages. In the first stage, partial nitrification and anammox were established in the reactor I and reactor II, respectively. In the second stage, simultaneous partial nitrification and anammox processes were established without further inoculation with biomass. During the second stage of the operation, nitrogen loading rates were changed from 0.5 to 2.0 kg-N m^{-1} d^{-1} (reactor I) and from 1.2 to 3.6 kg-N m^{-1} d^{-1} (reactor II), respectively; and the aeration rates were changed from 20 to 50 air-mL reactor-mL^{-1} m^{-1} (reactor I) and from 10 to 50 air-mL reactor-mL^{-1} m^{-1} (reactor II), to achieve a maximal total nitrogen removal rate in each reactor. The reactor performance was evaluated according to the maximal nitrogen removal rate and the stability of the process operation.

The synthetic medium was composed of $(NH_4)_2SO_4$ (40–300 mg-N L^{-1}), $KHCO_3$ (500–1500 mg L^{-1}), KH_2PO_4 (27 mg L^{-1}), $MgSO_4$ $7H_2O$ (300 mg L^{-1}), $CaCl_2{\cdot}2H_2O$ (180 mg L^{-1}), and 1 mL of trace element solutions I and II, as described previously by van de Graaf et al. (1996). Hydroxylamine (250 μM) was added to the medium of reactor I to promote partial nitrification in the first stage (Kindaichi et al., 2004). An equal molar amount of nitrite and ammonium was added to the feeding solution for the reactor II during the first stage to support activity and growth of the anammox bacteria.

2.2. Analytical procedure

The concentrations of NH_4^+-N, NO_2^--N and NO_3^--N in the influent and effluent were analyzed three times a week to determine the removal rate of total inorganic nitrogen, which was defined as the sum of the concentrations of NH_4^+-N, NO_2^--N and NO_3^--N in the reactors. The nitrogen removal rate was calculated by the difference of the total nitrogen concentration between the influent and effluent. The concentrations of NH_4^+-N, NO_2^--N, and NO_3^--N were determined using an ion chromatograph (DX-100, DIONEX, Sunnyvale, CA) equipped with an IonPac CS3 cation column and IonPac AS9 anion column. The samples were filtered through 0.2-μm pore size membranes (ADVANTEC, Tokyo, Japan) before the analysis. The dissolved oxygen of the effluent was measured using a DO meter (KRK DO-5Z, Japan), and pH of the influent and the effluent was measured using a pH meter (Horiba B-212, Japan) three times a week. The concentration of free ammonia was calculated based on the relationship between the concentration of free-ammonia and pH as described previously by Anthonisen et al. (1976).

2.3. Fluorescence in situ hybridization (FISH)

Biofilm samples were taken from both reactors on day 70 and 220, and fixed immediately for 2–3 h with 4% paraformaldehyde. Spatial distribution of AOB and anammox bacteria in the biofilm was analyzed using fluorescence in situ hybridization (FISH). FISH analysis was carried out as described previously by Tsushima et al. (2007a). The 16S rRNA-targeted oligonucleotide probes used in this study was Nso1225 (5′-CGCCATTGTATTACGTGTGA-3′) which hybridizes specifically with aerobic *β-proteobacterial* AOB (Mobarry et al., 1996) and Amx820 (5′-AAAACCCCTCTACTTAGTGCCC-3′) which hybridizes specifically with Candidatus *Brocadia anammoxidans* and *Kuenenia stuttgartiensis* (Schmid et al., 2001). A model LSM510 confocal laser-scanning microscope (CLSM, Carl Zeiss, Oberkochen, Germany) equipped with an Ar ion