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Presence and activity of anammox and denitrification process in low ammonium-fed bioreactors

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Abstract

A combination of anammox and denitrification process was studied for 300 days in low ammonium-fed bioreactors under the support of organic carbon. Nutrient profiles, ^{15}N-labelling techniques and qualitative fluorescence *in situ* hybridization (FISH) probes were used to confirm the nitrogen removal pathways and intercompetition among different bacteria populations. About 80% of nitrogen removal was achieved throughout the study period. The results confirmed that anammox bacteria were absent in the bioreactor inoculated with anaerobic granules only but they were present and active in the central anoxic parts of biopellets in the bioreactor inoculated with mixed microbial consortium from activated sludge and anaerobic granules. It also showed that the anammox bacteria were successfully enriched in the low ammonium-fed bioreactors. Results of this study clearly demonstrated that anammox and denitrification processes could coexist in same environment and anammox bacteria were less competitive than denitrifying bacteria.

Keywords: Anammox; Anaerobic granules; Denitrification; Immobilized microbial consortium

1. Introduction

Recently, the hydrosphere has become the main sink for excess nitrogen as a result of human activity (Galloway et al., 2003). Nitrogen in its various forms can deplete dissolved oxygen (DO) levels in receiving waters, stimulate aquatic growth, exhibit toxicity towards aquatic life, present a public health hazard and affect the suitability of wastewater for reuse (Smith, 2003; Wolfe and Patz, 2002).

Nitrogen removal is one of the costly elements in wastewater treatments and simultaneous bionitrification and biodenitrification (Hsieh et al., 2003; Khin and Annachhatre, 2004; Ruiz et al., 2006) have been commonly used as the main processes to remove nitrogen from water and wastewater. The addition of external carbon source to the anoxic zone of treatment plants facilitates denitrification process. For a long time, this combination was considered the only way to remove ammonium from wastewater. More recently, shortcut processes by replacing nitrate with nitrite (van Dongen et al., 2001), or application of the anaerobic oxidation of ammonia (anammox) process (Jetten et al., 2005) have been considered as novel nitrogen removal process. The anammox process has recently been used extensively in ammonium-rich wastewater (Guven et al., 2004; Jetten et al., 2005; Third et al., 2005) and has also investigated in natural water (Kuypers et al., 2003). However, application of the anammox process in low ammonium nitrogen has been still limited. In the anammox process, ammonium is oxidized anaerobically to N_2 by autotrophic bacteria using nitrite as the electron acceptor.

According to Van de Graaf et al. (1996), the anammox process produces nitrate which is about 10% of the influent ammonium. This means that the anammox process removes only 90% of the incoming nitrogen leaving behind

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10% in the effluent. In order to meet stringent effluent standards, an improved process has to be explored. Although the control over the physiological requirements is crucial because of the involvement of two different groups of bacteria (autotrophic and heterotrophic), a combination of both anammox and denitrification would give the complete solution to nitrogen removal. This combined method offers significant economical advantages such as reduction in energy and low carbon substrate. Therefore, the aim of this work was to examine the simultaneous presence and activity of anammox and denitrification processes in low ammonia-fed single bioreactors under the support of external carbon substrates.

2. Methods

2.1. Biopellets fabrication

Biomass from the activated sludge of a domestic wastewater treatment plant and anaerobic granules from full-scale upflow anaerobic sludge blanket (UASB) reactors treating brewery wastewater in Ibaraki, Japan were collected separately. The collected activated sludge was entrapped in polyethylene glycol (PEG) prepolymer gel as biopellets (Pathak et al., 2005). The composition of the immobilized material was 10% (w/v) PEG prepolymer and 2% (w/v) activated sludge. The final size of the cubic biopellets was 3 mm.

2.2. Reactor setup and operation

Two upflow bioreactors, namely AG (anaerobic granules) and BAG (biopellets and anaerobic granules) were designed such that each had an effective volume of 100 mL. The AG bioreactor was inoculated with anaerobic granules to 50% of the total volume bioreactor and the BAG bioreactor was inoculated with a mixture of biopellets and anaerobic granules to 20% of the total volume of bioreactor. A 20 g aliquot of caprolactone $[(C_6H_{10}O_2)n]$ (solid biodegradable plastic), equivalent to 30% of the effective volume of the bioreactor was supplied to the BAG bioreactor as an additional carbon source. Water samples with total nitrogen content of 1.47 ± 1.00 mg/L (>95% in the form of nitrate nitrogen) were retrieved from Shiokawa Reservoir (Yamanashi, Japan). Ammonium nitrogen and nitrite nitrogen were added to understand the nitrification and denitrification processes more precisely under oxygen limiting conditions. The modified water was supplied to the both bioreactors continuously at the rate of 200 mL/day using peristaltic pumps. All experiments were performed for 300 days at 20 °C in temperature-controlled bioreactors (Eyelatron FL1-301NH, Japan) under 12 h hydraulic retention time (HRT).

Nutrient profiles, ^{15}N-labelling techniques and fluorescently labelled rRNA probes were used to identify the nitrogen removal pathway. The details of these methods are described in Schmid et al. (2005).

2.3. Nutrient analysis

Grab samples of influents and effluents were collected twice a week from all bioreactors for chemical analyses. Samples were filtered using Whatman GF/F filters (0.45 μm) and ammonium nitrogen (NH_4^+–N), nitrate nitrogen (NO_3^-–N) and nitrite nitrogen (NO_2^-–N) concentrations were measured colorimetrically as described in APHA (1998). Nitrification performance was calculated based on the change in ammonium concentration in the influents and effluents with respect to the initial NH_4^+–N concentration. Summation of the differences in NO_3^-–N and NO_2^-–N in the influents and effluents was used to determine the extent of denitrification. The nitrogen removal efficiency was calculated as $(N_{in} - N_{out})$, where N_{out} and N_{in} are the nitrogen concentrations (mg/L) in the effluent and influent, respectively. The nitrogen removal rate (g-N/m^3 day) was calculated as daily nitrogen load removed [daily flow rate $\times (N_{in} - N_{out})$] per unit volume of reactors. Dissolved organic carbon (DOC) concentration in the effluent was measured using a total organic carbon analyser (Shimadzu – TOC-VCSH, Japan). Although DOC inside the reactors and in effluent could be slightly different, the expected concentration was presumed closely similar and comparable.

2.4. ^{15}N-labelling techniques

To identify the nitrogen removal pathway in the bioreactors, water samples retrieved from the reservoirs were modified by adding equal concentrations of 99% $^{15}NO_2^-$–N (Cambridge Isotope Laboratories, USA) and native NH_4^+–N. The modified water sample was applied as influent for experimental time of 266–296 days. The gas produced in the bioreactor was carefully collected using a syringe and transferred to a bottle containing saturated NaCl. The bottle was carefully filled with saturated NaCl to prevent sample contamination by atmospheric gases and any possible bubble entrapment. The bottle was sealed with thick butyl rubber stoppers and kept in refrigerator in an inverted position until delivery to the laboratory at Shoko Co. Ltd. (Saitama, Japan) for isotope analysis. The concentration of $^{14}N^{14}N$, $^{14}N^{15}N$ and $^{15}N^{15}N$ were determined using a Hitachi RM1-2 mass spectrometer using atmospheric air was as standard. The experiments were performed in triplicate.

2.5. Microbial analysis using florescence in situ hybridization (FISH)

Granules samples from the AG bioreactor as well as biopellet and granule samples from the BAG bioreactor were collected on 300 days of experiment. The oligonucleotide probes for domain bacteria (EUB338/EUB338II/EUB338III), archea (ARC915), anammox bacteria (AMX820) and planctomycete (PCL46) were used as described by Daims et al. (1999), Stahl and Amann