Bioresource Technology 139 (2013) 285–291

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Development and characterization of the partial nitrification aerobic granules in a sequencing batch airlift reactor

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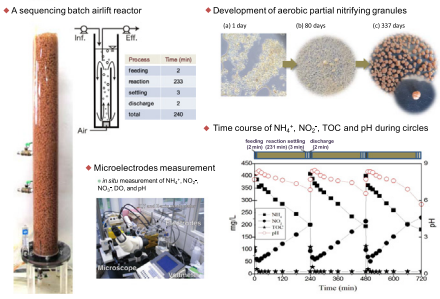
HIGHLIGHTS

- An aerobic granule reactor was developed for partial nitrification.
- Mature granules have a settling velocity of 103 m h^{-1} and SVI of 60 ml g^{-1}.
- Net NO_2^- production rate of 0.64 kg-N m^{-3} day^{-1} was obtained.
- Simultaneous NH_4^+ oxidization and NO_2^- reduction occurred near the granule surface.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 February 2013
Received in revised form 3 April 2013
Accepted 5 April 2013
Available online 20 April 2013

Keywords:
Partial nitrification
Aerobic granules
Denitrification via nitrite
Microelectrodes
FISH

ABSTRACT

In this study, partial nitrifying (PN) aerobic granules were developed in a sequencing batch airlift reactor by controlling the airflow rate and NH_4^+ loading rate. The PN reactor produced an effluent with a NO_2^-/NH_4^+ ratio of approximately one and with an NH_4^+ conversion rate of 1.22 kg N m^{-3} day^{-1}. More than 95% of the total organic carbon was removed during the process. On the basis of clone library analysis and fluorescence *in situ* hybridization, ammonia-oxidizing bacteria (AOB) closely related to *Nitrosomonas eutropha* and putative heterotrophic denitrifiers were mainly present near the surface of the PN aerobic granules. Microelectrode measurements revealed that both NH_4^+ and NO_2^- were consumed near the surface (<200 μm), whereas no nitrate (NO_3^-) accumulation was observed throughout the granules. These results indicate that PN by AOB and nitrite denitrification by heterotrophs, but not nitrite oxidation, simultaneously occurred near the surface of the PN aerobic granules.

1. Introduction

Partial nitrification (PN) followed by anaerobic ammonium oxidation (anammox) is a promising approach for removing nitrogen from wastewater (Van Hulle et al., 2010). The nitrogen compounds present in most wastewaters are in the form of ammonium (NH_4^+). In the PN reaction, half of the NH_4^+ in wastewater is converted to nitrite (NO_2^-) by ammonia-oxidizing bacteria (AOB). In the anammox process, dinitrogen gas (N_2) is produced from NH_4^+ and NO_2^- (Van de Graaf et al., 1995). Advantages of the PN-anammox process over conventional nitrification–denitrification process include the following: no requirement for external organic carbon, 63% reduction in oxygen demand, less sludge production, and low emissions of carbon dioxide (CO_2), nitrous oxide (N_2O), and nitric oxide (NO).

Currently, the low NO_2^- production rate in the PN system limits the overall nitrogen removal rate (Okabe et al., 2011a,b; Tsushima et al., 2007; Van Hulle et al., 2010). To obtain a high NO_2^- production rate, PN reactors need to be developed that enhance the growth of AOB while inhibiting the growth of nitrite-oxidizing bacteria (NOB). Because favorable growth conditions for AOB and NOB are different, operation conditions for the PN reactor should be set to enhance the growth of AOB and inhibit the growth of NOB. For example, previously developed PN reactors used low dissolved

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http://dx.doi.org/10.1016/j.biortech.2013.04.018

oxygen (DO) concentrations, high free NH_4^+ and nitrous acid (HNO_2) concentrations (Anthonisen et al., 1976; Okabe et al., 2011b; Park and Bae, 2009), high temperature, and short sludge retention time (Hellinga et al., 1998). In particular, a single reactor system for high activity ammonium removal over nitrite (SHARON) has been used in several PN reactors (van Kempen et al., 2001). However, these systems use suspended biomass; therefore, they have problems, such as a large footprint, long liquid–solid separation times, and, more importantly, a low PN efficiency compared with the anammox rate (Cho et al., 2011; Tsushima et al., 2007).

In contrast, aerobic granule reactors have attracted attention because of their rapid sludge settling property, long sludge retention time, tolerance to shock loading, and no sludge bulking (Liu and Tay, 2004). Several researchers have developed PN aerobic granule reactors. Tokutomi (2004) first developed PN aerobic granules by mixing methanogenic anaerobic granules and nitrifying activated sludge in continuous airlift reactors. However, sequencing batch airlift reactors (SBAR) are used more commonly to develop aerobic granules (Liu and Tay, 2004). PN aerobic granules have been successfully developed using SBAR by feeding NH_4^+ without a C source (Kim and Seo, 2006; Vazquez-Padin et al., 2010) or using sucrose (Shi et al., 2009), glucose, and/or acetate (Shi et al., 2011; Wang et al., 2012; Xu et al., 2011). These researchers identified *Nitrosomonas*-related AOB in their PN aerobic granules, whereas no NOB were detected. However, the microbial population structures and their *in situ* microbial activities in PN granules have not been previously investigated. Thus, understanding the microbiology and ecology within PN aerobic granules could help us to control and improve the process performance.

Consequently, the objectives of this study were to: (1) develop PN aerobic granules in SBAR; (2) evaluate the performance of the PN aerobic granule reactor; (3) analyze the identity, localization, and activity of AOB and other microbial populations in PN aerobic granules.

2. Methods

2.1. Reactor set-up and operation

An internal-circulating SBAR with a working volume of 5 L (100 cm height and 9 cm inner diameter at the down-comer; 70 cm height and 4.2 cm diameter at the riser; the height:diameter ratio is 10) was designed as shown in Fig. S1, and used as a PN reactor. The composition of the synthetic wastewater was 200–600 mg N L^{-1} $(NH_4)_2SO_4$, 200 mg total organic carbon (TOC) L^{-1} sodium acetate, 24–72 mM $KHCO_3$ (varied to maintain the influent pH at 7.5–8.0), 0.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.2 mM $CaCl_2$, and 1 mL of the trace element solutions I and II per liter of the total solution volume, as described by van de Graaf et al. (1996). The NH_4^+ concentration was increased stepwise from 200 mg N L^{-1} to 600 mg N L^{-1}. The reactor was inoculated with 1.5 L of fresh activated sludge from the secondary settling tank of the municipal wastewater treatment plant located in Sosegawa, Sapporo, Japan.

The reactor was operated in successive cycling conditions, which comprised substrate (2.5 L) feeding, aeration, sludge settling, and effluent (2.5 L) withdrawal. During effluent withdrawal, only the upper half of the liquid was removed so that the settled sludge was retained in the reactor. For the first four days, each cycle comprised 2 min of substrate feeding, 360 min of aeration, 60 min of settling, and 2 min effluent withdrawal. Subsequently, the reactor was operated in cycles with a duration of 4 h, which comprised 2 min of substrate feeding, 3–30 min of sludge settling, 2 min of effluent withdrawal, and the remaining time was used for aeration. No aeration was applied during the substrate feeding, sludge settling, and effluent withdrawal periods. After the development of PN granules (after 83 days), the sludge settling time was fixed to 3 min. The airflow rate was manually controlled at 0.5–1 L min^{-1} to maintain a DO concentration of approximately 2 mg L^{-1}, and the superficial air velocity was 0.6–1.2 cm s^{-1}. The reactor was operated at room temperature (15–25 °C).

2.2. Analytical procedure and wastewater

The DO and pH were measured using a DO meter (KRK DO-5Z, Japan) and a pH meter (Horiba B-212, Japan), respectively. The NH_4^+, NO_2^-, and NO_3^- concentrations were measured using an ion-exchange chromatography system (DX-100, DIONEX, Sunnyvale, CA., USA) with an IonPac CS3 cation column and IonPacAS9 anion columns. The free ammonia (NH_3) and HNO_2 concentrations were calculated as functions of the NH_4^+ and NO_2^- concentrations, pH, and temperature using the equation reported by Anthonisen et al. (1976). TOC concentration was measured using a TOC-analyzer (TOC-5000A; Shimadzu, Kyoto, Japan).

Mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and sludge volume index (SVI) were determined according to standard methods (APHA, 1998). The settling velocity of the sludge was measured by recording the traveling time of individual granules from the top to the bottom of a cylinder (40-cm distance). The granule size was manually measured using a fine-scale ruler.

2.3. Batch experiments

Batch experiments were conducted to determine denitrification and anammox occurrence in the granules. Granules were taken from the PN aerobic granule reactor and washed three times with a synthetic medium without NH_4^+.

To analyze the denitrification activity, the granules were placed in 250-mL conical flasks and the air phase was replaced with N_2 gas. The granule samples were subsequently incubated with the synthetic medium supplemented with 100 mg N L^{-1} (final concentration) as NO_2^- or NO_3^-, and with or without 100 mg TOC L^{-1} (final concentration) as acetate. The culture was mixed by magnetic stirring, and liquid samples were taken at 30, 60, and 90 min after substrate addition, followed by analysis of the NH_4^+, NO_2^-, and NO_3^-concentrations.

To analyze the anammox activity, the granules were placed in 30-mL vials and the air phase was replaced with He gas. The granule samples were subsequently incubated using the synthetic medium supplemented with 100 mg N L^{-1} ^{15}N-labeled NH_4^+ and 100 mg N L^{-1} (final concentration) as unlabeled NO_2^-, and with or without 200 mg TOC L^{-1} (final concentration) as acetate. Gas samples were taken at 1, 2, 3, 4, 5, 6, 12, and 24 h after substrate addition and analyzed to determine their $^{29}N_2$ and $^{30}N_2$ content by gas chromatography/mass spectrometry (GCMS-QP2010, Shimadzu, Kyoto, Japan).

2.4. Clone library analysis

Genomic DNA was extracted from individual mature granules ($n = 3$) using a Fast DNA spin kit (Bio 101, Qbiogene, CA., USA). It was confirmed that the microbial community structures were similar in the three granules by denaturing gradient gel electrophoresis (DGGE) analysis, which was conducted as described previously (Ishii et al., 2011). The genomic DNA samples were pooled and used for the clone library analysis, as described below.

Near-full length 16S ribosomal RNA (rRNA) gene fragments were amplified using PCR with modified 27F and 1492R primers, as described previously (Ishii et al., 2011). The PCR products were ligated with pCR-4-TOPO vectors (Invitrogen, Carlsbad, CA., USA) and electrochemically transformed into competent *Escherichia coli*