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Development of Anammox Reactor Equipped with a Degassing Membrane to Improve Biomass Retention


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Abstract
In up-flow anammox reactors, one of the contributing factors of biomass wash-out is the adherence of nitrogen gas produced by anammox reaction to biomass. In this study, we operated an up-flow anammox reactor equipped with a degassing membrane to minimize the biomass wash-out from the reactor by separating the produced gas from the biomass. In addition, both the effect of degassing on the anammox reactor performance and the durability of the membrane submerged in the anammox reactor were investigated. The results show that the use of the degassing membrane in the anammox reactor could 1) Improve the biomass retention ability (by separating the produced gas from the biomass), and 2) Increase the component ratio of anammox bacteria in the reactor. In addition, degassing could reduce the N₂O emission produced in the reactor (for the gas selectivity of the degassing membrane). No membrane fouling was observed even after two months of operation without washing, indicating an advantage to the use of the degassing membrane.

Keywords
Anaerobic ammonium oxidation (anammox), degassing membrane, biomass retention

INTRODUCTION
The anaerobic ammonium oxidation (anammox) process is a novel and promising alternative in which ammonium is oxidized to nitrogen gas using nitrite as the electron accepter (Mulder et al., 1995). This process has advantages such as lower oxygen demanded and no requirement for external carbon sources, so the anammox process would allow the reduction of costs compared to the traditional nitrogen treatment process. However, the anammox bacteria grow so slowly, with doubling times of weeks in many ecosystems (Strous et al., 1998, 1999a, b), thus the application of anammox needs a long start-up time. Moreover, an additional problem is caused by the loss of a fraction of the sludge washed out with the effluent despite minimizing the wash-out of biomass from the reactor, and this becomes critical when slow-growing bacteria are used. For this reason, in order to avoid biomass wash-out with the effluent, an efficient system or operation strategy is required. In order to improve the biomass retention and the stability process, a sequencing batch reactor (SBR) was successfully used to grow anammox biomass (Strous et al., 1997, 1999; Dapena-Mora et al., 2004a). These reactors were operated with an additional mechanical stirring in order to improve the biomass retention and prevent the entrapment of nitrogen bubbles, therefore increasing the stability of the process. Other systems that have been used with success include a reactor containing non-woven media for biomass immobilization (Furukawa et al., 2003) and an up-flow system seed with anaerobic granular sludge (Imajo et al., 2004). However, a fraction of the generated biomass is inevitably washed out with the effluent in all these systems. Therefore, other approaches for improving biomass retention are required. In up-flow anammox reactors, one of the contributing factors of biomass wash-out is the adherence of nitrogen gas produced by anammox reaction to biomass. We hypothesized that the retention of anammox biomass in the reactor may be improved when the produced gas is separated from the biomass. Then, we focused on the degassing membrane to achieve separation of the produced gas from the biomass. As an example of the application of the degassing membrane to the biological treatment process, Bandara et al. (2011) operated a degassing membrane reactor, using degasification to recover the residual dissolved
methane in the effluent of an anaerobic wastewater treatment reactor.

In this study, we operated an up-flow anammox reactor equipped with a degassing membrane to minimize the biomass wash-out from the reactor by separating the produced gas from the biomass. In addition, the effects of degassing on the anammox reactor performance and the durability of the membrane submerged in the anammox reactor were investigated.

MATERIALS AND METHODS

Degassing membrane reactor
Two types of anammox reactors each equipped with degassing membrane modules, —a degassing reactor, and a control reactor—, were operated to compare the reactor and degassing performances in this study (Fig. 1). The membrane module involved three-layer hollow fiber membrane (MHF, Mitsubishi Rayon Co., Ltd., Tokyo, Japan) with a total membrane area of 1.766 m². To determine the effect of degassing, one membrane module was continuously degassed by using a vacuum pump (DAP-6D, ULVAC Inc. Chigasaki, Japan) at 0.09 MPa during the reactor operation. The reactor volume was 1340 cm³ (diameter, 8.9 cm; height 40 cm). The hydraulic retention time (HRT) was 5.1 h. Temperature was maintained at 37 °C. The anammox biomass was obtained from a fixed-bed biofilm column reactor, which was developed previously (Tsushima et al., 2007), and then 1.37 g (dry weight) of granular anammox biomass was directly inoculated into the reactor.

The synthetic nutrient medium consisted of (NH₄)₂SO₄ (35.7-214.3 mM), NaNO₂ (71.4-428.6 mM), KHCO₃ (5.0 mM), KH₂PO₄ (0.2 mM), MgSO₄·7H₂O (1.2 mM), CaCl₂·2H₂O (1.4 mM), and 1.0 ml/l of trace elements solution I and II (van de Graaf et al., 1996).

Sampling and analysis
The concentrations of NH₄⁺-N, NO₂⁻-N, and NO₃⁻-N were determined using ion-exchange chromatography (HPLC 10Avp; Shimadzu Co., Kyoto, Japan). Suspended solids (SS) in effluent were determined according to the Standard Methods (APHA, 1998). The amount of biomass wash-
out (SS) was measured as two different biomass: free biomass and granular biomass. The free biomass was periodically corrected from the effluent. On the other hand, granular biomass was trapped in the U-trap, and was measured at random times (as shown as arrows in Fig. 3). The doubling time of anammox bacteria was estimated based on the amount of biomass before and after the reactor operation. The exhaust gas was collected into the gas bag and the gas volume was measured by using a gas-meter (Shinagawa Co., Tokyo, Japan). The aspirated gas was directly collected in a syringe via the degassing port. To determine gas composition, N₂ and O₂ gas concentrations were measured utilizing a gas chromatograph equipped with a thermal conductivity detector (TCD) (GC-8A, Shimadzu), and the N₂O and CO₂ gases were determined by using a gas chromatograph equipped with an electron capture detector (ECD) (GC-14BPsE, Shimadzu).

Measurement of permeation rate
To evaluate the membrane fouling, permeation tests were conducted before and after the reactor operation. In this test, the permeation rates of five different gases (O₂, N₂, CO₂, N₂O, and CH₄) were measured separately to confirm the gas selectivity of the membrane. The permeation rate was calculated according to the following equation:

\[
P = \frac{V}{A \cdot p \cdot t}
\]

where, \(V\) is the permeated gas volume (m³), \(A\) is the total membrane area (1.766 m²), \(p\) is the suction pressure (0.09 MPa), and \(t\) is the suction time (h).

FISH analysis
A FISH analysis was performed according to the procedure described by Amann et al. (1995) and Okabe et al. (1999). The 16S rRNA-targeted oligonucleotide probes used in this study were EUB338 (Amann et al., 1990), EUB338-II (Daims et al., 1999) and EUB338-III (Daims et al., 1999), all used in a mixture (EUB338mix) for most bacteria, and Amx820 (Schmid et al., 2000) for anammox bacteria. The probes were labeled with fluorescein isothiocyanate (FITC) or tetramethylrhodamine 5-isothiocyanate (TRITC). A LSM5 PASCAL confocal laser scanning microscope (CLSM; Carl Zeiss) equipped with an Ar ion laser (488 nm) and a HeNe laser (543 nm) was used for microscopic observation. Image combining and processing were performed with the standard software package provided by Zeiss, as described previously (Kindaichi et al., 2004; Kindaichi et al., 2007; Kindaichi et al., 2011). For quantitative determination of the microbial composition in the reactor, the surface fractions of the Amx820 probe-hybridized cell area and the EUB338mix probe-hybridized cells (total biomass) were determined after simultaneous in situ hybridization. The average surface fraction was determined from at least 10 randomly-chosen CLSM projection images obtained from each of the duplicate anammox biomass samples by using image analysis software provided by Zeiss. The fraction was statistically compared using a Student’s t-test.

RESULTS AND DISCUSSION

Reactor performance
The simultaneous removal of NH₄⁺ and NO₂⁻ was observed shortly after the inoculation, suggesting that an anammox reaction was occurring in both the reactors. From Day 12, effluent was collected into a tank and SS was determined to evaluate the biomass concentration in effluent (i.e., biomass wash-out) from the reactors. The degassing was started in the degassing reactor on Day 24. After the degassing, the nitrogen removal rates in both the reactors were almost the same (Fig. 2). The
amounts of biomass in the effluent of both the reactors were clearly different after degassing started (Fig. 3). Generally, when an anammox reactor is operated with a high nitrogen loading rate, some produced gas attaches to the anammox biomass and then the floating biomass tends to overflow and is discharged from the reactor. On the other hand, when an anammox reactor is operated using the degassing process, the produced gas is separated from the biomass, suggesting that the degassing reduces the overflow of biomass from the reactor. The amount of biomass wash-out during the degassing period in the degassing reactor and the control reactor were 1.55 g and 2.22 g, respectively. These values are corresponding to 113% and 162% of inoculated biomass (1.37 g), and we found that the degassing reactor could reduce approximately 30% of biomass wash-out compared with the control reactor in this study. However, it is not clear why nitrogen removal rate in the degassing reactor was lower than that of the control reactor at present. It is possible that specific anammox activity was different between the degassing and the control reactor. The doubling time was estimated to be 11.8 days for both of the reactors. This doubling time estimated in this study was similar to the value of 11 days reported by Strous et al. (1998).

After the two-month operation, the rate of membrane permeability was determined and compared to the rate prior to the reactor’s operation. No differences in membrane permeability or selectivity before or after the reactor operation were observed, indicating that membrane fouling did not occur during the reactor operation (Table 1). This would be an advantage to the use of a degassing membrane.

![Fig. 2. Profile of nitrogen loading rate (NLR) and nitrogen removal rate (NRR).](image)

![Fig. 3. The amount of cumulative wash-out biomass. Arrows indicate measurement points of granules wash-out.](image)
Component ratio of anammox bacteria

At the start of the operation, the percentages of anammox bacteria in the degassing reactor and in the control reactor were 77.8% and 78.8%, respectively (Table 1). Two months later, the percentage in the control reactor was 82.1%, and in the degassing reactor was 87.6%. A statistical analysis using an independent t-test verified that the difference in the values before and after the reactor operation in the degassing reactor was statistically significant (P<0.05). On the other hand, with respect to the control reactor, there was no significant difference in the values before and after the reactor operations. As a result, the percentage of anammox bacteria increased. This result indicates that the use of the degassing membrane enhances the anammox bacterial population in granules and/or biofilms.

Gas composition

Gas samples were collected from gas bags and the degassing port of the degassing reactor on Day 70. The exhaust gas volume in the control reactor was 1.65 l/day, whereas the gas volume in the degassing reactor was 0.79 l/day, and thus, the gas recovery efficiency using the degassing was 48%. Concerning the components of the aspirated gas, N2 was 81.2%, O2 was 8.1%, CO2 was 9.1%, and N2O was 1.5%. The main component of both the exhaust and the aspirated gases was nitrogen gas in both of the reactors. Also, the exhaust gases contained N2O (0.8% in the degassing reactor, 1.0 % in the control reactor), which is known as a powerful greenhouse gas. The N2O conversion ratio per loaded nitrogen was 1.7% in the degassing reactor and 2.1% in the control reactor. These values are
approximately twenty times higher than that of previous study reported by Okabe et al. (2011). However, the microbial community composition of anammox bacteria reported by Okabe et al. (2011) was more than 90%, which is higher than that of this study (Table 1). One possible explanation is that coexisting denitrifying bacteria might be responsible for the N\textsubscript{2}O production. Therefore the N\textsubscript{2}O conversion ratio per loaded nitrogen might be high in this study. On the other hand, it is noteworthy that the N\textsubscript{2}O concentration in the aspirated gas was two times higher than the concentration in the exhaust gas. This result could be attributed to the high N\textsubscript{2}O selectivity of the membrane module, as shown in Table 2. This shows that the use of the degassing membrane condensed the N\textsubscript{2}O concentration in the aspirated gas, bringing about the reduction of N\textsubscript{2}O emission produced in the reactor.

In order to evaluate the inhibitory effect of N\textsubscript{2}O gas, the amounts of N\textsubscript{2}O gas emissions and the recovery were investigated. As shown in Fig. 4, 63% of N\textsubscript{2}O gas produced in the reactor was recovered by degassing under the operating condition in this study. Although the gas recovery efficiency in this study was 52%, if it can be improved, the recovery rate of N\textsubscript{2}O gas also can can be improved.

CONCLUSIONS
The use of the degassing membrane in the anammox reactor successfully improved biomass retention ability (by separating the produced gas from the biomass) and increased the component ratio of anammox bacteria in the reactor. This result indicates that the use of the degassing membrane may be suitable for the start-up of an anammox reactor. In addition, degassing could reduce the N\textsubscript{2}O emission produced in the reactor (for the gas selectivity of the degassing membrane). No membrane fouling was observed even after two months of operation without washing, indicating an advantage to the use of a degassing membrane.

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