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Comparison of Specific Anammox Activity and Nitrous Oxide Production of Enriched Anammox Cultures in Suspended-and Attached-Growth Systems

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Abstract

Anaerobic ammonia oxidizing (anammox) bacteria are able to remove nitrogen in both attached- and suspended-growth systems. Attached-growth or biofilm systems are better able to handle high nitrogen loading (total nitrogen concentrations (NH_4^+-N plus NO_2^--N) up to 80.5 mM) than suspended-growth systems. High nitrogen concentrations (along with constant concentrations of minor nutrients and trace elements) were fed to enriched anammox cultures in suspended-and attached-growth systems, contained in sequencing batch reactors (SBRs). For attached-growth a polystyrene sponge was used as the growth medium. The specific anammox activity (SAA) with attached-growth systems was higher than the SAA for suspended-growth systems. Nitrous oxide (N_2O) production was significantly greater in both reactors at pH 6.8 than at pH 7.3, 7.8, and 8.3. N_2O production from both reactors was significantly higher at an NH_4^+:NO_2^- ratio of 0.5:1 than at ratios of 0.75:1, 1:1, and 1:1.3. However, N_2O production from the attached-growth system was slightly lower than N_2O production from the suspended-growth system. The results from this research suggest that attached-growth reactors could provide operational advantages over suspended-growth reactors in field applications.

Keywords : Suspended Growth; Attached-Growth; Anammox

Introduction

In practice conventional nitrification-denitrification processes can be problematic and costly in treating wastewaters with high ammonium (NH_4^+) but low carbon concentrations, such as for treated effluent from anaerobic treatment of landfill leachate, sludge from domestic wastewater, piggery and poultry wastewater, and food industry substrates. A new biological approach, anaerobic ammonium oxidation (anammox) has been proposed. Anammox provides an efficient method for removal of nitrogen from these wastewaters. Moreover, this process has the following advantages over the conventional nitrification-denitrification process: saves energy during aeration, reduces carbon requirements, and decreases biomass produced.

However, the anammox process is difficult to manage and requires constant maintenance. Also, there are many significant complications during both inoculation and operation. For example, anammox cultures have low biomass yields and require very low dissolved oxygen concentrations. Furthermore, anammox bacteria are strictly autotrophic, using carbon dioxide (CO_2) as a carbon source with ammonium (NH_4^+) as the electron donor, so doubling times of anammox bacteria are quite long (>10 days) [1]. These significant obstacles have limited the research and application of the anammox process in the field. Most researchers assume that attached-growth or biofilm reactors would provide operational advantages over suspended-growth reactors because the attached-growth media increase retention of suspended solids which would better handle the high nitrogen loading.

There is increasing concern regarding the release of nitrous oxide (N_2O), a greenhouse gas with potential impacts that could be about 300 times that of carbon dioxide [2]. The denitrification process may produce N_2O at low C/N ratios in wastewater [3]. Moreover, N_2O can be produced at low concentrations of oxygen that may exist in a wastewater aeration tank [4]. Wunderlin, et al. 2012 [5] reported that ammonium oxidizing bacteria (AOB) may be the main microorganisms responsible for N_2O emissions in nitrifier denitrification under aerobic conditions when NO_2^- accumulates. However, information on N_2O production in the anammox process is not available.

For this reason, the results from this work are expected to provide useful information for the application of the attached-growth anammox process in wastewater treatment and commercial production of anammox biomass. In addition, this work studied N_2O production in the anammox process in both suspended-growth and attached-growth reactors at various pH (6.8, 7.3, 7.8, and 8.3) and with several NH_4^+:NO_2 ratios (0.5:1, 0.75:1, 1:1, and 1:1.32). Some of the results from this work could guide the application of the anammox process for the treatment of wastewater in the field.

Materials and Methods

Enriched stock anammox cultures of suspended- and attached-growths reactors

Two enriched stock anammox cultures, one suspended-and one attached-growth, were maintained in sequencing batch reactors (SBRs). Polystyrene sponge material was used as the attachment medium in the attached-growth reactor. Both of these SBRs (attached-growth SBR-A and suspended SBR-S) were cylindrical vessels that used 3.0 L working volumes of a synthetic wastewater. A decant:recycle ratio of 1:1 was maintained throughout. After withdrawal of 1.5 L supernatant liquid, an equal volume of synthetic wastewater was introduced. The pH of the influent and the effluent from both reactors was 7.8±0.4. The concentration ratio of NH_4^+:NO_2^-

was maintained at 1:1.32. The tops of the reactors were closed, but a pipeline was used to collect gas. A manually controlled SBR cycle consisted of four periods: fill (5 minutes), reaction time with mixing with a magnetic stir-bar at 120 rpm on a magnetic stirrer (23 hours), settle (1 hour), and decant (5 minutes). An inert gas mixture (95% Ar, 5% CO_2) was diffused through an air stone into the bottom of the SBRs for 5 minutes after filling in order to limit dissolved oxygen (DO). The composition of synthetic wastewater, which was used to feed enriched anammox cultures, consists of major substrates, minor nutrients and trace elements. The composition of the synthetic wastewater fed to the enriched stock anammox culture is shown in Table 1.

Nitrogen loading effects

To investigate the effects of nitrogen loading on the rate of N removal, the concentration of nitrogen in the synthetic wastewater recharge was gradually increased. Using the same SBR cycling described above, the concentration of NH_4^+:NO_2^- in wastewater refills was raised from 210:273 mg N/L (15:19.7 mM) to 490:637 mg N/L (35:45.5 mM) every seven days until the maximum total nitrogen concentration was reached. From the beginning of the experiment (day 0) to day 162, the total nitrogen concentration and all minor nutrients including trace elements were increased until the NH_4^+:NO_2^- ratio was 35:46.2 mM. At days 184, 191, 198, and 205, the individual major substrates (NH_4^+ and NO_2^-) were increased but all minor nutrients and trace elements were fixed for that used with the NH_4^+:NO_2^- ratio (15:19.5 mM). Immediately after refilling the SBR, a sample was taken to confirm starting nitrogen content. The rate of nitrogen removal was determined through measuring nitrogen concentrations for seven hours. The concentrations of NH_4^+ and NO_2^- from both influent and effluent were determined using the method described in APHA 2005 Standard Methods [8].

Table 1 Composition of Synthetic Wastewater

Constituent	Concentration	Unit
$NaNO_2$	273	mg N/L
$(NH_4)_2SO_4$	210	mg N/L
$KHCO_3$	1,250	mg/L
KH_2PO_4	18.75	mg P/L
$Na_2EDTA.2H_2O$	26.25	mg/L
$FeSO_4.7H_2O$	7.5	mg/L
$MgSO_4.7H_2O$	150	mg/L
$CaCl_2.2H_2O$	225	mg/L
Trace elements No.1	1.05	ml/L

Modified from van Dongen et al. [6] and Isaka et al. [7]

Trace element No.1 :0.06 mg/L $Na_2O_3Se.5H_2O$; 0.165 mg/L $MoNa_2O_4.2H_2O$; 0.187 mg/L $CuSO_4.5H_2O$; 0.322 mg/L $ZnSO_4.7H_2O$; 0.742 mg/L $MnCl_2.4H_2O$; 0.18 mg/L $CoCl_2.6H_2O$; and 0.142 mg/L $NiCl_2.6H_2O$

Specific anammox activity (SAA)

NH_4^+ and NO_2^- concentrations were determined every hour from 0 to 7 hours in SBR-A or SBR-S after filling with new synthetic wastewater. Total nitrogen concentration (NH_4^+ plus NO_2^- concentrations) was plotted versus time (hour). The rate of total nitrogen removal from each experiment was calculated based on the slope of a fit line for the whole data set and quantified for the mixed liquor volatile suspended solids (MLVSS) of anammox culture of each run. This calculated rate (mg N/mg VSS-day) is called specific anammox activity (SAA).

Nitrous oxide analysis

Nitrous oxide (N_2O) gas samples from each SBR were collected in 1 L Tedlar bags (SKC, PA, USA) with a single polypropylene fitting. The bags contained a Teflon syringe port lines septum and a hose connection that functioned as a shut-off valve for incoming and outgoing gas. SKC air sampling pump (No. 224-PCXR8, PA, USA) was used to pump samples into Tedlar bag. Low flow 100 mL/min was applied and connected with midget impinger standard nozzle (SKC, PA, USA) in order to limit moisture from reactor. Gas samples (500 µL) were analyzed for nitrous oxide using gas chromatography with 5975C mass spectrometer (Agilent Technologies, 7890A). The GCMS used an electron capture detector (ECD) and the column was a 0.320 mm capillary with 30 cm length 30 cm (GC-Carbonplot). The detector, column, and injection temperatures were 230, 110, and 35°C, respectively. The run time was 5 minutes.

Nitrous oxide production

The production of nitrous oxide (N_2O) was quantified under various NH_4^+:NO_2^- ratios and different starting pH with batch experiments using the enriched anammox stock cultures. N_2O production from both SBR-S and SBR-A was observed with NH_4^+:NO_2^- ratios of 0.5:1, 0.75:1, 1;1, and 1:1.3. In a separate set of experiments the starting pH was adjusted to 6.8, 7.3, 7.8, and 8.3 with additions of HCl or NaOH solutions before conducting the standard batch experiment. For both sets of experiments, the reactors were operated for one week after inoculation before N_2O production was measured.

Results and Discussion

Nitrogen loading effects

The specific anammox activity (SAA) declined in the long run as shown in Figure 1. From the beginning of experiment to day 162, it was shown that the SAA decline was due to the increase of total nitrogen concentration up to 1,127 mg N/L (490 mg N/L NH_4^+ and 637 mg N/L NO_2^- or NH_4^+:NO_2^- ratio, 35:45.5 mM). During this phase of experimentation all minor nutrients and trace elements were increased in proportion to the increase in TN. Figure 1 shows that the SAA of the attached-growth (SBR-A) was higher than the SAA of the suspended-growth (SBR-S). After day 162, the NH_4^+ :NO_2^- ratio was decreased back to the starting 15:19.5 and the SBRs were maintained at this ratio for 10 days. From this new starting point only the major substrates were increased to the final NH_4^+:NO_2^- ratio (35:46.2 mM). All minor nutrients and trace elements were fixed at the amount used for the starting TN amounts. The experimental results from days 184, 191, 198, and 205 day, as shown in Figure 1, demonstrated that SAA is dependent on TN and on ratio of major:minor substrates. It is concluded that the attached-growth system is more tolerant of the high nitrogen loading than is the suspended-growth system. This result is similar to that reported by Sobotka et al. [9] who studied enriched anammox cultures under granular sludge in an SBR over a long-term (>330 days), at high temperature (30°C) and with high NH_4^+ and NO_2^- concentrations (200-1,150 mg N/L) with fixed minor nutrients and trace elements.

Also, the maximum SAA observed in this current research, 1.35 mg V/mg VSS-day is quite similar to seen by Sobotka et al [9], at 1.5 mg N/mg VSS-day SAA maximum. This result suggests that attached-growth systems could be superior to suspended growth systems in the field.

Nitrous oxide production

Nitrous oxide (NO_2^-) productions at different pH from SBR-S and SBR-A at NH_4^+:NO_2^- ratio of 1:1.3 are shown in Figure 2.

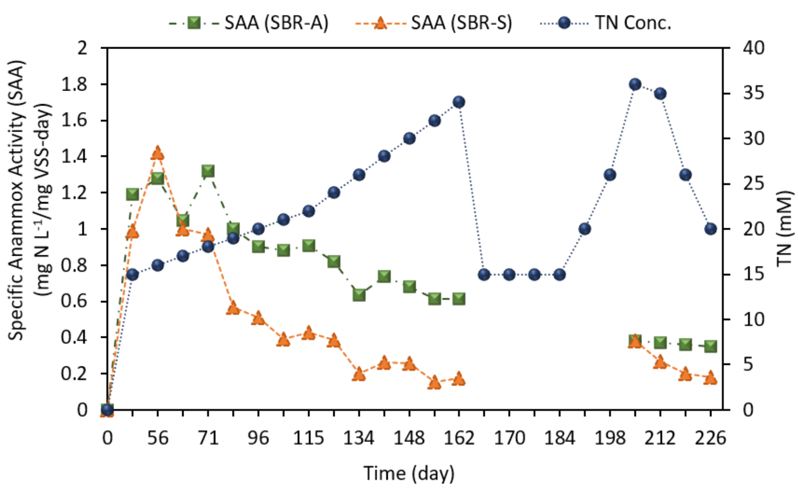

Figure 1 Specific anammox activity (SAA) from SBR-S and SBR-A and total nitrogen (TN) concentration in synthetic wastewater

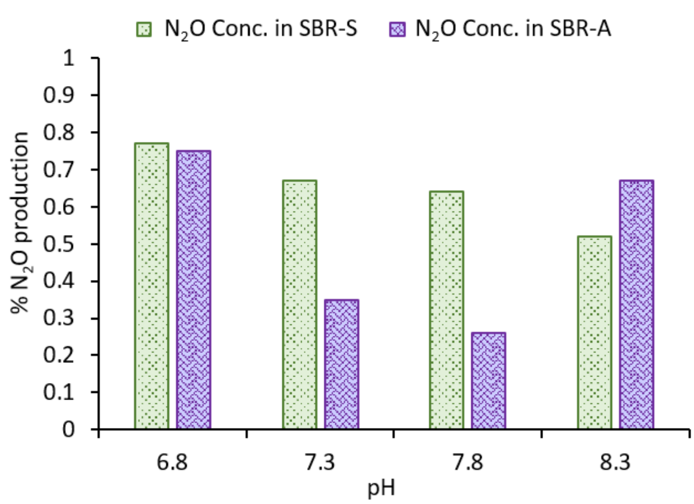

Figure 2 Nitrous oxide (N_2O) production at different pH in SBR-S and A at NH_4^+:NO_2^- ratio of 1:1.3 after 7 hours

Egli et al. [10] found that an anammox culture was active in a pH range from 6.5-9 with an optimum around 8. With a NH_4^+:NO_2^- ratio of 1:1.3, the N_2O production from both SBR-S and SBR-A was considerably high at pH 6.8 comparison with pH 7.3, 7.8, and 8.3.

Nitrous oxide concentrations gases collected at different NH_4^+:NO_2^- ratios from SBR-S and SBR-A at pH 7.8 are shown in Figure 3. At NH_4^+:NO_2^- ratios of 1:1.3 and 1:1, N_2O production in SBR-S was slightly higher than in SBR-A. Several reasons could be postulated for this behavior. First, the origin of the anammox culture in this work was from activated sludge from the anoxic tank of Nongkheam WWTP in Bangkok, Thailand, with potentially many nitrifying and denitrifying bacteria still alive and active. Some denitrifying cultures are able to oxidize nitrite to nitrous oxide under anoxic conditions and at very low C/N ratios [11-12]. Nitrifying bacteria are also capable of oxidizing nitrite to nitrous oxide under anoxic or very low dissolved oxygen conditions [13-14]. Although the anammox culture in SBR-A came from the same source as SBR-S, it generated slightly less nitrous oxide. Park et al. [15] suggested that biofilm or attached-growth systems offer high biomass retention and coexistence of metabolic activities in the same environment. Biomass in SBR-A could accumulate inside media (polystyrene sponge) and may have had more diverse metabolic activities.

With NH_4^+:NO_2^- ratio of 0.5:1 at pH 7.8, the N_2O production was meaningfully greater than with ratios of 0.75:1, 1:1, and 1:1.3 in both reactors. Nitrous oxide production was significant at a NH_4^+:NO_2^- ratio of 0.5:1 in both SBR-S and SBR-A. It could be postulated that the enriched anammox culture in SBR-A included more nitrifying bacteria than in SBR-S. Strous et al. [16] reported that N_2O production from lab-scale anammox cultures was around 0.03-0.06%.

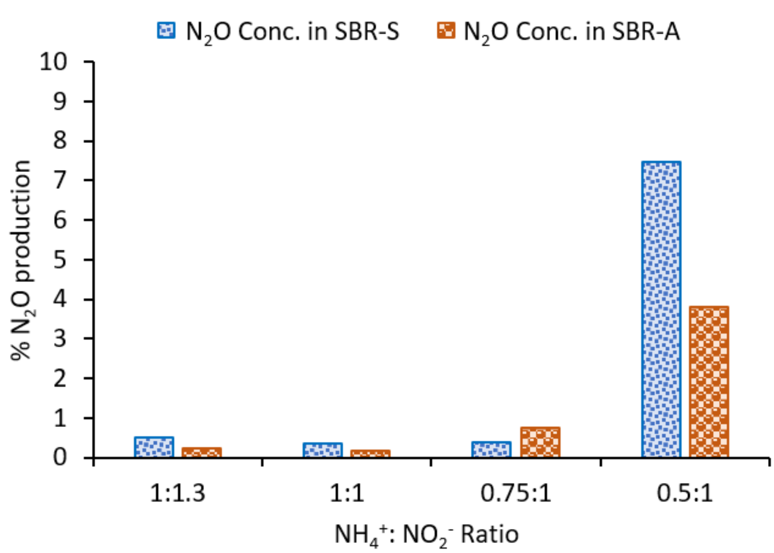

Figure 3 Nitrous oxide (N_2O) production at different NH_4^+:NO_2^- ratios in SBR-S and SBR-A at pH 7.8 after 7 hours

These results confirm the result of Wunderlin et al. [5], who found low N_2O emissions without significant nitrite accumulation under anoxic conditions. Therefore, to produce less N_2O during biological nitrogen removal, NH_4^+ and NO_2^- concentrations should be kept low by maintaining high solids retention time (SRT) or extended denitrification, and controlling sludge recycling depending on loads of substrates, organic matter and nitrogen in anoxic conditions.

At full scale, nitrous oxide production would be expected to increase. However, the amount of N_2O production was still less than 0.01% [17]. An SBR-A system could hold higher NH_4^+ and NO_2^- concentrations than the SBR-S. Furthermore, SBR-A produced lower quantities of N_2O than SBR-S. For this reason, SBR-A may provide significant operational benefits and would be recommended for implementation in the field.

Conclusions

The results from this work suggest that attached-growth systems show more promise for use in the field than suspended-growth systems. This is because they can better handle the higher NH_4^+ and NO_2^- concentrations than suspended-growth systems. Although high concentrations of total nitrogen (NH_4^+ plus NO_2^-) were added, all minor nutrients and trace elements were fixed. Attached-growth systems would produce less N_2O. With NH_4^+:NO_2^- ratio at 1:1.3 the N_2O production was significantly greater in both reactors at pH 6.8 than at 7.3, 7.8, and 8.3. These results demonstrate that the anammox process is likely to produce low amounts of N_2O gas. Nitrous oxide production from both suspended-and attached-growth reactors of anammox process was significantly higher at an NH_4^+:NO_2^- ratio of 0.5:1 than with ratios of 0.75:1, 1:1, and 1:1.3 (all at pH 7.8).

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