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Screening of Anaerobic Ammonium Oxidation (Anammox) Potentials in Biomass from a Variety of Wastewater Treatment Processes

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Abstract

A variety of biomass samples were examined for their anaerobic or anoxic ammonium oxidation (Anammox) potentials. The biomass samples were collected from various types of wastewater treatment processes including a conventional activated sludge process, nitrification/denitrification processes (one sludge systems), a biological P removal process, denitrification processes and an anaerobic digestion process. Two denitrifying biomass samples exhibited significant removals of both NH_4-N and NO_x-N (NO_2-N or NO_3-N) under an anoxic condition without organic carbon when both N constituents co-existed, suggesting the occurrence of an Anammox phenomenon. The one was biomass obtained from an autotrophic denitrification process using sulfide as the electron donor, and the other from a denitrifying process using the BOD which is absorbed onto a primary sludge. An Anammox potential seems to be able to be found in the biomass which is continuously exposed to anoxic and no or low organic carbon conditions, though it cannot be ubiquitously detected in normal wastewater treatment processes.

Key words : Anammox (anoxic ammonium oxidation), screening tests, wastewater treatment biomass, denitrifying sludge

INTRODUCTION

Since widely-adopted wastewater treatment techniques, such as conventional activated sludge processes, cannot efficiently remove nitrogen (N), N in the effluent from existing wastewater treatment facilities have caused serious eutrophication problems in closed water bodies. At present, combinations of nitrification and denitrification have been generally utilized for N removal.

In these processes, after organic N (Org-N) is converted into ammonium N (NH_4-N), it is further converted into nitrite N (NO_2-N) and, subsequently, into nitrate N (NO_3-N) (Table 1, eqs. 1 and 2). After then, NO_2-N and NO_3-N (NO_x-N) are reductively converted to gaseous N, mainly dinitrogen (N_2), under anoxic conditions, leading to removal of N from aqueous phase (eqs. 3 and 4).

Biological N removal processes through nitrification/denitrification are relatively cost-effective compared with physico-chemical processes like ammonia stripping and ion-exchange. However, the cost problems still exist in that nitrification requires very efficient oxygen supply causing high energy consumption, and in that denitrification generally needs organic carbon as an electron donor. If the target wastewater contains less amount of organic carbon compared with N, and/or if organic carbon are consumed

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Table 1 Biological N transformation processes

Nitrification
$NH_4^+ + 3\ 2O_2 \longrightarrow NO_2^- + H_2O + 2H^+$ ($\Delta G = -65$ kcal mol) (eq. 1)
$NO_2^- + 1\ 2O_2 \longrightarrow NO_3^-$ ($\Delta G = -18$ kcal mol) (eq. 2)

Denitrification
$2NO_3^- + 2\ (H_2) \longrightarrow 2NO_2^- + 2\ H_2O$ (eq. 3)
$2NO_2^- + 3\ (H_2) \longrightarrow N_2 + 2H_2O + 2OH^-$ (eq. 4)
*(H_2) electron donors can be given by organic compounds, sulfur, hydrogen etc.

Anammox
$NH_4^+ + NO_2^- \longrightarrow N_2 + 2H_2O$ ($\Delta G = -358$ kcal mol) (eq. 5)
$5\ NH_4^+ + 3\ NO_3^- \longrightarrow 4N_2 + 9H_2O + 2H^+$ ($\Delta G = -297$ kcal mol) (eq. 6)

during nitrification process, we have to add expensive, external carbon sources like methanol. When autotrophic denitrifiers are utilized, sulfur, sulfide or hydrogen can replace the organic carbon, while it should be externally added or be contained in the wastewater. If denitrification step precedes nitrification step, nitrified wastewater should be recirculated to denitrification step, leading to a requirement for additional pumping systems and energy consumption.

In early 1990's a novel biological N removal process, "Anammox" (ANaerobic or ANoxic AMMonium OXidation), was reported by the research group of Technical University Delft (TU-Delft), Netherlands, which seems ideal for very cost-effective N removal[1, 2]. In short, the Anammox refers to NH_4^+ oxidation into N_2 using NO_2^- as oxidant, or denitrification of NO_2^- using NH_4^+ as the electron donor under anoxic conditions (eq. 5). Because it requires neither oxygen supply for NH_4^+ oxidation and nor organic carbon for denitrification of NO_2^-, energy cost for oxygen supply may be reduced and the external organic carbon addition for denitrification can be omitted. The potential existence of autotrophic bacteria capable of oxidizing NH_4^+ into N_2 with NO_3^- (eq. 6), which is thermodynamically possible, was predicted by Broda[3], however, such biological reactions had not been demonstrated for a long time.
Although there has been a report demonstrating that well-known nitrifiers *Nitrosomonas* sp. could also catalyze an Anammox-like reaction, denitrification of NO_2^- into NO and N_2 using NH_4^+ as an electron acceptor, when grown under oxygen limitation[4], the reaction efficiency was extremely low and the reaction seems non-specific.

The TU-Delft group published a series of papers on the microbial aspects of the Anammox originally discovered in a denitrifying fluidized bed reactor (FBR)[5-9], and tentatively identify the Anammox microbe as a special species of Planctomycetes[10]. They also proposed a new concept for N removal system using the Anammox reaction, and experimentally investigated its feasibility in labo-scale reactors[11, 12]. Based on the results, they concluded that the Anammox can be a more cost-effective N removal process than conventional nitrification/denitrification systems. However, real or full-scale application of the Anammox process has never been attempted until now, and it is still a tough challenge[12].

The greatest reason for the delay in the Anammox practice might be that it is very difficult to find or obtain Anammox microbes. Anammox phenomena had never been reported outside the TU-Delft for a long time, before a few other groups made successes to discover the Anammox biomass very recently[13, 14]. In this article, a screening of Anammox potentials was tried from a variety of biomass sampled from different wastewater treatment facilities so as to answer a greatest riddle of Anammox, in what kind of environment and how abundantly Anammox potentials or microbes exist.

MATERIALS AND METHODS

Biomass samples Biomass samples examined for Anammox potentials were

listed in Table 2. TU-AS (1) and (2) were collected from a pilot-scale plant operated as a conventional activated sludge process treating a domestic wastewater. Se-ND and BB-ND were the activated sludge samples obtained from practical plants adopting nitrification/denitrification processes with circulation of nitrified wastewater. Ka-BioP sludge was sampled form an anaerobic/aerobic sequencing batch reactor treating a food-processing wastewater containing a high concentration of phosphate (biological P removal). TU-MeD, Ar-EcoD and Sc-AutD were the biomass specialized to denitrification. TU-MeD was from a pilot-scale denitrifying sludge blanket reactor which treat a NO_3-N-supplemented domestic wastewater with continuous addition of methanol. Ar-EcoD was a denitrifying biomass which used the primary sludge (BOD absorbed onto the sludge) as the source of electron donor. Sc-AutD was collected from a wastewater treatment plant treating a wastewater from a petrochemical industry in which a sulfide-dependent autotrophic denitrification was performed. Judging from the smell and appearance, Sc-AutD seemed to contain a considerable amount of sulfide salts. BB-SD was collected from an anaerobic digestion process treating an excess activated sludge produced in a domestic wastewater treatment plant. The biomass samples were condensed by gravitational settling or centrifugation to give suspended solid (SS) concentrations around 20,000 mg/*l*, stored at 4 ℃, and utilized for the screening test within 2 days after sampling.

Media Composition of the basal medium (BM) used in this study is shown in Table 3(A). For preparing a series of media used in the tests for screening Anammox potentials, NH_4-N (as NH_4Cl_2), NO_2-N (as $NaNO_2$) and/or NO_3-N (as $NaNO_3$) was/were supplemented to BM to give each final concentration of ca. 100 mg/*l*. The pH of all the media were adjusted to 8.0.

Screening tests design and evaluation For evaluating Anammox potentials in biomass samples, a series (6 kinds) of 22-m*l* serum bottles each containing 10 m*l* of the test media were prepared (Table 3). The blank system contained BM without any N-supplements, while NH_4, NO_2, and NO_3 systems contained BM with NH_4-N, NO_2-N, and NO_3-N supplements, respectively. The NH_4/NO_2 system contained BM supplemented with NH_4-N and NO_2-N, while the NH_4/NO_3 system with NH_4-N and NO_3-N. All the systems were inoculated with 0.1 % (0.1 m*l*) of biomass sample. The media inoculated with biomass were flushed with N_2 gas for 15 min to remove dissolved oxygen (DO), and the bottles were sealed with Teflon-coated rubber septums and aluminum crimps. To completely remove the remaining oxygen in the system, the head space of the bottles was replaced by N_2 gas, and 3 drops (ca. 0.05 m*l*) of 10 g/*l* Na_2SO_3 solution was added to each bottle. The series of bottles were prepared in triplicate for each biomass sample, and

Table 2 Biomass samples used in this study

sample	specialized to	wastewater	note (sampling date)
TU-AS(1)	conventional activated sludge process (pilot)	domestic	on 14, July, 1998
TU-AS(2)	conventional activated sludge process (pilot)	domestic	on 6, October, 1998
Se-ND	nitrification/denitrification (one sludge system)	domestic	on 4, August, 1998
BB-ND	nitrification/denitrification (one sludge system)	domestic	on 18, September, 1998
Ka-BioP	biological P removal (A/O-SBR*1)	industrial (food processing)	on 23, September, 1998
TU-MeD	denitrification on methanol (A-SBR*2) (pilot)	synthetic NO_3^-	on 16, November, 1998
Ar-EcoD	denitrification on primary sludge	domestic	on 19, October, 1998
Sc-AutD	autotrophic denitrification on sulfide	industrial (oil refinery)	on 20, August, 1998
BB-SD	anaerobic sludge digestion	domestic	on 18, September, 1998

*1, anoxic/oxic sequencing batch reactor
*2, anoxic sludge blanket reactor

Table 3 Design of the screening test

(A) Composition of Basal Medium (BM)

$NaHCO_3$	2.35 g
$Na_2H_2PO_4 \cdot 12H_2O$	57.7 mg
Trace element solution	1 m*l*
Tap water	1000 m*l*

(B) Composition of trece element solution

$ZnSO_4 \cdot 7H_2O$	1 g
$MnCl_2 \cdot 4H_2O$	0.3 g
$Co(NO_3)_2 \cdot 6H_2O$	2 g
$CuCl_2 \cdot 2H_2O$	0.1 g
$NiSO_4 \cdot 6H_2O$	0.2 g
$NaMoO_4 \cdot 2H_2O$	0.5 g
H_3BO_3	3 g
Distilled water	1000 m*l*

(C) Designed N concentrations

system	biomass*1	medium (approx. N concentration : mg/*l*)			purpose (evaluation of N loss)
		NH_4-N	NO_2-N	NO_3-N	
Control system	−	100	100	100	abiotic changes of N
Blank system	+	0	0	0	N elimination from biomass
NH_4 system	+	100	0	0	NH_4^+ assimilation
NO_2 system	+	0	100	0	NO_2^- assimilation + denitrification
NO_3 system	+	0	0	100	NO_3^- assimilation + denitrification (*via* NO_2^-)
NH_4/NO_2 system	+	100	100	0	Anammox on NO_2^-*2
NH_4/NO_3 system	+	100	0	100	Anammox on NO_3^-(*via* NO_2^-)*2

*1, inoculation of biomass (SS concentration = ca. 200 mg/*l*)
*2, in addition to N assimilation and denitrification of NO_x

statically incubated at 20-22 ℃. One series (6 kinds) of the bottles for each biomass sample were opened after 2-4 hours and N concentrations were assayed, while the remaining two series after 4 weeks as duplicate samples. The control system, which is a serum bottle containing BM added with NH_4-N, NO_2-N and NO_3-N, was also prepared, and incubated without biomass for evaluating abiotic changes of N under the experimental condition. DOC (dissolved organic carbon) concentration in all the test systems were lower than 5 mg/*l*.

Theoretically, increases of N concentration in the blank system refers to the N elimination due to the decomposition of the biomass. Total N (NH_4-N plus NO_x-N) decrease in the NH_4 system should attribute to NH_4^+ assimilation. Since nitrification is impossible under anoxic conditions, total N loss due to denitrification via NO_x-N cannot occur in this system. Total N decrease in the NO_2 and NO_3 systems can occur through denitrification, because the biomass inoculation could introduce a small amount of DOC (and sulfide for the Sc-AutD) which can serve as an electron donor. The electron donor could be also given by the biomass autolysis. The assimilation of NO_x-N can be another reason of a N loss in these systems. On the other hand, loss of total N in the NH_4/NO_2 system can be caused by Anammox using NO_2^- in addition to assimilation of N (NH_4^+ and NO_2^-) and denitrification. In the NH_4/NO_3 system, total N loss can be due to NO_3^-- and/or NO_2^--dependent Anammox as well as to N assimilation and denitrification. Thus, if an Anammox potential exists in a biomass, the following results should come out :

- (NH_4-N loss in the NH_4/NO_x system) > (NH_4-N loss in the NH_4 system)
- (NO_x-N loss in the NH_4/NO_x system) > (NO_x-N loss in the NO_x system)
- (Total N loss in the NH_4/NO_x system) > (Total N loss in the NH_4 system) + (Total N loss in the NO_x system)

Here, the N losses refer to the differences of N concentrations after 2-4-hour and 2-week incubation in the screening tests, and the differences between the values of left and right sides of the above inequalities refer to the N losses which were presumably caused by Anammox.

Analytical procedures NH_4-N, NO_2-N and NO_3-N concentrations were colorimetrically measured using Dr. Lange Küvetten-Test kits: LCK304 and 305 for NH_4-N, LCK341 and 342 for NO_2-N and LCK339 and 340 for NO_3-N (Dr. Bruno Lange GmbH Berlin, Düsseldorf, Germany) according to the instructions. The principles of the analyses by the kits are similar to DIN 38405-D9 for NH_4-N, DIN 38405-D10 for NO_2-N and DIN 38406 for NO_3-N (DIN-Normen; Deutschen Institut für Normung). DOC was assayed using a TOC analyzer system, TOCOR2 with an autosampler MPA2 and a gas analyzer UNOR6N (Maihak AG, Hamburg, Germany). SS was determined after drying the centrifuged sample (pelleted fraction) at 105 ℃ for about 1 hour.

RESULTS

Any Control systems in the screening tests did not show considerable changes in N concentrations during the 4-week experimental period (data not shown), suggesting there had been no abiotic effect on NH_4-N, NO_2-N and NO_3-N concentrations in the test systems. Thus, small changes of the N concentrations observed in the Control systems refer to experimental errors including the medium preparation and chemical analyses. The maximal changes observed on the NH_4-N, NO_2-N and NO_3-N concentrations in the Control systems were 2.38 mg/*l* (decrease from 95.81 mg/*l*), 1.11 mg/*l* (increase from 99.20 mg/*l*) and 1.87 mg/*l* (increase from 92.65 mg/*l*), respectively. As for Total N, 3.14 mg/*l* decrease from 294.51 mg/*l* in a Control system was observed as the maximal change. These indicate that experimental errors for each test system could be 2.5 % at maximum. For evaluating the experimental results, when the difference between 2 values of N concentration was more than 2.5 % of the assay, it was defined as a significant or substantial difference. All the Blank systems also showed no or very little increase/decrease of N concentrations, indicating negligible effect of biomass decomposition on the results of the screening tests. The blank systems initially contained less than 1.5 mg/*l* total N, and its change after 4 weeks was less than 1.0 mg/*l* for all biomass samples.

Table 4 shows the changes of NH_4-N concentration in the test systems during 4 weeks. As for the NH_4 systems, the TU-AS(1), Se-ND, Ka-BioP and Sc-AutD samples showed 3.51-5.04 mg/*l* losses of NH_4-N, while no significant loss (change) of NH_4-N was caused by the other biomass samples. In the NH_4 systems for the TU-AS(1) and Se-ND samples, increases in the NO_x-N concentration were observed corresponding to the decreases of NH_4-N (NO_x-N data not

Table 4 NH_4-N decreases in the screening tests

sample	NH_4-N decreases in the screening test system			presumable Annamox	
	NH_4	NH_4/NO_2	NH_4/NO_3	NH_4/NO_2*1	NH_4/NO_3*2
TU-AS(1)	4.62	3.50	4.40	−1.12	−0.22
TU-AS(2)	0.18	−0.14	0.33	−0.32	0.15
Se-ND	3.96	3.91	4.54	−0.05	0.58
BB-ND	−0.79	−0.72	−1.16	0.07	−0.37
Ka-BioP	5.04	4.30	6.09	−0.74	1.05
TU-MeD	0.95	1.63	1.11	0.68	0.16
Ar-EcoD	−2.52	3.60	1.44	6.12	3.96
Sc-AutD	3.51	7.11	6.46	3.60	2.95
BB-SD	0.08	0.76	−1.53	0.68	−1.61

Values are indicated as the decreased NH_4-N mg/*l* in the test systems (negative values indicate the increases of NH_4-N), and the averages of duplicate experiments.

*1, (NH_4-N loss in the NH_4 + NO_2 system) − (NH_4-N loss in the NH_4 system) ; the NH_4-N loss presumably depends on the Anammox using NO_2^-

*2, (NH_4-N loss in the NH_4 + NO_3 system) − (NH_4-N loss in the NH_4 system) ; the NH_4-N loss presumably depends on the Anammox using NO_3^- (*via* NO_2^-)

shown), suggesting the occurrence of nitrification probably due to insufficient oxygen removal from the medium. On the other hand, the other samples showed no increase of NO_x-N in the system, therefore, oxygen seems to be completely removed in most cases. The NH_4-N losses in the NH_4/NO_2 and NH_4/NO_3 systems were almost same as those observed in the NH_4 systems for most biomass samples. However, Ar-EcoD showed a substantially higher NH_4-N loss in the NH_4/NO_2 system than in the NH_4 system. It showed a slightly higher NH_4-N removal in the NH_4/NO_3 system than in the NH_4 system as well. Sc-AutD also showed slightly higher NH_4-N decreases in the NH_4/NO_2 and NH_4/NO_3 systems than in the NH_4 system. These extensive NH_4-N removal depending on the co-presence of NO_2^- and/or NO_3^- suggested the occurrence of Anammox by Ar-EcoD and Sc-AutD.

Tables 5 and 6 show the comparison of NO_x-N losses in the NO_2 and NH_4/NO_2 systems, and that in the NO_3 and NH_4/NO_3 systems, respectively. Significant NO_2-N (NO_x-N) decreases occurred in the NO_2 systems, when the medium was inoculated with Ka-BioP, TU-MeD, Ar-Eco-D or Sc-AutD. In the NO_3 systems, those 4 biomass samples also showed substantial NO_3-N decreases, suggesting that denitrification occurred in those systems using organic carbon or sulfide which was introduced by the biomass inoculation. Although Ar-EcoD and Sc-AutD accumulated about 12 and 3 mg/*l* of NO_2-N in the NO_3 systems, respectively, the others showed no or little NO_2^- accumulation after 4-week incubation. Ar-EcoD showed significantly higher removals of NO_x-N in the NH_4/NO_2 system than in the NO_2 system, and in the NH_4/NO_3 system than in the NO_3 system. Sc-AutD also lowered NO_x-N more extensively in the NH_4/NO_2 system than in the NO_2 system. These extensive losses of NO_x-N can be elucidated by assuming the denitrification using NH_4^+ (Anammox). On the other hand, the Ka-BioP showed a much lower removal of NO_x-N in the NH_4/NO_2 system than in the NO_2 system, though the reason was not clear.

Table 7 summarizes Total N losses in the screening test systems. The Total N losses which presumably depended on Anammox were calculated and shown in the table. Significant losses of Total N in the NH_4/NO_2 and/or in the NH_4/NO_3 systems were observed for Ar-EcoD and Sc-AutD samples. Considering the results on the NH_4-N and NO_x-N losses, these 2 biomass samples seemed to possess Anammox potentials.

DISCUSSION

Possible microbial processes for N removal from water phases are (i) uptake or assimilation of NH_4^+ and NO_x by biomass, (ii) denitrification of NO_x and (iii) Anammox and similar anoxic NH_4^+ removal catalyzed by autotrophic nitrifiers[15]. Based on this consideration, the screening test system was designed to be able to find an occurrence of Anammox in the NH_4/NO_2 and/or NH_4/NO_3 systems.

From the experimental results, Ar-EcoD and Sc-AutD were considered to possess NO_2^--dependent Anammox potentials. Although Ar-EcoD showed extensive N decreases also in the NH_4/NO_3 system compared with in the NH_4 and NO_3 systems, the presumable Anammox seemed to depend on NO_2^-not on NO_3^-. Because a considerable amount of NO_2^- was accumulated in the NH_4/NO_3 system, and the N losses in the NH_4/NO_3 system were less than those in the NH_4/NO_2 system. All the previously-reprted Anammox reactions were considered to depend on NO_2^-, and NO_3^- could not be utilized as the electron acceptor[5, 13, 14]. The extensive N losses (in the NH_4/NO_2 system) which were presumably caused by the Anammox potential in Ar-EcoD during 4 weeks were estimated at about 6 mg/*l* for NH_4-N, 5 mg/*l* for NO_x-N and 13 mg/*l* for Total N, respectively. Those by Sc-AutD were estimated at 4 mg/*l*, 5 mg/*l* and 12 mg/*l*, respectively. If we assume that the Anammox biomass reported by TU-Delft was applied to the screening test performed in this study, the Anammox-dependent Total N loss in the NH_4/NO_2 system after 4 weeks could be estimated by simple calculation at between 31 mg/*l* (the biomass from the original FBR[2]) and 130 mg/*l* (a highly enriched biomass[8]). On the other hand, Anammox-like reaction by the autotrophic

Table 5 NO_x-N decreases in the screening tests (1) NO_2 and NH_4/NO_2 systems

sample	NO_x-N (NO_2-N) decreases in the screening test system				presumable Anammox*	
	NO_2		NH_4/NO_2			
TU-AS(1)	0.82	(0.83)	−2.91	(−3.00)	−3.73	(−3.83)
TU-AS(2)	1.20	(1.18)	2.94	(2.95)	1.74	(1.17)
Se-ND	0.68	(1.33)	−2.07	(−1.59)	−2.75	(−2.92)
BB-ND	0.91	(0.91)	0.36	(0.30)	−0.55	(−0.61)
Ka-BioP	13.54	(12.82)	5.35	(4.98)	−8.19	(−7.84)
TU-MeD	7.34	(7.31)	6.75	(6.65)	−0.59	(−0.66)
Ar-EcoD	6.78	(6.59)	11.84	(11.40)	5.05	(4.81)
Sc-AutD	30.64	(30.56)	35.95	(35.75)	5.31	(5.19)
BB-SD	−0.42	(−0.42)	−3.64	(−3.55)	−3.22	(−3.15)

Values are indicated as the decreased NO_x-N mg/*l* in the test systems (negative values indicate the increases of NO_x-N), and the averages of duplicate experiments. Values in parentheses are the decreases in the NO_2-N concentrations.

*, (NO_x-N (or NO_2-N) loss in the NH_4/NO_2 system) − (NO_x-N (or NO_2-N) loss in the NO_2 system) ; the NO_x-N (or NO_2-N) loss presumably depends on the Anammox using NO_2^-

Table 6 NO_x-N decreases in the screening tests (2) NO_3 and NH_4/NO_3 systems

sample	NO_x-N (NO_3-N) decreases in the screening test system				presumable Anammox*	
	NO_3		NH_4/NO_3			
TU-AS(1)	1.07	(1.20)	−0.78	(1.20)	−1.85	(0.00)
TU-AS(2)	2.00	(2.05)	1.46	(1.41)	−0.54	(−0.64)
Se-ND	0.97	(1.01)	−1.35	(1.51)	−2.32	(0.50)
BB-ND	−0.63	(0.45)	−0.43	(0.37)	0.20	(−0.08)
Ka-BioP	6.14	(6.12)	7.23	(9.24)	1.09	(3.12)
TU-MeD	4.18	(5.38)	5.66	(8.70)	1.48	(3.32)
Ar-EcoD	2.92†	(14.83)†	7.32	(20.68)	4.40	(5.85)
Sc-AutD	16.68†	(19.56)†	13.69	(17.00)	−2.99	(−2.56)
BB-SD	1.90	(1.93)	1.70	(−0.89)	−2.79	(−2.82)

Values are indicated as the decreased NO_x-N mg/*l* in the test systems (negative values indicate the increases of NO_x-N), and the averages of duplicate experiments. Values in parentheses are the decreases in the NO_3-N concentrations. †, Ar-EcoD and Sc-AutD accumulated a considerable amount of NO_2-N, about 2 and 3 mg/*l*, respectively, in the NO_3 system.

*, (NO_x-N (or NO_3-N) loss in the NH_4/NO_3 system) − (NO_x-N (or NO_3-N) loss in the NO_3 system) ; the NO_x-N (or NO_3-N) loss presumably depends on the Anammox using NO_3^- (*via* NO_2^-)

Table 7 Total N decreases in the screening tests

sample	Total N decreases in the screening test system					presumable Anammox	
	NH_4	NO_2	NO_3	NH_4/NO_2	NH_4/NO_3	NH_4/NO_2*1	NH_4/NO_3*2
TU-AS(1)	0.40	0.82	1.06	0.59	3.62	−0.63	2.16
TU-AS(2)	0.22	0.78	1.69	2.80	1.79	1.80	−0.12
Se-ND	0.25	0.37	0.74	1.84	3.19	1.22	2.20
BB-ND	−0.66	0.70	−1.10	−0.36	−1.53	−0.40	0.23
Ka-BioP	5.61	10.75	6.15	11.65	13.32	−4.71	1.56
TU-MeD	0.98	5.66	3.02	8.38	6.77	1.74	2.77
Ar-EcoD	−2.07	4.46	0.86	15.44	8.76	13.05	9.97
Sc-AutD	3.69	27.3	13.01	43.06	20.15	12.07	3.45
BB-SD	0.07	−0.01	1.76	−2.98	−2.42	−2.96	−4.25

Values are indicated as the decreased Total N mg/*l* in the test systems (negative values indicate the increases of Total N), and the averages of duplicate experiments.

*1, (Total N loss in the NH_4/NO_2 system) − {(Total N loss in the NH_4 system) + (Total N loss in the NO_2 system)} ; the Total N loss presumably depends on the Anammox using NO_2^-

*2, (Total N loss in the NH_4/NO_3 system) − {(Total N loss in the NH_4 system) + (Total N loss in the NO_3 system)} ; the Total N loss presumably depends on the Anammox using NO_3^- (*via* NO_2^-)

nitrifiers[4] could account for only 1.3 mg/*l* of Total N loss under the experimental condition, therefore, cannot be detectable in the screening test system.

The common property of Ar-EcoD and Sc-AutD biomass was that they had been specialized to denitrification. Ar-EcoD used BOD which is absorbed onto a primary sludge as the electron donor for the denitrification, while Sc-AutD autotrophically denitrified NO_x using sulfide. Although Sc-AutD had been treating the wastewater containing a considerable amount of COD, the main components were considered to be biologically refractory substances based on the chemical analyses (data not shown), therefore, to unable to act as the electron donor for the denitrification. The TU-MeD was also a denitrifying biomass, but an easily-available organic carbon, methanol, was fed as an electron donor for the denitrification. Although Se-ND, BB-ND and Ka-BioP should have denitrifying activity also, they are different from Ar-EcoD and Sc-AutD in that they were obtained from one sludge N removal processes, therefore, they were exposed to alternate aerobic/anaerobic conditions. Thus, it appears that Anammox potentials can be possibly detected in the biomass which is continuously exposed to the environmental condition that is anoxic and poor in the biologically available organic carbons. This estimation accords to that the Anammox was originally discovered in an anaerobic denitrifying FBR with sulfide as the limiting electron donor[2]. Another Anammox sludge was acclimated using a denitrifying sludge which is continuously exposed to anoxic condition with organic carbon-limitation[14]. However, Siegrist *et al.*[13] have found Anammox-like reaction in a nitrifying rotating contactor which was operated under at least partially aerobic condition.

CONCLUSIONS

From the experimental results, it may be concluded that Anammox potentials can be found in the environment which is kept under anoxic and no or low organic carbon conditions, though seem not to be ubiquitously detected. Therefore, when an intensive screening of Anammox potentials is performed for various biomass samples obtained from such environments, there seems to be a certain possibility to obtain a good seed for developing Anammox enrichments. The screening system used in this study can be a useful tool to easily detect the Anammox potential from biomass samples.

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