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DEVELOPMENT OF A NOVEL NITROGEN REMOVAL PROCESS USING ANAMMOX AND PARTIAL NITRITATION AND ITS APPLICABILITY TO LANDFILL LEACHATE

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Abstract
In this paper, development and some characteristics of a novel nitrogen removal process named SNAP (Single-stage Nitrogen removal using Anammox and Partial Nitritation) are presented. The SNAP process was developed on reactors packed with acryl-resin fiber biomass carriers and seeded with nitrifying activated sludge during long-term partial nitritation. Continuous experiments on synthetic landfill leachate containing 240 mg NH_4-N/*l* showed stable ammonium conversion of 85~90% and N-removal of 75~80% at loading rate of 0.6 kg-N/m^3/d. Operation of another reactor fed with 500 mg NH_4-N/*l* also confirmed the treatment capability, with 80% N-removal at loading rates up to 1.0 kg-N/m^3/d. SNAP was a less sludge producing process with sludge yield of 0.045 mg-VSS/mg-N removed. MPN tests revealed the co-existence of aerobic ammonium oxidizers (AOB), anaerobic ammonium oxidizers (anammox bacteria) and nitrite oxidizers (NOB) in the SNAP sludge. Nitrifiers closely relative to *Nitrosomonas europaea, Nitrospira sp.* and anammox bacteria similar to KU2 and KSU-1 strains were identified in the SNAP sludge by 16S rDNA analyses. AOB and anammox bacteria are dominant under operational conditions of the SNAP process.

Key words: anammox, landfill leachate, nitrogen removal, SNAP

1. Introduction
Conventional nitrogen removal technology is based on the combination of nitrification and denitrification steps. The first step consumes large amounts of oxygen for oxidizing ammonium to nitrate while the second step requires addition of external organic carbon source for reducing nitrate to dinitrogen gas. These requirements make nitrification-denitrification systems expensive, especially for the treatment of high strength ammonium wastewaters, such as digester supernatant and landfill leachate. It is known that leachate from a mature landfill site or secondary-treated leachate contains relatively low degradable organic matter but high ammonium nitrogen (Reinhart and Caroline, 1997).

Since discovery of anammox reaction in mid-1990s, development of novel nitrogen removal processes based on the use of this reaction has gained much attention (Jetten *et al.*, 2001). In this approach, ammonium is converted ultimately to dinitrogen gas by two sequential reactions: partial nitritation (equation 1) and anammox (equation 2). The overall reaction of this process is given in equation 3.

$$2\,NH_4^+ + 1.5\,O_2 \rightarrow NH_4^+ + NO_2^- + 2\,H^+ \qquad (1)$$
$$NH_4^+ + 1.32\,NO_2^- \rightarrow 1.02\,N_2 + 0.26\,NO_3^- + 2\,H_2O \qquad (2)$$
$$NH_4^+ + 0.85\,O_2 \rightarrow 0.44\,N_2 + 0.11\,NO_3^- + 1.43\,H_2O + 1.14\,H^+ \qquad (3)$$

This approach eliminates the addition of external organic carbon source and reduces the requirement of oxygen supply. Therefore, the novel processes have some advantages over the traditional nitrification-denitrification process and was recognized as potential technology for high-ammonium and low-organic wastewaters such as sludge digester effluent and landfill leachate (Jetten *et al.*, 2001). Combination of the two conversion steps in wholly autotrophic processes can be accomplished in separate reactors or in a single reactor. In the later category, processes like OLAND (Oxygen-Limited Autotrophic Nitrification-Denitrification) and CANON (Completely Autotrophic Nitrogen removal Over Nitrite) are widely known. The OLAND process was first developed by the application of oxygen-limited condition to a sequencing batch reactor (SBR) seeded with an enriched nitrifying sludge (Kuai *et al.*, 1998). Afterwards, the OLAND process was described in a mixed community biofilm of a lab-scale rotating biological contactor (RBC) (Pynaert *et al.*, 2003). The CANON process was originally developed in a SBR using a specific start-up pattern consisting of anoxic inoculation with anammox biomass followed by oxygen supply to develop nitrifying population (Slierkers *et al.*, 2002). To date, numerous studies have been undertaken for improving the performance of wholly autotrophic nitrogen removal process. Under oxygen-limiting conditions with high ammonium concentrations, the competition between ammonium-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) is minimized. The use of biomass carriers would increase the sludge retention time thereby increase the treatment efficiency of a wholly autotrophic nitrogen removal processes. Some biomass carriers made of acryl-resin fiber have demonstrated effectiveness in nitrification and partial nitritation processes in our previous studies (Furukawa *et al.*, 2003; Lieu *et al.*, 2004). In this study, development of a single-stage nitrogen removal using anammox and partial nitritation (SNAP) process and its performance during the treatment of simulated landfill leachate with an acryl fiber biomass carrier were investigated.

2. Methods

Reactor and influent

Two identical 5-L reactors, named SN-2 and SN-3 and made of acrylic resin, were used in this study. Each reactor, as shown in Fig.1, was designed to allow for control in HRT, pH, temperature and aeration rate. A net-type acryl resin fiber material (BX, NET Co. Ltd., Japan) was used as the biomass carrier at package ratio of 10 g-material/L-reactor. Each reactor was seeded with 13 g (as SS) of nitrifying activated sludge. Synthetic wastewater simulating pre-treated landfill leachate was used for feeding the reactors after start-up phase. Composition of the influents used for the two reactors is shown in Table 1. Potassium hydrogen phthalate (KHP) or mixture of KHP and humic acid (Hum) (m.p. > 300°C) at KHP-to-Hum ratio of 10:1 were used as organic carbon additives to simulate landfill leachate.

Experimental plan

Both two reactors had operated more than 200 days of start-up and nitritation treatment phase before achieving SNAP process. Ten experimental periods with various sets of operational conditions including HRT, pH, temperature, and aeration rate were assigned to investigate the responses of SNAP performance in reactor SN-2. Operation was extended to 5 periods for testing the process stability. Experimental conditions of these periods are shown in Table 2. Reactor SN-3 was operated separately under the typical operational conditions obtained from reactor SN-2 for investigation of performance at higher nitrogen loads and effect of influent organic carbon on the SNAP process. Sludge samples were withdrawn from reactors at certain times (operational days 127, 298, 335, 344, 442) for determination of MPN and bacterial composition.

MPN tests

MPN tests for enumeration of AOB and NOB in the SNAP sludge consisted of 10-fold serial dilutions, from 10^{-3} to 10^{-7}, in five culture tubes. The medium used in this study were adopted from Lipponen *et al.* (2002). Test tubes were incubated at 28.0 ± 0.5°C for 30 days. After incubation, activities of AOB and NOB were determined by bromothymol blue and Griess-Ilosvay reagents, respectively. The results were calculated as MPN per g-VSS.

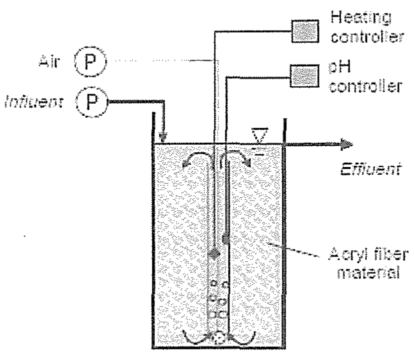

Fig.1 Schematic diagram of the reactor

Table 1. Composition of synthetic influents (in mg/L)

Composition	Reactor SN-2	Reactor SN-3
1. NH_4Cl	916 (240 as N)	1908.5 (500 as N)
2. KH_2PO_4	43.4	43.4
3. $NaHCO_3$	630	1480.5
4. $KHCO_3$	750	1762.5
5. $MgSO_4.7H_2O$	328	328
6. $CaCl_2.2H_2O$	235.2	235.2
7. $FeSO_4.7H_2O$	16	16
8. Na_2.EDTA	16	16
9. KHP$^{(a)}$	37.5	37.5~45$^{(b)}$
10. Humic acid	0	4.5~5.0$^{(b)}$

$^{(a)}$*KHP: Potassium Hydrogen Phthalate* ($C_8H_5O_4K$)
$^{(b)}$*Applied in study on effect of organic carbon.*

Bacterial composition analyses

16S rDNA analyses were performed to examine the existence of nitrifying and anammox bacteria in the SNAP sludge. For anammox bacteria, a simple analytical procedure was applied which included DNA extraction, DNA amplification with anammox specific primers, direct DNA sequencing and homology search using BLAST at NCBI databases. The specific primers were Ana-5' (5'-TAGAGGGGTTTTGATTAT-3') and Ana-3' (5'-GGACTGGATACCGATCGT-3'), whose sequences correspond respectively to positions 811 to 828 and 1004 to 1022 in the 16S rDNA of anammox KSU-1 strain. For detection of nitrifiers, DNA was extracted and amplified with bacterial primer set 357F-534R (corresponding to positions 341 to 534 in 16S rDNA regions *E.coli*), and GM5F-907R (used for amplifying the 16S rDNA of members belonging to the domain Bacteria (Muyzer *et al.*, 1993, 1995). DGGE was performed for amplified DNA fragments, excised bands were sequenced, and sequences obtained were searched for homologies.

Table 2 Experimental periods for reactor SN-2

Period (term)	Operational conditions			
	HRT (h)	Temperature (°C)	pH	Aeration rate (vvm)
1 (0~17)	6	35	7.5	0.10
2 (18~31)	6	35	7.5	0.06
3 (32~45)	8	35	7.5	0.06
4(46~67)	8	35	7.8	0.06
5 (68~82)	10	35	7.8	0.10
6 (83~88)	10	35	≥ 8.0[a]	0.10
7 (89~103)	10	32.5	7.8	0.06
8 (104~113)	10	35	7.5	0.14
9 (114~127)	10	35	7.5	0.10
10 (128~191)[b]	10	35	7.5	0.10
11 (192~209)[c]	-	-	-	-
12 (210~242)[d]	12~10	30~35	7.5	0.06~0.10
13 (243~298)	6	35	7.5	0.10
14 (299~322)	10	35	7.5	0.10
15 (323~344)[e]	10	35	7.8	0.10
16 (345~464)	10	35	7.5~7.8	0.06~0.10

[a] *Not controlling pH but increasing influent bicarbonate*
[b] *After removing loosely attached sludge*
[c] *Rest phase (Stop operation, store SNAP sludge in refrigerator)*
[d] *Restart-up reactor, operational conditions varied*
[e] *Stop operation, then restart within the day 344.*

Chemical analyses

Determinations of NO_2-N and NO_3-N were in accordance with Standard Methods (1999), by using the colorimetric method (4500-NO_2^-B) and UV screening method (4500-NO_3^- B), respectively. In case of NO_3-N determination, the interference of NO_2^- was quantified experimentally by using NO_3-N standards added with known concentrations of NO_2-N. A modification of the standard phenate method was applied for NH_4-N as reported by Kanda (1995). Absorbance was measured using a U-2010 Spectrophotometer (HITACHI). A Mettler 320 pH meter (TOLEDO) was used for measurement of pH. TOC was quantified using a TOC-5050 Analyzer (SHIMADZU) and DO was measured with a 782 Oxygen Meter (STRATHKELVIN INSTRUMENTS). Alkalinity was determined using titration method (2320.B-, Standard methods).

3. Results and discussion

Development of the SNAP process

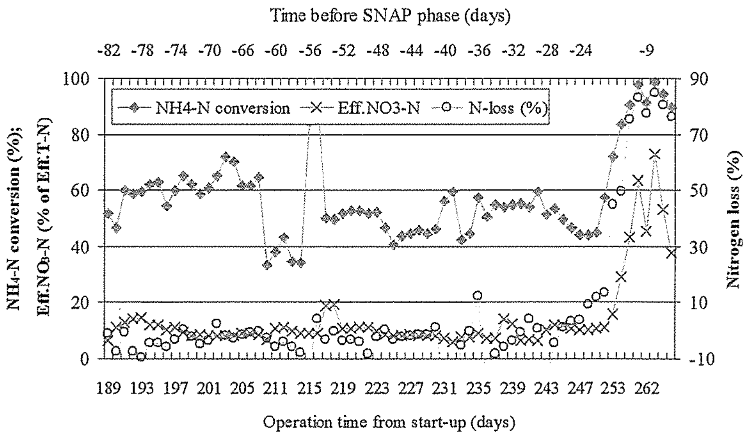

Fig. 2 Occurrence of anammox reaction at the end of partial nitritation phase in reactor SN-2.

The reactor SN-2 had operated with influent containing organic carbon (TOC = 25~30 mg/L) to achieve partial nitritation for about 240 days after starting-up (Lieu *et al.*, 2004). Nitrogen balance was insignificant during this nitritation phase. Then nitrogen losses more than 80% and simultaneous removal of ammonium and nitrite were observed as shown in Fig.2. Sludge color turned from dark yellow to reddish. It was suggested that anammox bacteria grew together with AOB in the sludge attached on biomass carrier. A single-stage nitrogen removal using anammox and partial nitritation (SNAP) process was established.

Performance of the SNAP process under various operational conditions

The variations in ammonium conversion, N-removal and effluent nitrate concentrations during 10 experimental periods of SNAP phase are shown in Fig.3. Higher values of ammonium conversion and N-removal were obtained with longer HRTs (periods 5~10). This can be understood considering low growth rates of AOB and anammox bacteria. The SNAP process performance was enhanced when pH increased from 7.5 to 7.8 (period 3 to period 4), but not higher than 8.0. Both ammonium conversion and N-removal decreased at pH equal or greater than 8.0 in period 7, possibly due to the inhibition of AOB and anammox bacteria by higher free ammonia concentration. An increase in aeration (period 8) made ammonium conversion increasing, but also did effluent nitrate concentrations. This might be due to the favored condition for nitrite oxidation at higher oxygen concentrations (bulk DO in this period was about 2.2 to 2.5 mg/L compared to 0.5 to 2.0 mg/L in other periods). Higher N-removals were obtained at 35°C than at 32.5°C. The changes in both HRT and aeration rate from period 4 to period 5 led to a pronounced change in SNAP performance. The best SNAP performance was obtained in period 5 with the average ammonium conversion of 88.1 ± 3.1% and N-removal of 78.5 ± 2.8% (n = 15). The operational conditions in this period were 10 h HRT, 35°C, pH 7.8 and 0.10 vvm aeration rate.

After removing loosely attached sludge from reactor SN-2 on day 128, ammonium conversion and N-removal continuously decreased, then ammonium conversion was almost stable around 65% while N-removal varied and dropped to about 20%. The existence of loosely attached sludge and amount of sludge in the reactor seemed to have an effect on process performance. Comparison of the SNAP performance with other single-reactor autotrophic processes is made in Table 3. Ammonium conversion and N-removal of the SNAP process are higher, in terms of percentage, and much higher, in terms of loading rate, than the original CANON and OLAND processes. A modified OLAND process on RBC showed a little better performance. The CANON gas-lift reactor could operate at a considerably higher loading rate than other processes, but both ammonium conversion and N-removal on a percentage basis were still low. These parameters are important for evaluation of treatment efficiency. Performance data of extended periods in comparison with previous ones having the same operational conditions are shown in Table 4.

In general, there were no significant differences in ammonium conversion and nitrogen removal efficiencies being observed. Data obtained in extended periods were a little higher than those obtained in the previous ones. This fact may be attributed to the long-term adaptation and compositional optimization of the SNAP sludge. Average ammonium conversion of 89.2 ± 6.2% and N-removal of 76.3 ± 7.2% were obtained for 60 days of operation under conditions of 35°C, pH 7.5~7.8, 0.06~0.10 vvm aeration rate at loading rate of 0.6 kg-N/m^3/d.

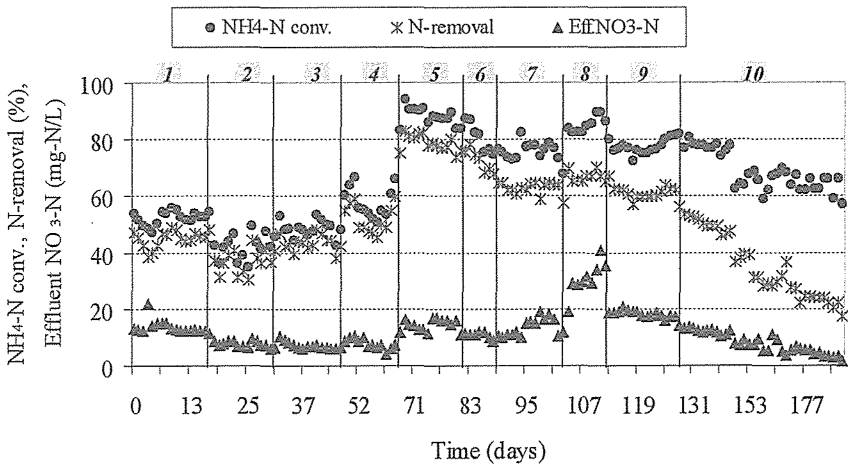

Fig. 3 Effects of operational conditions on the SNAP process performance (reactor SN-2).

Table 3 Performance data of SNAP and other single-reactor processes

System	Loading rate (kg-N/m^3/d)	NH$_4$-N conversion		N-removal		Reference
		kg-N/m^3/d	%	kg-N/m^3/d	%	
SNAP (SN-2)	0.58	0.51	88.1	0.45	78.5	This study
SNAP (SN-3)	1.00	0.66	65.9	0.56	56.3	This study
CANON (SBR)	0.131	0.075	57.2	0.064	48.9	Slierkers *et al.*, 2002
CANON(Gas-lift)	3.70	1.50	42.0	1.44	39.0	Slierkers *et al.*, 2003
OLAND (SBR)	0.25	0.07	26.2	0.04	15.2	Kuai and Verstraete, 1998
OLAND (RBC)	1.189	1.135	95.5	1.058	89.0	Pynaert *et al.*, 2003

Table 4 Stability in performance of the SNAP process (reactor SN-2)

	Period 1	Period 13	Period 9	Period 14	Period 5	Period 15
NH$_4$-N conversion, %	52.2 ± 2.4	58.9 ± 5.2	77.0 ± 2.4	76.4 ± 11.2	88.1 ± 3.1	89.8 ± 6.2
N-removal, %	44.9 ± 2.8	51.4 ± 4.8	60.9 ± 1.7	63.4 ± 10.8	78.5 ± 2.8	77.4 ± 7.3

In reactor SN-3, the SNAP process occurred from day 200 after which N-removal continuously increased. The 50-day averages for ammonium conversion and N-removal were 65.9 ± 12.0 % and 56.3 ± 12.2 %, respectively. The highest N-removal of about 80% was obtained in several days at an applied loading rate of 1.0 kg-N/m^3/d.

SNAP process with influent containing organic carbon

Figure 4 shows the performance of the SNAP process in reactor SN-3 before and after adding organic carbon to the influent with a TOC/NH$_4$-N ratio of 0.10-0.15 at a nitrogen loading rate of 0.6 kg-N/m^3/d. These results show that both ammonium conversion and nitrogen removal efficiencies were increased after organic carbon was added. The average ammonium conversion and nitrogen removal efficiencies during the last 60 days of operation were 93.7 ± 5.6 % and 84.4 ± 6.9 %, respectively. The maximum efficiencies were 100 % and 94.8 % for ammonium conversion and nitrogen removal, respectively. The average influent TOC was 30.0 ± 2.5 mg/L and average effluent TOC was 7.3 ± 0.7 mg/L, resulting in an average TOC removal of 75.7%. An important observation was that effluent nitrate concentrations were almost unchanged after adding organic carbon. This indicates that denitrification did not occur and the anammox reaction was maintained. Thus, the increase in nitrogen removal might be attributed to denitritation by denitrifiers or unknown microorganisms.

Data on TOC removal (average reduction of 22.7 mg/L) and nitrogen removal (average 84.4% of 240 mg-N/L) demonstrate a ratio of 0.11 mg-TOC/mg-N removed for this experiment. The theoretical carbon requirement for denitrification was estimated to be about 1.1 mg-TOC/mg-N (Tchobanoglous *et al.*, 1991; Kayser, 2005). The carbon requirement for denitritation is 60% of denitrification, or about 0.7 mg-TOC/mg-N. Thus, the TOC consumption in this experiment roughly corresponded to 1/7 of the total nitrogen removal . This means denitritation had contributed about 14% to the overall nitrogen removal when the SNAP process operated with influent containing organic carbon. This value is very consistent with the increase in nitrogen removal efficiencies after adding the KHP-Hum mixture. Compared with sludge from a reactor fed with influent that was free of organic carbon, aerobic activity was almost unchanged whereas

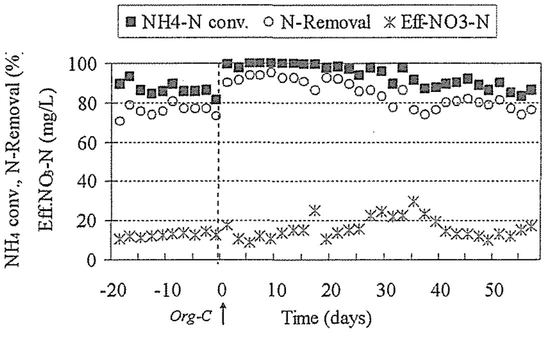

Fig. 4 Performance data of reactor SN-3 before and after adding organic carbon to the influent.

anaerobic activity increased by a factor of 1.5. The ratio of nitrite consumption rate to ammonium consumption rate was 1.28. These results support the conclusion that anammox bacteria are not strongly suppressed by the presence of organic carbon in the influent. However, the mechanisms of nitrogen removal in this case are thought to be very complicated, via a combination of various processes such as nitritation, heterotrophic denitritation, anammox and others. Figure 5 shows a suggestion of nitrogen removal mechanisms when the SNAP process operated with influent containing semi-refractory organic carbon. The limitation level of TOC/NH_4-N with positive effect to the SNAP performance should be futher considered.

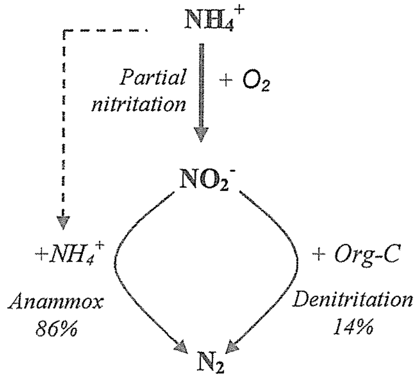

Fig. 5 Nitrogen removal mechanisms for the SNAP operation with influent containing organic carbon.

Characterization of SNAP process and sludge

DO and alkalinity consumption

The bulk DO of reactor SN-2 and SN-3 varied between 0.5 to 2.5 mg/L, while the DO inside biomass carrier block and close to biomass surface was consistently almost zero. These DO values indicated the oxygen-limited condition. Although changes in air flow rate clearly affected liquid circulation, DO levels did not changed significantly. Alkalinity consumption during the SNAP phase was calculated as 3.50 ± 0.78 mg $CaCO_3$/mg NH_4-N converted or 4.2 ± 1.0 mg $CaCO_3$/mg-N removed. These data are in good agreement with the theoretical value (4.07 mg $CaCO_3$/mg-N removed). The alkalinity consumption in SNAP and other wholly autotrophic processes was about half of the theoretical value for nitrification or nitritation (7.1 mg $CaCO_3$/mg NH_4-N converted). Saving in alkalinity consumption is also an advantage of wholly autotrophic processes together with the saving in oxygen demand.

Sludge concentration and sludge yield

During the steady operation, sludge concentration in reactor SN-2 was determined to be about 7.0 g-SS/L-reactor or a specific sludge attachment of 0.52 g-SS/g-biomass carrier. This is an advantage of acryl-fiber material to carry large sludge amount and maintain high sludge concentration. Based on the mass balance, sludge yield of SNAP process was calculated to be as low as 0.045 mg-VSS/mg-N removed. Sludge age of the SNAP process was estimated as 189 d. As a low sludge production process, the handling of excess sludge would thus be minimized. Corroboratively, only a small amount of excess sludge was removed from reactor SN-2 after about 200 days of operation, which was almost consistent with the calculated value.

Bacterial compsition of the SNAP sludge

Table 5 shows the results of 16S rDNA analysis for anammox bacteria. Anammox bacteria similar to the KU2 strain were detected in all sludge samples from reactors SN-2 and SN-3 and a bacterium identified as a KSU-1 strain was detected in a sample from reactor SN-2. These anammox strains were previously detected in column reactors packed with a non-woven biomass carrier in our laboratory (Fujii *et al.*, 2002; Imajo *et al.*, 2004). It was found that all sequences homologizing with KU2 bacterium also homologize at same identities with planctomycete KOLL2a strain, which is detected in a RBC treating ammonium-rich leachate (Egli *et al.*, 2001).

Results of BLAST searches for sequences of excised DGGE bands with respect to nitrifiers are summarized in Table 6. The existence of *Nitrospira* in samples SN-2 (day 442) and SN-3 (day 335) was confirmed later on by DGGE analysis with modifications in denaturant gradient and polyacrylamide gel to overcome the runoff and elution of amplified DNA fragments. Microorganisms close to *Nitrosomonas europaea* and *Nitrospira sp.* were identified as the bacteria responsible for oxidizing ammonium and nitrite, respectively,

Table 5 Homology search results for sequences amplified with anammox-specific primers

Sample	Highest homology (Accession No.)	Identity (%)
SN-2 (day 335)	Uncultured anoxic sludge bacterium KU2 (AB054007)	104/105 (99%)
SN-2 (day 344)	Uncultured anoxic sludge bacterium KU2 (AB054007)	116/116 (100%)
SN-2 (day 442)	Planctomycete KSU-1 (AB057453)	116/116 (100%)
SN-3 (day 344)	Uncultured anoxic sludge bacterium KU2 (AB054007)	131/131 (100%)
SN-3 (day 442)	Uncultured anoxic sludge bacterium KU2 (AB054007)	105/106 (99%)

Table 6 Homology search results for sequences amplified with bacterial primers with respect to nitrifiers

Sample	Highest homology (Accession No.)	Identity (%)
All SN-2 and SN-3	*Nitrosomonas europaea* (AJ245759)	99/104 (97%)
SN-2 (day 442)	*Nitrosomonas europaea* (BX321856)	515/531 (97%)
SN-2 (day 442)	*Nitrospira sp. clone b2* (AJ224038)	446/464 (96.1%)
SN-2 (day 442)	*Nitrospira sp. clone b30* (AJ224041)	524/533 (98.3%)
SN-3 (day 442)	*Nitrospira sp. clone b30* (AJ224041)	528/537 (98.3%)

in the SNAP process. From results of the MPN tests, as shown in Fig. 6, concentrations of *Nitrosomonas europaea* were always much higher than that of *Nitrospira sp.* in the SNAP sludge. This means the complete inhibition of *Nitrospira sp.* was not achieved. All of the above results of bacterial analyses revealed the co-existence of anammox bacteria, AOB and NOB in the SNAP sludge that are consistent with activity tests and MPN tests. In the CANON process, presence of *Nitrobacter* and *Nitrospira* was detected and interaction and competition between these groups of bacteria were suggested (Third *et al.*, 2001). In the OLAND process, the existence of small numbers of NOB was also assumed and then demonstrated with the presence of predominant AOB (*Nitrosomonas*-like) and anammox bacteria (close relatives to *Ca.* Kuenenia stuttgartiensis) (Pynaert *et al.*, 2003).

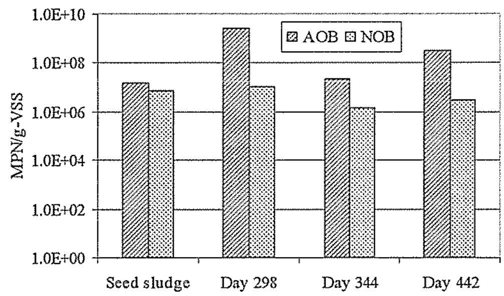

Fig. 6 MPNs of AOB and NOB in the seed and SNAP sludges versus time.

4. Conclusions

From the results of this study, the following conclusions were drawn:

- During long term partial nitritation treatment, anammox bacteria grew inside of the sludge attached on the acryl-fiber biomass carrier and enabled the short-cut conversion of ammonium to dinitrogen gas using only one reactor. This novel removal process was named as Single-stage Nitrogen removal using Anammox and Partial nitritation (SNAP).
- Continuous studies proved good treatment performances of the SNAP process for nitrogen removal from synthetic landfill leachate containing high levels of ammonium (up to 500 mg-N/L) and other inorganic salts. The SNAP process achieved a stable nitrogen removal of about 80% at loading rates of 0.6 kg-N/m^3/d. Similar removal rates was also obtained at higher loading rates up to 1.0 kg-N/m^3/d.
- The presence of semi-refractory organic matters in the influent at TOC/NH_4-N ratios of 0.10 - 0.15 did not affect the SNAP performance, even a little increase in removal efficiency was observed. Denitritation was estimated to occur and contribute to the nitrogen removal process.
- Coexistence and symbiosis of aerobic and anaerobic ammonium oxidizing bacteria on acryl fiber carrier was the key concept of the SNAP process. Three groups of bacteria including AOB, which are close relatives of *Nitrosomonas europaea*; NOB, which are close relatives of *Nitrospira sp.*; and anammox bacteria, which are close relatives of KU-2 and KSU-1 strains were observed in the SNAP sludge. AOB and anammox bacteria were dominant and play the key roles in SNAP process.

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