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Rapid startup and high rate nitrogen removal from anaerobic sludge digester liquor using a SNAP process

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Abstract In this study, a single-stage autotrophic nitrogen removal reactor, packed with a novel acrylic fiber biomass carrier material (Biofix), was applied for nitrogen removal from sludge digester liquor. For rapid start-up, conventional activated sludge was added to the reactor soon after the attachment of anammox biomass on the Biofix carriers, which allowed conventional activated sludge to form a protective layer of biofilm around the anammox biomass. The Nitrogen removal efficiency reached 75% within 1 week at a nitrogen loading rate of 0.46 kg-N/m³/day for synthetic wastewater treatment. By the end of the synthetic wastewater treatment period,

the maximum nitrogen removal rate had increased to 0.92 kg-N/m³/day at a nitrogen loading rate of 1.0 kg-N/m³/day. High nitrogen removal rate was also achieved during the actual raw digester liquor treatment with the highest nitrogen removal rate being 0.83 kg-N/m³/day at a nitrogen loading rate of 0.93 kg-N/m³/day. The thick biofilm on Biofix carriers allowed anammox bacteria to survive under high DO concentration of 5–6 mg/l resulting in stable and high nitrogen removal performance. FISH and CLSM analysis demonstrated that anammox bacteria coexisted and surrounded by ammonium oxidizing bacteria.

Keywords Single-stage autotrophic nitrogen removal · Partial nitrification · Anammox · Sludge digester liquor · Low C/N ratio

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Introduction

Anaerobic digester liquor of excess sludge in municipal wastewater treatment plant contains high concentration of ammonium accounting for approximately 20% of the nitrogen load in a wastewater treatment plant (van Kempen et al. 2001; Wett and Alex 2003). Discharge of such kind of wastewater to the receiving environment is undesirable due to potential adverse impacts including toxicity to fish and other aquatic organisms, dissolved oxygen (DO) depletion, eutrophication and potential public health hazards (Obaja et al. 2003).



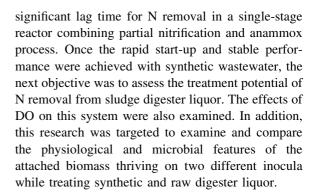
During the past few decades, a number of techniques to overcome the disadvantages of conventional nitrogen removal technology (nitrification–denitrification) have been investigated, including the shortcut biological nitrogen removal (SBNR) process and the entirely autotrophic process that is based on the application of anammox reaction (Turk and Mavinic 1986; Mulder et al. 1995; Yoo et al. 1999). In the latter approach, ammonium is converted ultimately to dinitrogen gas by two sequential reactions: partial nitrification plus the anammox reaction as shown in Eqs. 1–2.

$$2N{H_4}^+ + 1.5O_2 \rightarrow N{H_4}^+ + N{O_2}^- + H_2O + 2H^+ \eqno(1)$$

$$N{H_4}^+ + 1.32N{O_2}^- \rightarrow 1.02N_2 + 0.26N{O_3}^- + 2{H_2}O$$
(2)

This approach requires two distinct and separate unit processes for nitrogen removal from wastewater. Full-scale partial nitritation-anammox process for the treatment of ammonium rich wastewater with satisfactory performance has been reported, however, large footprint was necessarily required for the sequential two-stage process (Van Dongen et al. 2001). Due to evident benefits, partial nitrificationanammox in a single stage process has been investigated through various methods such as CANON, OLAND, and SNAP processes (Kuai and Verstraete 1998; Slierkers et al. 2002; Furukawa et al. 2006). Most of these studies only used synthetic wastewater as the target wastewater for nitrogen removal with very few results reported for raw ammonium wastewater. One study by Pynaert et al. (2004) achieved a relatively low nitrogen removal rate of 0.5 kg-N/m³/ day from an actual sludge digester liquor. In that research, Pynaert et al. (2004) introduced a procedure of sequential addition of ammonium oxidizing bacteria and anammox biomass for the start-up of the single-stage autotrophic N removal reactor, however there was no any N removal observed during the first 100 days of operation only for cultivation of ammonium-oxidizing bacteria. It is, therefore necessary to investigate and develop a quicker and more reliable approach for start-up and subsequent removal of nitrogen from raw ammonium rich wastewaters by a single-stage autotrophic nitrogen removal process.

The basic purpose of this study was to investigate the possibilities of rapid start-up procedure without



Materials and methods

Experimental setup

A column type Single-stage Nitrogen removal using Anammox and Partial nitritation (SNAP) reactor was used in this study as shown in Fig. 1. The reactor had an inner diameter of 15 cm and a height (to effluent port) of 28 cm providing a reaction zone of 5.0 l. Temperature was controlled at about 33 ± 2 °C by a heater. A pH controller (NPH-6900, Nissin Co. Ltd., Japan) was used for controlling the pH levels of reactor at 7.5 by adding NaHCO₃ (1 M). Hydrophilic net-type acrylic resin fiber material (Biofix, NET Co., Ltd., Japan) was packed in the reactor at 10% packing rate. This material had specific surface area of 146.5 m²/m³, yarn diameter of 2 mm, and specific weight of 980 kg/m³. In addition, this material proved to be able to provide a suitable environment for the coexistence of ammonia-oxidizing and anammox bacteria (Furukawa et al. 2006).

Influent composition and seed sludge

Synthetic wastewater was used during the start-up of the reactor and was mainly made up of $(NH_4)_2SO_4$. After the reactor start-up, sludge digester liquor was used, which was taken from the Kumamoto East Wastewater Treatment Plant (Kumamoto, Japan). This kind of sludge digester liquor was pretreated by centrifugation, so the SS concentration was not so high. Water quality parameters of the synthetic and the raw digester liquor are reported in Table 1. The SNAP reactor was initially seeded subsequently with anammox biomass and conventional activated sludge (MLVSS of 1920 and 1080 mg/l, respectively).



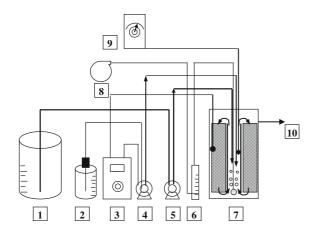


Fig. 1 Schematic diagram of SNAP reactor. *1* Influent, 2 NaHCO₃ (1M), 3 pH controller, 4 Buffer pump, 5 Influent pump, 6 Air flowmeter, 7 Reactor, 8 Air pump, 9 Heater, *10* Effluent

Table 1 Water quality of the synthetic and the actual raw anaerobic digester liquor

Components	Concentration (mg/l)			
	Synthetic	Raw digester liquor		
BOD ₅	-	100-210		
NH ₄ -N	100-500	600-1010		
NO ₂ -N	0	ND		
NO ₃ -N	0	ND		
SS	0	10-100		
pН	6.5-8.3	7.6–9.1		

ND not detected

Analytical methods

Nitrate–nitrogen (NO₃–N) and nitrite–nitrogen (NO₂–N) were measured by UV cadmium reduction method and colorimetric method, respectively (APHA 1995, 4500-NO₂–B, 4500-NO₃–E;). Ammonium-nitrogen (NH₄–N) was quantified by the method described by Kanda involving use of *o*-phenylphenol as a substitute for liquid phenol (Kanda 1995). Suspended solids (SS) content was determined according to Standard Methods (APHA 1995, 2540 D). Total sludge content of the reactor was estimated as mixed-liquor suspended solids (MLSS) while its biological portion as MLVSS according to Standard Methods (APHA 1995). The pH level was measured by electrometric method

using a pH meter (IM-22P; TOA Electronics, Ltd., Tokyo, Japan). DO was measured using a DO meter (HORIBA, pH/DO meter D-55).

FISH analysis

To construct FISH probes, the 3'-end of Nso190 (Mobarry et al. 1996) for detection of AOB was labeled with fluorescein isothiocyanate (FITC)-12-dUTP (Invitrogen, Tokyo), by using the DNA Tailing Kit (Roche Diagnostic Co. Ltd., Tokyo). In addition, the 3'-end of Amx820+ (5'-CAAAACCCCTCTAC TTAGTGCCC-3') revised from Amx820 for anammox bacteria also labeled with Alexa Fluor 568-5-dUTP (Invitrogen). Hybridizations of the fixed biofilm samples were performed in 20 mM Tris-HCl buffer, pH 7.2 containing 0.9 M NaCl, 0.01% SDS, 30% formamide, and the labeled probes as described by Amann et al. (1996) at 46°C, and then followed by washing with 20 mM Tris-HCl buffer, pH 7.2 containing 0.112 M NaCl, and 0.01% SDS.

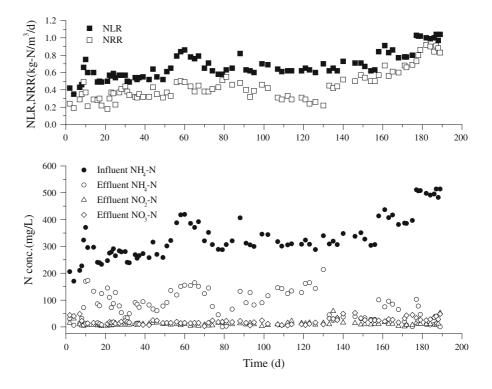
Results and discussion

Synthetic wastewater treatment performance

Figure 2 illustrates nitrogen removal performance of the SNAP reactor with synthetic wastewater. The hydraulic retention time (HRT) was kept constant about 12 h and the nitrogen loading rate (NLR) was increased only by enhancing the concentration of NH₄⁺-N in the influent. Rapid acclimatization and satisfactory interaction between the AOB and the anammox bacteria could be confirmed through evident nitrogen removal from the onset of the experiments. The nitrogen removal efficiency reached 75% within a week and the nitrogen removal rate (NRR) ascended to 0.35 kg-N/m³/day at a NLR of about 0.46 kg-N/m³/day. Subsequently, the relatively narrow space between the carrier material and the reactor wall was filled up due to the proliferation of biomass, which severely impaired the oxygen transfer and ammonium conversion. This resulted in accumulation of ammonium (average of 145 mg-N/l) between day 53 and 72. In order to improve oxygen transfer, the air flow rate was increased gradually to 0.6 l/min on day 73. This improvement in oxygen transfer resulted in



Fig. 2 Synthetic wastewater treatment performance



rehabilitating the reactor performance as high nitrogen removal efficiencies were soon achieved accompanied with almost 100% ammonium conversion rate. However, identical problems occurred again due to clogging of the air stone as a result of severe attachment of large quantity of biomass. The depletion of DO resulted in the accumulation of ammonium for about 40 days from day 88 and the effluent ammonium concentration reached its highest level of 214 mg/l on day 131. After backwashing the reactor and cleaning the air stone, the reactor performance improved dramatically, e.g., the effluent ammonium concentration decreased from 214 to 45 mg-N/l and the TN removal efficiency increased from 31 to 73%. In the following, an average TN removal efficiency of 74% was obtained at a NLR of about 0.6 kg-N/m³/day. Stable and consistent reactor performance continued thereafter even though the NLR was increased to about 1.0 kg-N/m³/day. The NRR increased to its highest level of 0.92 kg-N/m³/day and the average TN removal efficiency reached 84% at this NLR.

Raw digester liquor treatment performance

Considering the presence of refractory organics and high SS, the raw digester liquor was initially diluted by approximately 10 times and the HRT was shortened to 3 h to maintain the NLR close to the previous NLR of 1.0 kg-N/m³/day during the synthetic wastewater treatment experiments, shown in Fig. 3. During the first week of acclimatization, a nominal increase in nitrogen removal efficiency of about 10% was observed, which increased to 60% (NRR of 0.54 kg-N/m³/day) under NLR of 0.9 kg-N/ m³/day after 20 days of operation. In the subsequent 2 weeks (day 210-223), stable nitrogen removal performance was observed with an average removal of 55% at the same NLR. When the dilution rate was changed to five times and HRT was prolonged to 6 h, the removal performance did not show significant fluctuation as the removal performance remained steady at an average removal efficiency of 54%. In view of the fact that the nitrogen removal efficiency did not drop, the dilution rate was further decreased to two times while extending the HRT to 8.3 h. The nitrogen removal efficiency increased slightly to 63%. When the raw digester liquor without dilution was introduced to the SNAP reactor, the nitrogen removal efficiency increased significantly reaching at about 80%. These results demonstrated the achievement of substantial acclimatization and proliferation of responsible biomass on the sludge digester liquor.



Fig. 3 Time courses of raw digester liquor treatment

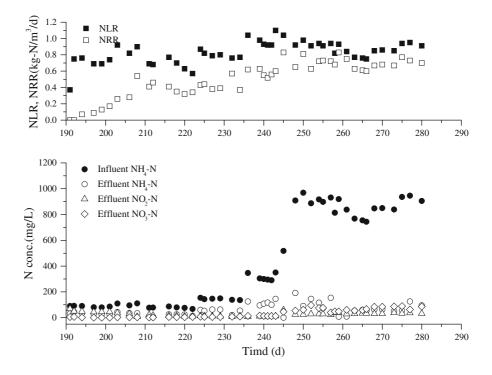


Table 2 compares the nitrogen removal performances of single-stage autotrophic processes such as CANON and OLAND. Although, Slierkers et al. achieved high nitrogen removal rate of 1.44 kg-N/m³/ day in a gas-lift reactor by applying 95% Ar/5% CO₂ and compressed air at a maximum gas flow of 0.2 l/min, the overall nitrogen removal efficiency was much lower in comparison to this study (42% vs. 91.3%). Pynaert et al. (2003, 2004) used a rotating biological contactor (RBC) for nitrogen removal from synthetic wastewater and achieved fairly high nitrogen removal rate of 1.8 kg-N/m³/day with DGGE and real-time PCR analysis demonstrating that the RBC biofilm was dominated by members of the genus Nitrosomonas and close relatives of Kuenenia stuttgartiensis. However, when the sludge wastewater was fed to the RBC, the nitrogen removal rate dropped to 0.42 kg-N/m³/day under a NLR of 0.91 kg-N/m³/day due to the depressed ammonium conversion rate of about 50% (Pynaert et al. 2004). In contrast, high nitrogen removal efficiency of 91.3% in this study indicated that the SNAP reactor with novel biofix packing material performed superior to other singlestage autotrophic N removal systems since the robust biofilm formed could provide suitable environment for the co-existence of AOB and anammox bacteria. Immediate N removal of 57% observed soon after the introduction of influent into the reactor indicated prospects of effective startup of the SNAP reactor. This method was more effective as compared to separate addition of AOB and anammox bacteria reported by Pynaert et al. (2004), since there was almost no N removal observed during first 100 days of operation. The overall results of the startup of the SNAP reactor suggested that the stepwise acclimatization approach was effective since no inhibition of anammox biomass was observed and high nitrogen removal was obtained even with the raw digester liquor without any dilution.

DO effect

DO is a key factor in single-stage autotrophic N removal process since the limiting step of partial nitritation is mainly dependent on the DO levels. Without cautious regulation of DO and associated NLR adjustments, nitrogen removal may be impaired due to large amount of unconverted ammonium or excessive accumulation of nitrite in the reactor. The later can totally inhibit the activity of anammox bacteria if the nitrite concentration is over 100 mg NO₂–N/l (Strous et al. 1999). Therefore, it is



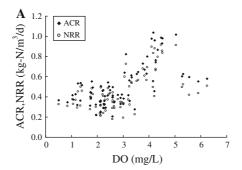
Table 2 Comparison of the performances of single-stage systems for nitrogen removal

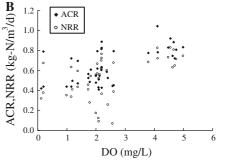
	System	NLR (kg-N/m³/day)	ACR (kg-N/m³/day)	Nitrogen removal		Reference
				%	kg-N/m³/day	
Synthetic wastewater	CANON (SBR)	0.13	0.075	57.2	0.064	Slierkers et al. (2002)
	CANON (Gas-lift)	3.70	1.50	42.0	1.44	Slierkers et al. (2003)
	OLAND (SBR)	0.13	0.08	62.0	0.05	Kuai and Verstraete (1998)
		0.25	0.07	26.2	0.04	
	OLAND (RBC)	1.19	1.14	89.0	1.06	Pynaert et al. (2003)
	OLAND (RBC)	2.04	1.82	88.0	1.80	Pynaert et al. (2004)
	SNAP	0.58	0.51	88.1	0.45	Lieu et al. (2005)
	SNAP	1.04	1.04	91.3	0.92	This study
Raw wastewater	OLAND (RBC)	0.91	0.50	46.5	0.42	Pynaert et al. (2004)
	SNAP	0.94	0.92	89.7	0.83	This study

ACR Ammonium Conversion Rate

necessary to adjust the DO levels in the reactor to an optimal value associated with the variable NLRs for the maximum nitrogen removal. However, Hao et al. (2002) reported that variation of DO concentration in a small range (0.2 mg/l) had no significant influence on the performance of CANON process, therefore the requirements on electrode sensitivity and a DO control scheme were not too stringent. It was demonstrated in that study that maximum N removal of 82% was achieved at a biofilm thickness of 1 mm and ammonium surface loading of 2 g NH₄⁺-N/m²/ day, which was associated with the optimal DO level of 1.3 mg/l. In our study, the air flow rate was carefully regulated in order to prevent the effluent nitrite concentration of over 50 mg/l since anammox bacteria was more sensitive to nitrite than ammonium. The relationship between ammonium conversion rate (ACR), NRR and DO is illustrated in Fig. 4. During both the synthetic and the raw digester liquor treatment periods, ACR and NRR were increased with increasing DO levels except the initial acclimatization period of raw digester liquor. Especially after backwashing the reactor on day 131, sudden increase in the DO concentration from 1.3 to 5.6 mg/l caused the ammonium conversion rate to increase sharply from 31 to 86% at a NLR of 0.7 kg-N/m³/day. Substantial ammonium conversion rates demonstrated to be a guarantee for high N removal performance during both periods of synthetic and raw digester liquor treatment. Although high DO concentration is in favor of the growth of NOB, no peak of nitrate was observed throughout the entire experimental period, which could be attributed to the inhibition of NOB by high operational temperature and pH. The bulk DO concentration in the reactor reached 6.2 and 5.0 mg/l in both periods of synthetic and raw digester liquor treatment, respectively, which were much higher than the DO levels reported in other single-stage autotrophic nitrogen removal processes. This phenomenon might be explained by the thick biofilm formation in the SNAP reactor. It was postulated that the conventional activated sludge,

Fig. 4 Relationship between ACR, NRR and DO concentration during the synthetic and the raw digester liquor treatment periods (*Note* a results of synthetic wastewater treatment period, b results of raw digester liquor treatment period)







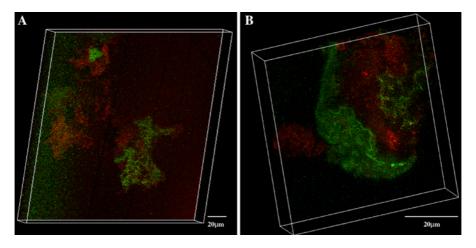


Fig. 5 FISH and CLSM images of SNAP biomass taken on days 190 (a) and 280 (b)

added as seed sludge, formed a protective layer outside the anammox biomass, which consumed the excessive DO and provided substrate transfer. However, the exact mechanism of DO consumption and nutrient transfer inside the biofilm requires further investigation. Denitrification reaction could be considered negligible due to fairly high nitrogen removal rate, no addition of organics in the synthetic wastewater and little organics removal (about 20%) observed in the raw digester liquor treatment.

FISH analysis of the biofilm

In order to visualize the localization of the bacterial strains in the biofilm, FISH analysis using the anammox (Amx820+) and Nitrosomonas DNA (Nso190) probes labeled with Alexa Fluor 568 and FITC, respectively, was performed with the samples taken on day 190 and 280, illustrated in Fig. 5. Imaging using epifluorescence microscopy revealed a high density of cells growing in clusters and emitting red fluorescence, indicating the dominance of anammox within the biofilm of the samples of day 190. A comparatively lesser surface area was stained green, indicating a lesser density of Nitrosomonas in the biofilm (Fig. 5a). Based on the image of samples of day 280, more strong green signals were observed, which meant that the proportion of Nitrosomonas had increased during the sludge digester liquor treatment. For more detailed localization, sectional imaging of samples using a confocal laser scanning microscope (CLSM) was performed using Amx820+ labeled with Alexa Fluor 568 and NSO190 labeled with FITC as hybridization probes. Thirty and thirty two horizontal planes were scanned at 0.4-1am intervals at a biofilm depth of 16 lain from which sectional images were reconstituted. The image of sample taken on day 190 showed that Nitrosomonas cells spread into the enclosing of anammox cells or they mixed with each other and a small agglomeration of Nitrosomonas cells was totally surrounded by anammox cells. The CLSM image kept the same accordance with FISH image that anammox cells accounted for more proportion than that of Nitrosomonas in the biofilm on day 190. On the other hand, CLSM image on day 280 elucidated more clearly the spatial location of anammox and Nitrosomonas cells compared with FISH image. In Fig. 5b, most anammox cells agglomerated in ellipse-similar shape and Nitrosomonas formed as a thick layer around anammox cells. The Nitrosomonas cell layer might serve to eliminate molecular oxygen from the anammox habitat and provide the substrate to anammox cell. In consequence, the cluster formation by anammox cells could maintain the high metabolic activity observed.

Conclusion

In this paper, experimental results of nitrogen removal from raw digester liquor using SNAP process involving novel biofix biomass carrier material are reported. The results demonstrated that the SNAP process was very effective for nitrogen removal from high ammonium-rich sludge digester liquor. Addition of conventional activated sludge and



anammox biomass proved to be a feasible startup means for the single-stage autotrophic nitrogen removal systems. When the sludge digester liquor was introduced into the SNAP reactor, high nitrogen removal rate of 0.83 kg-N/m³/day was achieved at a NLR of 0.94 kg-N/m³/day. The relatively thick biofilm formed on the biomass carriers allowed reactor operation at DO levels as high as 6 mg/l without impairing nitrogen removal performance indicating easy operation of the SNAP process. FISH and CLSM images illustrated that anammox biomass were surrounded by AOB in the later part of the actual digester liquor treatment and this co-existence of AOB and anammox biomass was considered to be the main factor for high nitrogen removal performance. Based on the results of this study, SNAP offers high potential for ammonium-rich wastewater treatment.

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