Anammox treatment of high-salinity wastewater at ambient temperature

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Abstract

The present study aims to provide a realistic understanding of how the anammox bacterial community and nitrogen removal performance are affected by increasing salt concentrations at ambient temperature. A laboratory-scale investigation was conducted for 92 days, during which the reactor was fed with synthetic inorganic wastewater composed mainly of NH_4-N and NO_2-N. A stable NRR of 4.5 ± 0.1 kg-$Nm^{-3}day^{-1}$ was obtained at a NaCl concentration of 30 g/L, suggesting that the enriched anammox consortium adapted to high salt concentrations. This NRR level is the highest level ever reported at high salt concentration. The addition of salt in the influent was expected to improve the physical properties of the anammox biomass. The anammox bacterium KU2 strain, which was confirmed to adapt to high salt concentrations, was considered to be responsible for the stable nitrogen removal performance. The successful application of anammox technology in this study provides an alternative for the treatment of wastewater containing high concentrations of salt and ammonium nitrogen.

Key words: Nitrogen removal; Anammox; Ambient temperature; High salinity; 16S r RNA

Introduction

Excessive nitrogen contained in the wastewater discharges from municipal and industrial systems pose a threat to the ecological health of water resources due to excessive algal growth is potential of causing ecological decline (Yang et al., 2010b). Feasible mechanisms and pathways by which nitrogen in its various forms can be transformed in and removed from wastewater have been proposed and supported by researches carried out in many parts of the world. Nitrogen from wastewater can be removed by a variety of physicochemical and biological processes, but biological processes are preferred base on the characterization of being more environmentally friendly and cost-effective (Furukawa et al., 2009). Conventional wastewater treatment systems for nitrogen removal require a lot of energy to create aerobic conditions for bacterial nitrification, and also use organic carbon to help remove nitrate by bacterial denitrification (Kartal et al., 2010). Since this treatment approach involves requirement of oxygen supply in nitrification process and organic carbon

source in denitrification process, development of cost effective and economical ammonium removal processes is required. By offering an alternative to traditional application, a novel biological process of anaerobic ammonia oxidation (anammox) can help to associate economical concerns. The Anammox process consists of the anaerobic oxidation of ammonia, using nitrite as electron under anaerobic conditions (Strous et al. 1999) (Eq. (1)).

$$\mathbf{NH_4^+ + 1.31NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2H_2O} \quad \mathbf{(1)}$$

Salinity is an important parameter for wastewater treatment because many industrial wastewaters rich in ammonium also contain high salt concentrations (Kartal et al., 2006). Only Anammox species belongs to *Candidatus Scalindua* was detected in natural saline ecosystems (Dalsgaard T. and Thamdrup B., 2002). Several recent publications have stressed the adaption of freshwater Anammox species to the high salt concentrations. Kartal et al. (2006) reported that the freshwater Anammox species of *Candidatus Kuenenia stuttgartiensis* and *Candidatus Scalindua wagneri* can adapt to the high salt concentrations of 30 mg/L (Kartal et al., 2006). Liu et al. (2008) demonstrated stable nitrogen removal rate (NRR) of 1.7 kg-$Nm^{-3}d^{-1}$ for 65 days under a salt concentrations of 30 mg/L in an anammox fixed-bed reactor with non-woven biomass carrier. Although the salt concentration was almost sea level, the freshwater anammox bacteria, *KU2*, were detected (Liu et al., 2008). In order to meet the increasing intensity of effective nitrogen removal, the high NRR is expected. However, NRR was slow during previous operation of reactor under steady-state conditions due to the species of Anammox bacteria and potential limitation by high salt concentration.

We have previously demonstrated the enrichment of anammox microorganism of this reactor under sufficient inorganic carbon (IC) source and ambient temperature (Yang et al., 2010a). To develop environmentally conscious operational practices, a realistic understanding of how bacteria community is affected by an increase concentration of salt is required. Besides, understanding the associated operational and required maintenance characteristics of inhibition by different salt concentration is also an important part of the selection process for facility operators and designers alike. This study was conducted to investigate the characterization of coexistent bacteria community in the Anammox reactor fed with high salinity wastewater under room temperature. The research that we report on in this paper was also undertaken to evacuate the high rate nitrogen removal from high salinity wastewater and the recovery ability after salinity inhibition.

Materials and Methods

Experimental set-up and operational strategy

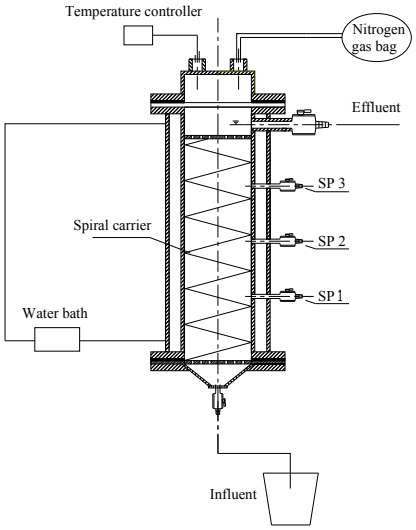


Fig. 1 Diagram of up-flow anammox column reactor in this study

This study was conducted in an up-flow column reactor, which had an apparent gross volume of 7.0 L and effective volume of 5.8 L (Fig. 1). Important characteristic of note is the spiral structure with eight pitches which was used as gas solid separator (GSS). Water temperature inside the reactors was monitored using a submerged temperature probe (B-211, Horiba, Japan). The variation in operational temperature in this study ranging from 23±2℃ to 26±1℃ was mainly due to the variation in room temperature. The reactor was always equipped with a black-vinyl sheet enclosure to inhibit the growth of photosynthetic bacteria as well as the reduction of Anammox growth rate by the light. The reactor was operated with up-flow mode and the influent was introduced from the bottom part by a peristaltic pump (CASSETTE TUBE PUMP SMP-21). The feed vessel was flushed with nitrogen gas to maintain influent DO concentration below 0.5 mg/L. In this study, samples were taken over a 92-day period and data used were selected from the dataset based on steady state conditions defined as constant feed rates for at least 7 days and relatively stable TN concentrations for at least 3 days.

Seed anammox sludge and feeding media

Prior to this study, the Anammox reactor had been operated for 202 days and high NRR of 17.5 kg-$Nm^{-3}d^{-1}$ was obtained under the operational temperature of 23±2℃.The dominant bacteria species in the consortium was detected as *KU2* with a high level of specific Anammox activity (SAA) of 0.8 g-N (g-MLSS)$^{-1}$$day^{-1}$, due to the continuous cultivation (Yang et al., 2010b). And then part of the Anammox sludge

was taken out of the reactor with remaining sludge concentration in the reactor of about 11g-MLSS/L, which was utilized to carry out the subsequent experiment. The reactor was fed with synthetic medium with a nitrite to ammonium molar ratio of 1.0–1.1. The composition of synthetic wastewater was as follows: NH_4-N 180–290 mg/L, NO_2-N 190–310 mg/L, NaCl 4-30 g/L, $KHCO_3$ 1500 mg/L, KH_2PO_4 54 mg/L, $FeSO_4 \cdot 7H_2O$ 9 mg/L, EDTA 5 mg/L, trace element solution 1 mL/L ($CuSO_4 \cdot 5H_2O$ 0.25, $ZnSO_4 \cdot 7H_2O$ 0.43, $CoCl_2 \cdot 6H_2O$ 0.24, $MnCl_2 \cdot 4H_2O$ 0.99, $NaMoO_4 \cdot 2H_2O$ 0.22, $NiCl_2 \cdot 6H_2O$ 0.19, $NaSeO_4$ 0.11, H_3BO_3 0.014) .

SEM observation

The surface and inner parts of the Anammox granules were observed using scanning electron microscope (SEM). Samples were first washed in a 0.1 M phosphate buffer solution (pH 7.4) for 5 min. The samples were then hardened for 90 min in a 2.5% glutaraldehyde solution prepared with the phosphate buffer solution. Next, samples were washed in the buffer solution three times for 10 min each and fixed for 90 min in a 1.0% OsO_4 solution prepared using the phosphate buffer solution. After washing samples three times for 10 min each in the buffer solution, they were dewatered for 10 min each in serially graded solutions of ethanol at concentrations of 10, 30, 50, 70, 90 and 95%. SEM observations were conducted using a scanning electron microscope (JEOL, JSM-5310LV, Japan).

Analytical method

NH_4-N was measured by the modified phonate method using ortho-phenyl phenol (OPP) (Kanda, 1995). NO_2-N and NO_3-N were analyzed by the colorimetric method. Bulk liquid dissolved oxygen (DO) concentration was measured using a DO probe (D-55, Horiba) and pH was determined using a pH meter (B-211, Horiba, Japan). Suspended solids were measured according to Standard Methods (Rand et al., 1978).

DNA analysis

DNA extraction and PCR amplification

The granular sludge sample was first grinded with a pestle under liquid nitrogen. Meta-genomic DNA was extracted using an ISOIL kit (Wako, Osaka, Japan) according to the manufacturer's instruction. The amplification of 16S rRNA gene was performed with Phusion High-Fidelity DNA polymerase (FINNZYMES, Finland) using conserved eubacterial primers 6F (forward primer: 5'-GGAGAGTTAGATCTTGGCTCAG-3') (Tchelet, R., 1999) and 1492r (reverse primer: 5'-GGTTACCTTGTTACGACT-3') (Lane, D.J，1991). PCR was carried out

according to the following thermocycling parameters: 30 sec initial denaturation at 98°C, 25 cycles of 10 sec at 98°C, 30 sec at 51°C, 20 sec at 72°C, and 5 min final elongation at 72°C. The amplified products were electrophoresed on a 1% agarose gel and the excided fragments were purified using Wizard SV Gel and PCR Clean-Up System (Promega, U.S.A.).

Cloning and sequencing of 16S rRNA gene

The purified fragments were ligated into the EcoRV site of pBluescript II KS+ (Stratagene, U.S.A.), and E. coli DH10B was transformed using the constructed plasmids. White colonies including the insert were randomly chosen and the plasmids were extracted by the alkaline method. The nucleotide sequences were determined with 3130xl genetic analyzer and BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, U.S.A.). The sequences determined in this study were compared with the sequences in the nr-databese using basic local alignment search tool (BLAST) program on the NCBI web site.

5.3 Results and Discussion

5.3.1 Nitrogen removal performance

Experimental data were collected over a period of 92 days. A summary of the operational conditions for the anammox reactor under high salinity is presented in Table 1. Continuous treatment results are shown in Fig. 1. The volumetric TN loading rate was varied within the range of 5.2 to 8.2 kg-$Nm^{-3}day^{-1}$ during the operation, and the nitrogen removal performance under different influent salt concentrations was evaluated. The effluent NO_2-N concentrations, which are a control item for the anammox process, were typically below 20 mg/L, except for some occasional spikes.

Table 1 Operational characteristics of the spiral anammox reactor

Items	Period I	Period II	Period III
Time (days)	0-14	15-57	58-92
HRT (hours)	1.74	1.74	1.74
Temperature (℃)	23±2 (15)	25-30	28±2 (35)
NaCl concentration (mg/L)	4-20	14-30	30

Note: average concentrations ± standard deviation (n)

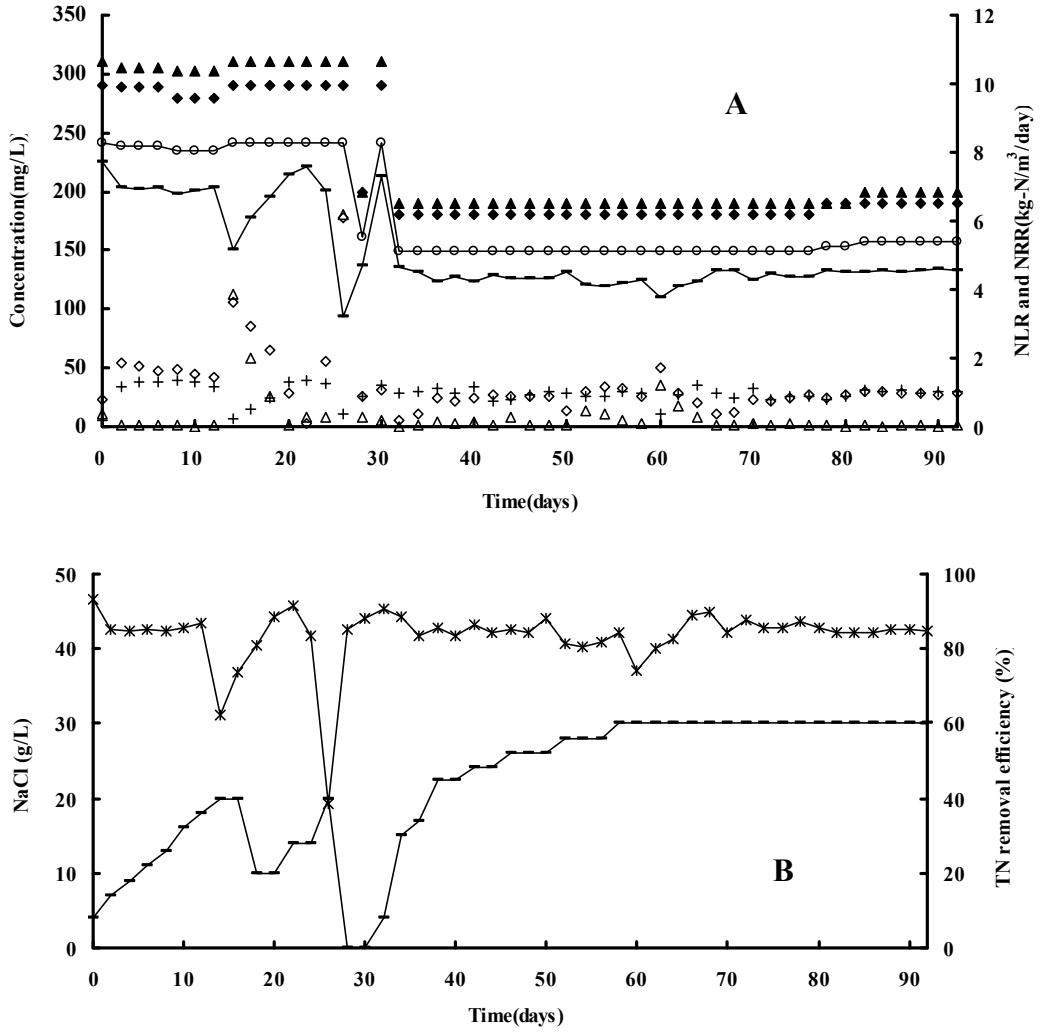


Fig. 1 Time courses of influent and effluent nitrogen concentrations, NLR and NRR (A), NaCl concentration and TNRR (B) of the reactor.
Symbols for A: ◆, Inf. NH_4-N; ◇, Eff. NH_4-N; ▲, Inf. NO_2-N; △, Eff. NO_2-N; ＋, Eff. NO_3-N; ○, NLR; -, NRR. Symbols for B: —, NaCl; ＊, TN removal efficiency.

Period I

As noted in the materials and methods section, the spiral-type anammox reactor fed with synthetic inorganic wastewater had been in operation for 202 days, during which a maximum NRR of 17.5 kg-$Nm^{-3}day^{-1}$ was achieved at ambient temperature (Yang et al., 2010b). Although half of the sludge was taken out from the reactor and the rest sludge was exposed to the air for several hours, this had no obvious influence on the nitrogen removal performance under the constant NLR of 8.2 ± 0.1 kg-$Nm^{-3}day^{-1}$. From day 0 to day 12, influent salt concentration was progressively increased from 4 g/L to 18 g/L. Potential reasons for the high effluent NO_2-N concentration of day 0 were short-term adaptation to low salinity of 4g/L as well as the exposure to air of the seed sludge. To avoid the potential inhibition under high

NLR and the increasing of influent salt concentration in the influent, we endeavor to decrease the NLR during the entire operation as well as increasing salt concentration, simultaneously. Stable performance was obtained with effluent NO_2-N concentrations below 2 mg/L, except for day 0, when the effluent NO_2-N concentration was greater than 10 mg/L. Potential reasons for the high effluent NO_2-N concentration of day 0 were short-term adaptation to salinity as well as the exposure to air of the seed sludge. The nitrogen removal efficiency of 85 ± 2% was steadily maintained during this period, with a corresponding NRR of 6.9 ± 0.2 kg-$Nm^{-3}day^{-1}$.

Period II

The increasing salt concentration was expected to risk destabilization of the anammox process. In this study, the anticipated inhibition was not observed with the constant NLR of 8.2 kg-$Nm^{-3}day^{-1}$ until the salt concentration reached to 20 g/L on day 14. High NO_2-N accumulation (greater than 110 mg/L) was observed, suggesting significant inhibition by the high salt concentration. Initially, we raised the operational temperature to 29±1℃ in an attempt to recover the anammox activity. However, the effluent NO_2-N concentrations were greater than 50 mg/L from day 16 to day 19, indicating that the improvement in the NRR from the elevated temperature is limited. To prevent the potential destabilization of the biological system, the influent salt concentration was decreased from 20 g/L to 10 g/L. The anammox activity quickly recovered, and the reactor experienced stable operational periods under salt concentrations of both 10 g/L and 14 g/L in the subsequent 7 days, with the effluent NO_2-N concentration always less than 10 mg/L.

Complete inhibition of anammox process was observed when the salt concentration was sharply increased from 14 g/L to 20 g/L on day 26, with a spike in the NO_2-N concentration of 280 mg L^{-1}. Compared with the previous inhibition by salt concentration of 20 g/L during the gradual adaption process, the inhibition by the salinity shock of 20 g/L was more serious. The desired recovery was not obtained, even though diluted (dilution time of 2/3, the influent TN concentration was 400 mg/L) influent was introduced into the reactor, and the effluent NO_2-N removal efficiency was only 10% (the effluent NO_2-N concentration of 180 mg L^{-1}). From day 28 on, the NLR was increased by reducing the influent TN concentration to 100 mg/L without the addition of salt to recover anammox activity. Correspondingly, the NO_2-N removal efficiencies spiked to 100%. In the following four days, stable nitrogen removal performance was obtained, even though the NLR was progressively enhanced by increasing influent TN concentration from 100 mg/L to 600 mg/L at a constant hydraulic retention time. The effluent NO_2-N concentrations were always

less than 10 mg/L, indicating the recovery of the anammox activity.

The temporary fractional inhibition value of the salt concentration was evaluated as 20 g/L in this study. As mentioned previously, the high SAA obtained was considered a possible reason for the high fractional inhibition value. It is also important to note that the inhibition caused by salinity shock was more significant than that caused by adaption to the high salt concentration, possibly because the microorganisms may be protected from shrinking by gradual adaption to the high salt concentration.

From day 32 to day 56, we progressively increased the influent salt concentration to 30 g/L under a NLR of around 5.1 kg-$Nm^{-3}day^{-1}$. The effluent NO_2-N concentrations were always less than 10 mg/L during this period, indicating the adaption of the anammox bacteria to the high salt concentration. Although the room temperature fluctuated during this period, the nitrogen removal performance did not vary significantly.

Period III

From day 58 to day 92, we monitored the stable nitrogen removal capacity of the anammox reactor under influent salt concentration of 30 g/L with an NLR of 5.2 ± 0.2 kg-$Nm^{-3}day^{-1}$. Stable performance was still obtained with effluent NO_2-N concentrations below 10 mg/L, except for on days 60 and 61, when the effluent NO_2-N concentrations exceeded 35 mg/L due to the high influent DO concentration (2 mg/L). Owing to the excellent tolerance of anammox sludge to the high salt concentration of 30 g/L, a NRR of 4.5 ± 0.1 kg-$Nm^{-3}day^{-1}$ was obtained under room temperature.

Comparison of different reactors operated under high salinity

Table 2 Performance of anammox reactor under high salt concentration

The spike NRR ($kg/m^3/day$)	MLSS (MLVSS)	Salt concentration (g/L)	Anammox bacteria	References
4.6	35 g MLSS L^{-1}	30	*AnDHS-2 and KU2*	This study
0.3		10		Dapena-Mora et al. (2004)
1.0		30	*Kuenenia Stuttgartiensis, Scalindua wagner*	Kartal et al. (2006)
1.7		30	*AnDHS-2 and KU2*	Liu et al. (2009)

0.3	0.9gVSS L^{-1}	30	*Kuenenia Stuttgartiensis*	Dapena-Mora et al. (2010)

A great deal of work has been devoted to understanding the adaption of anammox sludge to high salinity (**Table 2**). Dapena-Mora et al. (2004) applied anammox treatment to the digester liquor from industrial fish canning using a sequencing batch beactor (SBR), and an NRR of 0.3 kg-$Nm^{-3}day^{-1}$ was obtained at a salt concentration of 10 g L^{-1}. Kartal et al. (2006) operated a SBR for anammox treatment under salt concentration of 30 g L^{-1}, and the reported NRR was 1.0 kg-$Nm^{-3}day^{-1}$. The relatively lower NRRs obtained by Dapena-Mora et al. (2004) and Kartal et al. (2006) may be attributed to the fact that water density is increased by increasing salt concentration, and the lower-density sludge might have been washed out of the SBR due to interaction of gravity and buoyancy (Liu et al., 2009). Dapena-Mora et al. (2010) recently found that an anammox reactor was able to treat wastewater with a nitrite loading rate of around 0.32 kg-$Nm^{-3}day^{-1}$, containing 15 g L^{-1} of NaCl, with nitrogen removal efficiencies of 99%.

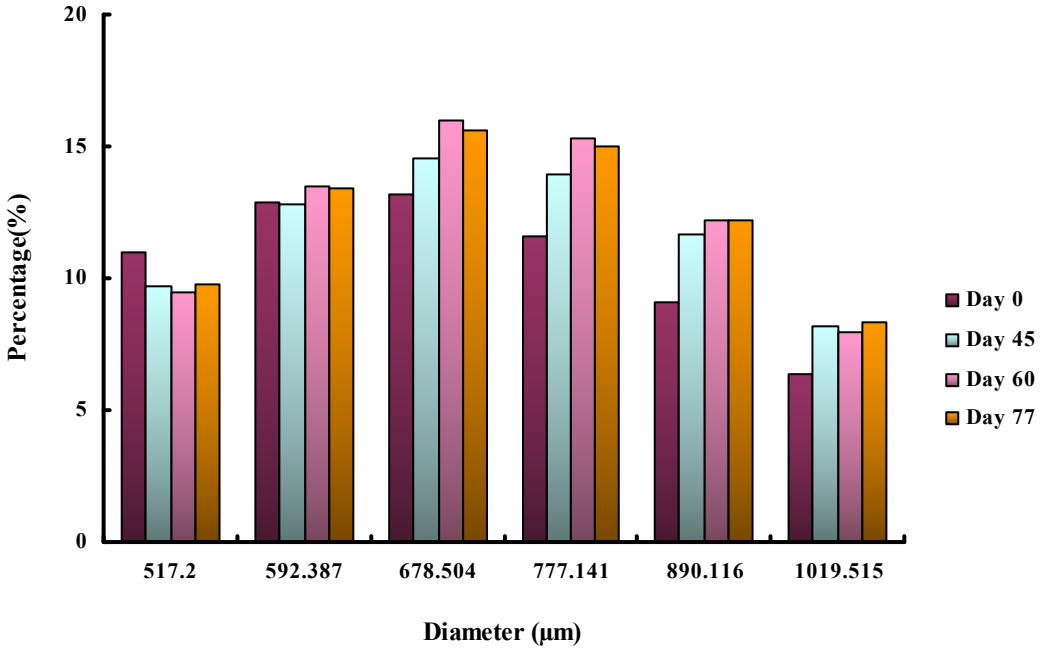
With respect to the nitrogen removal performance in this study, a high NLR of 4.5 ± 0.1 kg-$Nm^{-3}day^{-1}$ was obtained at a salt concentration of 30 g/L, as shown in Table 5-2. This result is most likely due to the high initial sludge concentration and the high level of SAA. The high concentration of anammox sludge, about 11 g-MLSS L^{-1}, provided the foundation for stable performance at the high salt concentration of 30 g/L (Liu et al., 2009). The high SAA of 0.8 g-N $(g\text{-MLSS})^{-1}day^{-1}$ is considered to be beneficial for the adaption of anammox sludge to the high salt concentration (Kartal et al., 2006). However, we cannot exclude the possibility that the special structure of the reactor contributed significantly to its stable nitrogen removal performance. It was suggested that the novel GSS provides hydrodynamic shear stress and avoids the washing out of sludge. In the reactor with spiral GSS, shear stress arose from both the nitrogen bubbles produced by the anammox process and the special inner geometry of the reactor (Liu and Tay, 2002). The anammox biomass moved along the spiral-shaped channel, which led to repeated friction and collision between the granules and the channel (Yang et al., 2010a). Furthermore, the stable performance could be attributed to the bacterial community shift in anammox sludge, especially from day 58 to day 92, due to the constant environmental condition was beneficial to the competitive bacteria. DNA evidence for this shift will be discussed in a later section

Sludge characteristics

Table 3 Comparison of anammox sludge characteristics

Time	Diameter (μm)	SVI_{30} (mL/g)	MLVSS/MLSS
Seed sludge	629	19.1	0.78
Day 45	639	12.4	0.70
Day 90	638	8.4	0.42

Note: mixed liquor volatile suspended solid (SVI).



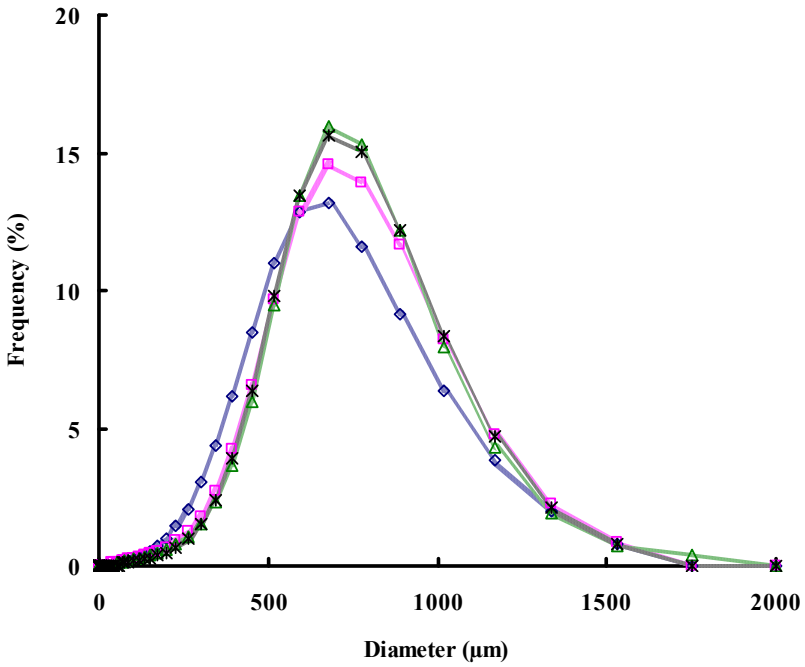


Fig. 2 Changes of granular size in anammox reactor on day 0, day 45, day 60 and day 77.
Symbols: ◇, Day 0; □, Day 45; △, Day 60; ✶, Day 77.

As anammox bacteria are vulnerable to being washed out from the reactor by intense gas production, especially at high NRR, challenges encountered during the operation must be addressed. The addition of salt in the influent was expected to improve the physical properties of anammox biomass. Investigation of the physical properties of anammox biomass over the course of operation could allow a better understanding of the reactor's ability to retain anammox sludge under high salinity. In this study, the granule size remained constant with no obvious changes in the granule size distribution, especially during the final 50 days of operation (Fig. 2).

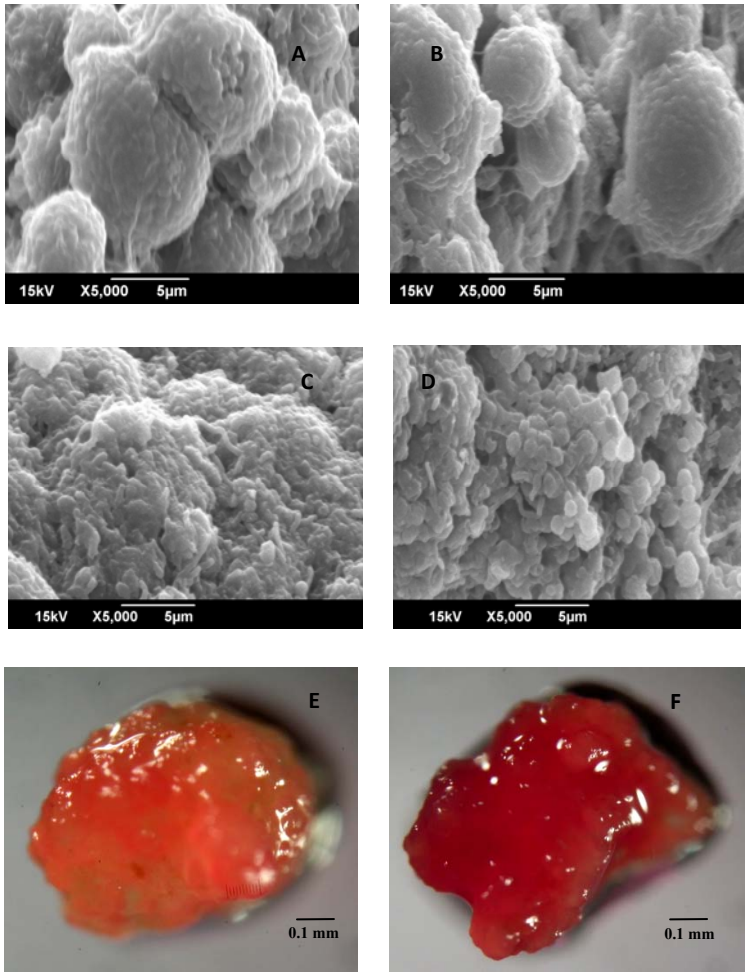


Fig. 3 Outer appearance (A) and Cross-section image (B) of an anammox granule on day 0; Outer appearance (C) and Cross-section image (D) of an anammox granule on day 90; Stereo-microscopic pictures of granules on day 0 (E) and day 90 (F)

Flotation of anammox sludge was previously observed by Strous et al. (1997) in a fixed-bed reactor, due to a combination of clogging and intense N_2 production, which caused nitrogen gas bubbles to become entrapped in the flocs, lifting up clusters of agglomerated beads. The formation of granules could benefit both the

anammox bacteria and the effluent quality by significantly enhancing the settling property of the biomass (Ni et al., 2010). Conditions under which the salt concentration as well as the substrate loading and conversion are constant contributed to the steady state of granules in this study. Such a steady state of granules is therefore responsible for the stable performance of the anammox process. It is possible that the granules are protected from intense gas bubbles by either the capture and degradation of dissolved salt in the media by the anammox biomass or by friction and collision between the granules and the dissolved salt. Consequently, the addition of salt was thought to be a somewhat effective method for the prevention of granule flotation. Dapena-Mora et al. (2010) demonstrated that the sludge volume index (SVI) decreased from 80 to 25 mL $gVSS^{-1}$ and the physical properties of the sludge changed from flocculent to granular when NaCl was added to the feeding media. A similar result was obtained in this study, with the SVI decreasing from 19.1 to 8.4 mLg^{-1} (Table 3). Besides, after 92 days of enrichment, the granules had a more well-defined and denser inner structure (Fig. 3 C) than the seed sludge (Fig. 3 A). Simultaneously, the mean diameter of the anammox granules remained constant compared with the seed sludge, indicating the high density of anammox granules. Within 90 days, the visual appearance of biomass changed from red to reddish-brown granular sludge and evolved into micro-granules (Fig. 3 E and Fig. 3 F). The changes in morphological characteristics of biomass were also verified by SEM. There were many obvious cavities inside and on the surface of the seed sludge (Fig. 3 A and Fig. 3 B), suggesting that nitrogen bubbles attached to the outer and inner layers of the granules. On day 90 (Fig. 3 C and Fig. 3 D), the sludge had a smooth surface and dense inner structure, indicating that well-defined particles were formed through enrichment culture. The mixed liquor volatile suspended solid (MLVSS) to MLSS ratio of biomass decreased from 0.78 to 0.42, suggesting the capture of inorganic salt by the anammox granules that were assumed to occur based on the self-aggregation of biomass (Dapena-Mora et al., 2010).

DNA analysis

Table 4 Microbial community in anammox reactor

OTU	Taxon	Accession	Identity (%)	Day 0	Day 92
1	*Uncultured anoxic sludge bacterium KU2*	AB054007	100-99	24 (24/32)	19 (19/52)
	Candidatus Kuenenia stuttgartiensis	CT573071	100-99		
2	*Uncultured bacterium clone: AnSal-04*	AB434256	100-99	0 (0/52)	5 (5/52)
	Uncultured bacterium clone: AnDHS-2	AB430333	99		
	Uncultured bacterium clone Ge64	FJ710714	99		
3	*Uncultured bacterium clone H2SRC239x*	FM213063	100-99	0 (0/52)	5 (5/52)
	Uncultured bacterium clone B38	EU234164	100-99		
	Uncultured bacterium clone KD3-123	AY188302	97		

An analysis of the microbial community by 16S rRNA was used to investigate the dominant species in the anammox reactor. Table 4 shows the cloning analysis of the 16S rRNA gene sequences of samples. Nineteen of 52 clones (36.5%) belong to *KU2* strains that are affiliated with the anammox bacteria after 92 days of culture in this study. Of the 52 unique sequences demonstrated by the DNA analyses, 5 were identified as clones of *AnSal-04* (AB434256), *AnDHS-2* (AB430333) and *Ge64* (FJ710714), which are possibly affiliated with *candidate division OP10*. A similar DNA result was also detected in an anammox reactor by Crocetti et al. (2002). The dominant *KU2* (AB054007) species strains in the microbial community, which belong to the genus *anammoxosome*, were assumed to be responsible for the high rate of nitrogen removal under high salinity conditions (Yang et al., 2010b). Kartal et al., (2006) also noted that the freshwater species *Candidatus Kuenenia stuttgartiensis* contributed to the activity of the biomass and could adapt to salt concentrations as high as 30 g/L and sustain anammox activity. However, after cultivating the microbial community with the addition of salt for 92 days, the population of *KU2* was substantially decreased, indicating that high influent salt concentration in the influent is not favorable for the enrichment of *KU2*. Besides, *unidentified bacteria* of operational taxonomic unit (OTU2), which perhaps belong to *Candidate division OP10*, were considered to have a competitive advantage over other bacterial strains present in the sludge at a salt concentration of 30 g/L (Liu et al., 2009) and have characteristics that appear to be beneficial to an anammox community capable of effective nitrogen removal. Long-term cultivation of these kinds of unidentified bacteria under high salt concentrations is still poorly understood and should be investigated in the near future.

Conclusion

In the present study, the treatment performance of anammox reactor and the characterization of its sludge under high salt concentration were investigated. With the addition of 30 g/L NaCl during the final 35 days of operation, a stable NRR of 4.5 ± 0.1 kg-$Nm^{-3}day^{-1}$ was maintained, indicating that the enriched anammox consortium successfully adapted to high salt concentrations. In addition, the experiments presented herein confirmed that high salt concentration could improve the physical properties of reactor biomass. Furthermore, DNA analysis illustrated that *KU2* and OTU2 were dominant species in the anammox reactor, suggesting that *KU2* and OTU2 had established symbiotic relationship under high salinity conditions. This successful operation of anammox reactor under high salt conditions shows that the anammox process is applicable to the treatment of high-salinity wastewater. The obtained high NRR and such high salt concentration (30 g/L) must have close relationship with initial sludge concentration, high SAA, special structure of the reactor and the microbial community.

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