[Japanese Journal of Water Treatment Biology Vol.46 No.2 109-117 2010]

High Ammonium Wastewater Treatment of Stirred Tank Anammox Reactor using Polyvinyl Alcohol/Alginate Gel as Biomass Carrier

LAI MINH QUAN*, TRAN THANH LIEM, DO PHUONG KHANH and KENJI FURUKAWA

Graduate school of Science and Technology, Kumamoto University
/2-39-1 Kurokami, Kumamoto 860-8555, Japan

Abstract

Appropriate biomass retention in the reactor is crucial to the operation of the anaerobic ammonium oxidation (anammox) process due to the extremely slow growth rate of the bacterial population. In the present work, anammox sludge, immobilized in a poly vinyl alcohol (PVA)/sodium alginate gel, was studied to improve anammox biomass retention in the reactor. 150 m*l* of concentrated anammox sludge (33.3 g suspended solids l^{-1}) was entrapped into a PVA/alginate aqueous solution (15% w/v PVA and 2% w/v alginate) at a volumetric ratio of 1:1 that was then solidified in a solution containing 50% w/v $NaNO_3$ and 2% w/v $CaCl_2$. The anammox activity increased gradually and reached a maximum nitrogen loading rate of 9.9 kg-N m^{-3} d^{-1}, with a removal efficiency of more than 80% even at a low hydraulic retention time of 2.67 h, after 250 days of operation when the bicarbonate concentration was increased to 1.0 g l^{-1} as $KHCO_3$ from day 145. A porous structure in the PVA/alginate immobilized biomass beads was observed by scanning electron microscopic (SEM) analysis. This study shows the potential for the treatment of wastewaters containing high ammonium and low carbon, such as digester liquor, using PVA/alginate gels for whole cell entrapment of anammox sludge.

Keywords: anammox; immobilized sludge; PVA/alginate gel; wastewater treatment

INTRODUCTION

The anaerobic ammonium oxidation (anammox) process was discovered recently as the microbial pathway by which ammonium is converted into nitrogen gas, with nitrite as the electron acceptor[1]. The reaction does not require addition of an external carbon source such as methanol and it leads to the reduction of excess sludge production. In addition, about 50% of influent ammonium should be oxidized to nitrite in pre-treatment to anammox process, reducing the amount of oxygen required by 50%. In view of these advantages, there is great interest in the development of improved nitrogen removal using a combination of partial nitritation and anammox process[2-5]. The equation for the anammox reaction was previously determined experimentally as follows[6]:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad \text{(Eq-1)}$$

However, the maintenance of a sufficient amount of anammox bacteria in the reactor is not easy due to its extremely slow growth rate, low biomass yield and vulnerability to being washed out from the reactor by intense N_2 gas bubble production. Because the anammox process produces large amounts of N_2 gas under high-rate nitrogen removal, gas bubbles become trapped in the anammox biomass, causing it to float[7]. Taking these factors into account, the use of immobilized

*Corresponding author

anammox biomass is a potential method for obtaining stable and high rates of nitrogen removal.

Gel entrapment techniques for activated sludge have been evaluated by other reports[8-11]. Nitrifying bacteria immobilized in a gel carrier are easily separated from the liquid in the reactor and this yields prolonged biomass retention time even with a short hydraulic retention time (HRT). The success of gel entrapment was shown by its application with activated sludge in 11 sewage treatment plants and 20 industrial water treatment plants in Japan and the USA[12]. In lab-scale experiments, whole cell entrapment of anammox sludge was studied using polymeric materials (poly ethylene glycol-PEG)[12, 13]. In these reports, high and stable anammox treatment performance was achieved with feeding not only of synthetic wastewater but real wastewater as digester liquor. In particular, unexpected results derived from treatment of digester liquor with a high suspended solid (SS) concentration of 3,000 mg l^{-1} [13] will open new application fields for anammox treatment of wastewater with high colloidal SS concentrations. However, application of immobilized anammox sludge using a poly vinyl alcohol (PVA) gel for nitrogen removal from wastewater has not been widely reported. In this study, a PVA gel for whole cell entrapment of anammox sludge was used. PVA is a promising synthetic polymer that is cheap and nontoxic to microorganisms. In addition, PVA gel beads have porous microstructures, which allows microorganisms to penetrate and colonize, thus providing favorable conditions for retention and cultivation of slow growing anammox bacteria. However, some problems emerged when using the PVA-boric acid, PVA-freezing/thawing method for immobilization of anammox cells, such as the agglomeration of PVA beads, the toxicity of boric acid, the negative effects of extremely low temperatures and the swelling performance. Improvement of PVA by combination with sodium alginate as a material for immobilization of anammox cells could solve these problems[14, 15].

In many reactors, such as fixed-bed or packed-bed reactors, much of the reactor volume is not effectively used due to either the high nitrite concentration near the inlet or very low substrate concentrations in other parts. In addition, clogging is a challenge in fixed bed reactors. Conversely, immobilized reactors with complete mixing conditions are powerful tools for solving these challenges. Consequently, the stirred tank reactor (STR) with complete mixing conditions was used. The PVA gel beads have improved contact with substrate under mixing conditions and N_2 gas is more easily separated from the process. The goal of this research was to evaluate the nitrogen removal capability of anammox sludge immobilized in a PVA gel.

MATERIALS AND METHODS

Seed anammox sludge Anammox sludge which had been cultivated in our laboratory was used as the seed sludge for anammox pellets. This enriched sludge was grown in 50 l fixed-bed reactor filled with a polyester non-woven fabric carrier at 36℃[16]. The enriched anammox sludge collected from the bottom of reactor was suspended into the effluent from the anammox reactor and was then concentrated by centrifuge. The concentrated anammox sludge characteristics were 33.3 g-SS l^{-1} and 25.2 g-VSS (volatile suspended solids) l^{-1}, respectively.

Immobilization technique PVA/sodium alginate was prepared with PVA-HC (100% saponification, Kuraray Co. Ltd, Osaka, Japan) concentration of 15% (w/v) and sodium alginate (Wako Pure Chemical Industries Ltd., Osaka, Japan) concentration of 2% (w/v). This mixture was heated by autoclave at 115℃ for 20 minutes until dissolved. The mixture was then cooled to room temperature and 150 ml of concentrated anammox sludge, equivalent to 5 g dry SS, was added slowly into the PVA solution to a final volumetric ratio of 1:1. The final concentration of PVA, sodium alginate and anammox sludge were 7.5% (w/v), 1% (w/v) and 1.67% (w/v), respectively. The mixture of PVA/sodium alginate and anammox sludge was dropped slowly into solidifying solution (50% w/v $NaNO_3$ and 2% w/v $CaCl_2$) by syringe to make spherical beads. PVA gel beads were then immersed in a solidifying solution for 4 h at room temperature to increase their

mechanical strength ("harden" the beads). The PVA gel beads were then washed with a large amount of distilled water and used for continuous experiments. Fig. 1 shows the whole cell entrapment procedure using PVA/alginate gel.

Synthetic medium A synthetic medium was used for the continuous feeding test. The medium contained (per liter): $(NH_4)_2SO_4$, 50–550 mg; $NaNO_2$, 50–550 mg; $KHCO_3$, 125–1,000 mg; KH_2PO_4, 27.2 mg; $MgSO_4$, 300 mg; $CaCl_2$, 180 mg; and 1 m*l* trace element solutions 1 and 2. Trace element solution 1 contained (per liter): ethylenediamine tetraacetic acid (EDTA), 5 g and $FeSO_4$, 5 g. Trace element solution 2 contained (per liter): EDTA, 15 g; $ZnSO_4.7H_2O$, 0.43 g; $CoCl_2.6H_2O$, 0.24 g; $MnCl_2.4H_2O$, 0.99 g; $CuSO_4.5H_2O$, 0.25 g; $NaMoO_4.2H_2O$, 0.22 g; $NiCl_2.6H_2O$, 0.19 g; $NaSeO_4.10H_2O$, 0.21 g and H_3BO_4, 0.014 g. The influent pH was adjusted in the range of 6.9–7.1 by adding 1N hydrochloric acid.

Reactor and experimental setup The stirred tank reactor (STR) with a total volume of 1.2 *l* and reaction volume of 1.0 *l* used for determination of hydraulic retention time (HRT) was used in continuous experiments (Fig. 2). Two reactors (Reactor 1 and Reactor 2) with the same characteristics and operational strategy except for influent bicarbonate concentration were used to confirm the effect of inorganic carbon concentration on anammox activity.

300 m*l* of gel carriers containing anammox sludge concentration of 1.67% w/v were placed inside the reactor. Considering the reaction volume was 1.0 *l* and the packing ratio of gel carriers was 30%, inoculated anammox sludge was 5 g-SS l^{-1} reactor. The reactor temperature was maintained at 33–35℃, controlled thermostatically with a water jacket. The reactor pH was not controlled. The gel beads were mixed continuously at an agitator speed of 80 rpm. Agitation was primarily necessary to mix the influent and to remove the nitrogen gas bubbles which formed on the surface of the gel beads. Purging with nitrogen gas was used to keep the dissolved oxygen (DO) level in the influent below 0.5 mg l^{-1}. In addition, small polystyrene balls were placed in the feed storage tank to retard oxygen transfer to the synthetic wastewater. Light is known to have a negative effect (30–50% reduction) on ammonium removal rate[17], so darkness was maintained using black vinyl sheet enclosures.

Chemical analyses Determinations of NO_2–N and NO_3–N were in accordance with Standard Methods[18], using the colorimetric (4500-NO^-_2 B) and UV (4500-NO^-_3 B) screening methods, respectively. Nitrite was determined to have an interfering response in the nitrate UV screening method of 25% of the nitrate response on a nitrogen weight basis, thus results were calculated by the following equation:

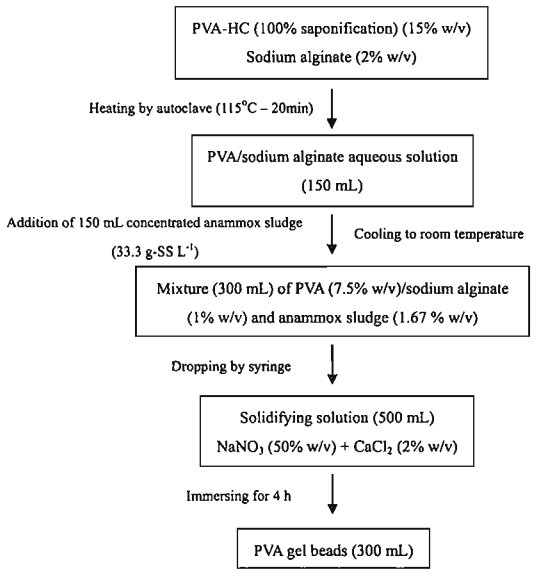

Fig. 1 Flow chart of whole cell entrapment procedure using PVA/sodium alginate

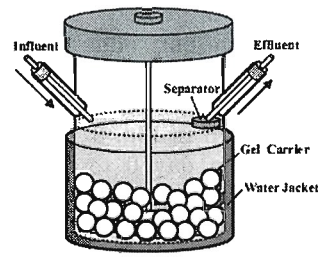

Fig. 2 Schematic of anammox reactor used for continuous treatment

$$[NO_3\text{–}N]_{corrected} = [NO_3\text{–}N]_{UV\text{–}measured} - 0.252 \times [NO_2\text{–}N] \quad \text{(Eq–2)}$$

Ammonium was quantified based on the indophenol reaction with ortho-phenylphenol (OPP)[19]. Total nitrogen was calculated as the sum of ammonium, nitrite and nitrate concentrations. Absorbance, pH, and DO were measured using a spectrophotometer (U–1900, Hitachi High Technologies Corporation, Tokyo, Japan), a pH meter (F–55, Horiba Ltd, Kyoto, Japan) and a DO meter (D–55, Horiba Ltd), respectively.

Scanning electron microscope (SEM) observation of porous structures of PVA gel beads A PVA gel was cut into 1–2 mm pieces and washed by 0.1M phosphate buffer (pH 7.4) twice for 5 min each. The PVA gel pieces were then fixed by 2.5% glutaraldehyde solution prepared with 0.1M phosphate buffer for 1–2 hours and washed by 0.1M phosphate buffer three times for 10 min each. The samples were then fixed by 1.0% OsO_4 solution prepared with 0.1M phosphate buffer and washed again by 0.1M phosphate buffer three times for 10 min each. Subsequently, the samples were dehydrated in serially graded ethanol solution at concentrations of 10, 30, 50, 70, 90 and 95% for 5–15 min each, and at a concentration of 99.5% twice for 30 min each. The samples were frozen in a freezer then dried by a freeze-drying device (JEOL JFD-300) and sputter-coated with gold for 100 s by an ion sputtering device (JEOL JFC–1100E). Finally, the samples were observed by SEM (JEOL JSM 6390LV).

RESULTS

Nitrogen removal performance The STR (Reactor 1) was started up with a feed nitrogen loading rate of 0.2 kg–N m^{-3} d^{-1}. The entire operation was divided into 2 phases, each with a different added bicarbonate concentration. The time courses of influent and effluent nitrogen concentrations during operation are shown in Fig. 3.

In phase 1 (from day 0 to day 145), the NLR was increased by adjusting the influent nitrogen concentration as well as the HRT to the desired level. For the first 25 days, the NLR was increased by an increase in influent concentration while the HRT was kept

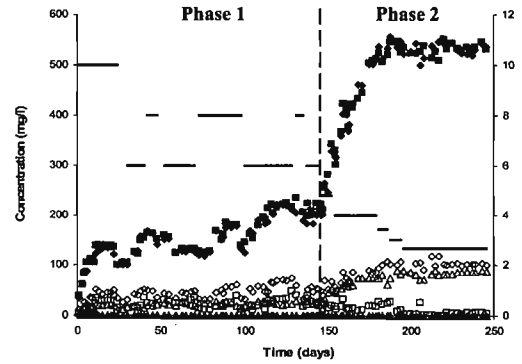

Fig. 3 Time courses of influent and effluent concentrations of nitrogenous compounds. Inf. NH_4–N (closed diamond); Inf. NO_2–N (closed square); Inf. NO_3 (closed triangle); Eff. NH_4–N (open diamond); Eff. NO_2–N (open square); Eff. NO_3–N (open triangle); HRT (solid line)

constant at 10 h. Anammox bacteria gradually adapted to the increase in NLR and a maximum removal rate of 0.45 kg–N m^{-3} d^{-1} was obtained. Consequently, the HRT was decreased to 6 h to increase the NLR. However, due to a poor adaptation of anammox activity to the change of HRT, the removal efficiency fluctuated around values less than 70%. Thus, the HRT was increased to 8 h to avoid the negative effects of the high NLR. After a very short time, the anammox activity recovered and a removal efficiency of more than 72% was obtained. Once again, the HRT was shortened to 6 h to enhance anammox performance. Unfortunately, from days 52 to the end of Phase 1, the nitrogen removal performance was not as high and stable as expected. In summary, a relatively low removal efficiency of 71% was obtained with a maximum removal rate of 1.4 kg–N m^{-3} d^{-1} at a NLR of 1.8 kg–N m^{-3} d^{-1}. In spite that the NLR and removal efficiency of the anammox reactor was not so high, the pH effluent variation of 7.3 – 8.6 was occurred. It could be considered as an inhibitory factor to anammox activity.

Due to the poor nitrogen removal performance from Phase 1, the bicarbonate concentration was increased from 0.125 g l^{-1} to 1.0 g l^{-1} as $KHCO_3$ from day 145 (Phase 2) to investigate the effect of inorganic carbon on anammox activity. Instantaneously, much better performance could clearly be seen

after a very short time. At the beginning of Phase 2, the NLR was 2.0 kg-N m^{-3} d^{-1} corresponding to influent total nitrogen (TN) of 400 mg-N l^{-1} and a HRT of 4.8 h. After 40 days of Phase 2, the influent TN concentration was increased to a maximum level of 1,100 mg-N l^{-1} while the HRT decreased to 2.7h corresponding to a NLR of 9.9 kg-N m^{-3} d^{-1}. A relatively high TN removal efficiency of more than 80% was obtained with ammonium and nitrite removal efficiencies of 80±3% and 95±4%, respectively. This condition was maintained until the end of the experiment. At the end of the experiment, the maximum removal rate of 8.2 kg-N m^{-3} d^{-1} was obtained at a NLR of 9.9 kg-N m^{-3} d^{-1}. The pH effluent value was range of 7.9 - 8.2 which was more stable compared to that of phase 1. The time courses of TN removal efficiency, loading rate and removal rate are shown in Fig. 4.

Stirred tank reactors have also been used as a strategy to obtain stable treatment at high TN concentrations in comparison with other reactors. In many reactors such as fixed-bed or packed-bed reactors, much of reactor volume is not effectively used due to either a high nitrite concentration near the inlet or very low substrate concentrations in other parts of the reactors. In this study, a very high influent TN concentration of 1,100 mg l^{-1} was treated stably without problems. This advantage can be explained by the complete mixing condition of the reactor, which provides a uniform substrate concentration inside the reactor. Consequently, anammox activity was not negatively affected by nitrite accumulation in the reactor.

To confirm the role of bicarbonate concentration on anammox performance, another reactor (Reactor 2) was operated with almost the same characteristics as Reactor 1 except that a bicarbonate concentration of 1.0 g l^{-1} as $KHCO_3$ was added from the beginning of the experiment. The same trend of nitrogen removal as seen in phase 2 of Reactor 1 was observed in Fig. 5. The initial NLR applied to Reactor 2 was 0.2 kg-N m^{-3} d^{-1}, the same as that of Reactor 1. Anammox bacteria adapted very quickly to the increase in NLR and reached a maximum removal rate of 8.2 kg-N m^{-3} d^{-1} at a NLR of 9.9 kg-N m^{-3} d^{-1} after only 120 days of operation.

Stereomicroscope and SEM observation of the porous structure of PVA gel beads Fig. 6 shows the immobilized anammox sludge in PVA gel beads. Compared with the original appearance of PVA beads, a large amount of anammox bacteria was attached on the surface as well as in the center of the beads after 200 days of operation. The extraordinary merit of this material for stable retention of anammox sludge is clearly shown.

Scanning electron microscopic analysis was also carried out to observe the outer and inner structure of PVA gel bead (Fig. 7). The SEM images of PVA gel bead entrapped anammox sludge were sampled on day 200.

By comparison with SEM images of PVA beads at the beginning of experiment, it can

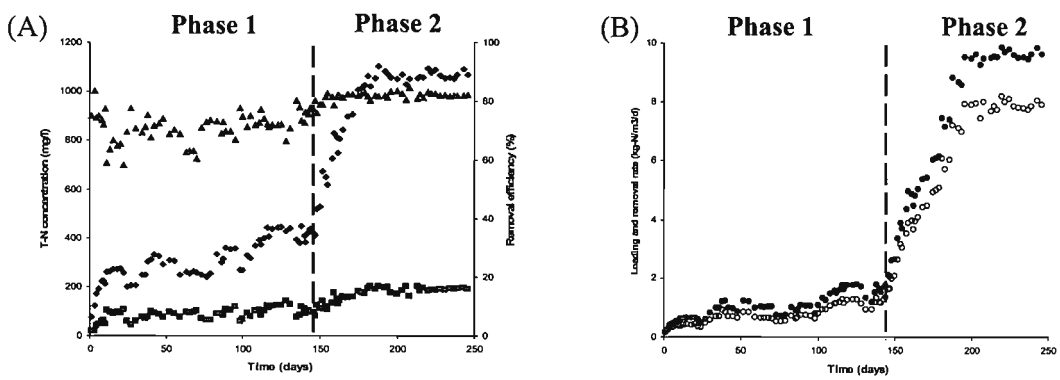

Fig. 4 Time courses of influent and effluent TN, TN removal efficiency (A), nitrogen loading and removal rate (B) of Reactor 1. Inf. TN (closed diamond); Eff. TN (closed square); Removal efficiency (closed triangle); Nitrogen loading rate (closed circle); Nitrogen removal rate (open circle)

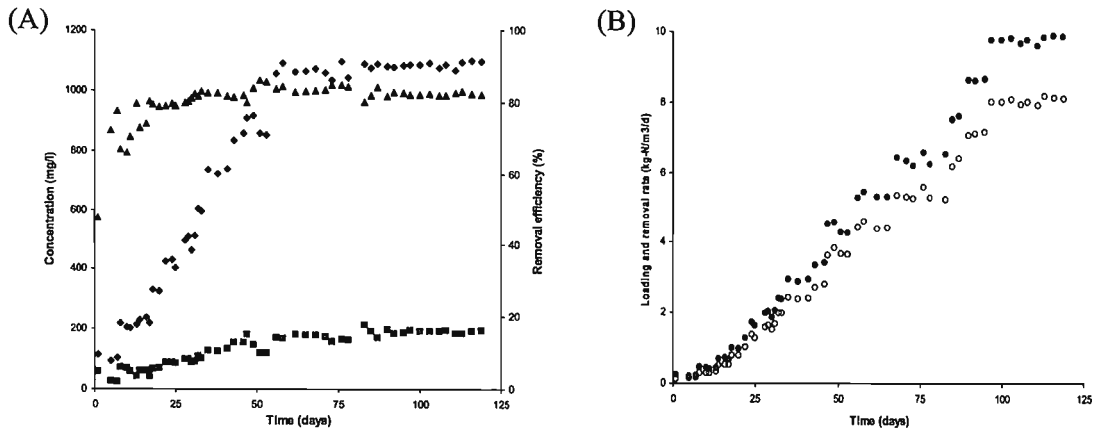

Fig. 5 Time courses of influent and effluent TN, removal efficiency (A), nitrogen loading and removal rate (B) of Reactor 2. Inf. TN (closed diamond); Eff. TN (closed square); Removal efficiency (closed triangle); Nitrogen loading rate (closed circle); Nitrogen removal rate (open circle)

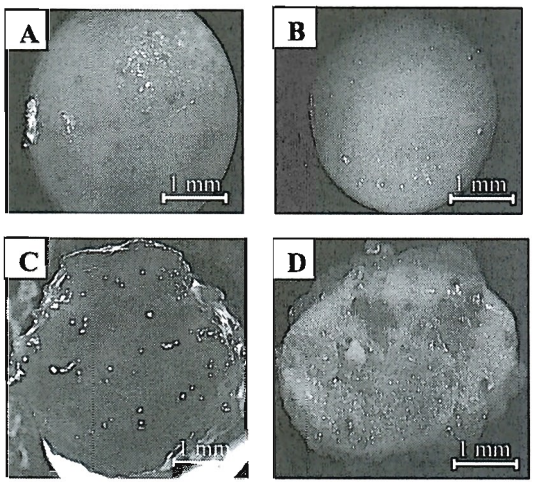

Fig. 6 Stereomicroscopic pictures of surface and cross-section of PVA gel bead at the beginning of experiment (A, B) and on day 200 (C, D)

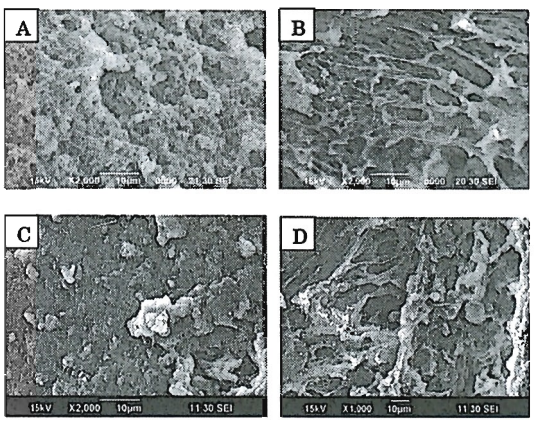

Fig. 7 SEM observation of surface part and inside of PVA bead at the beginning of experiment (A, B) and on day 200 (C, D)

be seen in the SEM images of PVA beads on day 200 that the microcosmic structure of the carrier is further optimized (Fig. 7). When the complex PVA/alginate hydrogels were dripped into $CaCl_2$ solution, Ca^{2+} ions in solution replaced Na^+ to form calcium alginate which would harden the complex beads. Because of the inter-solubility between sodium alginate and PVA, the calcium alginate gels could restrict the fluidity of PVA molecules, enhancing the agglomeration between PVA molecules and, to some extent, enhancing the density of crosslinking sites and controlling the pore size of PVA gels. As indicated in Fig. 7, many micropores, suitable for growth of immobilized anammox sludge, could be seen clearly.

DISCUSSION

In previous reports, anammox enriched sludge with different carriers, such as PVA gel beads, glass beads and PEG gel cubes, was used to start up the anammox reactor. Table 1 lists the immobilization methods and nitrogen removal performance in different anammox reactors. Tran *et al.* showed that a

Table 1 Comparison of anammox activity in different types of reactors

Reactors	Carrier	Initial sludge conc. (per L reactor)	HRT (h)	Conversion rate (kg-N m^{-3} d^{-1})	References
FBR (phase 1)	PVA gel beads	Not shown	12 – 9	1.3	20)
FBR (phase 2)	PVA gel beads	Not shown	9 – 3	3.0	20)
Fix bed reactor	Glass beads	Not shown	23 – 6	1.1	7)
Stirred tank reactor	PEG gel entrapment	0.72 g dry-SS	2	3.7	12)
Stirred tank reactor	PEG gel entrapment	4.7 g dry-SS	2	3.4	12)
Stirred tank reactor	PVA gel entrapment	5.0 g dry-SS	10 – 2.7	8.2	This study

FBR: fluidized bed reactor

successful nitrogen removal maximum of 3.0 kg-N m^{-3} d^{-1} was obtained in a fluidized bed reactor using PVA gel beads (Poval, Kuraray Co. Ltd, Osaka, Japan)[20]. However, it took more than one year to attach the anammox biomass to the surface of PVA gel beads in a packed-bed reactor and consequently, it took 500 days to reach this high removal rate in the fluidized bed reactor. On the other hand, Isaka *et al.* used PEG gels for whole cell entrapment of anammox sludge[12]. They reported that it took 67 days and 25 days to obtain a removal rate of 3.7 kg-N m^{-3} d^{-1} with an initial anammox sludge amount of 0.72 g and 4.7 g dry SS. According to another report, Hsia *et al.* used the PVA/alginate immobilization method but only conducted a batch experiment in their research so it is difficult to use their study for comparison[15]. In our study, an initial anammox sludge amount of 5.0 g dry SS, which is the same as that used by Isaka *et al.*[12], was fed to an anammox reactor and a removal rate of 3.7 kg-N m^{-3} d^{-1} was obtained after about 50 days (Fig. 5), slower than that of Isaka *et al.*[12]. However, we think that the aim of our study and theirs might be different. In our study, a high and stable performance was investigated but the potential maximum nitrogen removal after the shortest operational time was their goal. If we operated anammox reactor as their strategy, the same removal performance could be obtained. Finally, a maximum removal rate of 8.2 kg-N m^{-3} d^{-1} was confirmed after 250 days of operation in our study. This result demonstrates that whole cell entrapment by PVA gel is one of the most effective techniques for immobilizing anammox bacteria.

From Fig. 6, we can see that anammox sludge existed in the center of PVA gel bead from the beginning of experiment and significantly increased after 200 days of operation. This point indicated that anammox sludge not only was kept but also grew up inside gel bead which is important to attain sufficient amount of anammox sludge for start-up period. On the other hand, whole cell entrapment using PVA/alginate with presence of solidifying solution could make the spherical bead which is considered to be suitable for wastewater treatment reactor because the contact between gel bead and feeding substances is better. In addition, the strength of PVA bead under mixing condition was confirmed as almost of them was not broken during operational time. Taking these points into account, the use of PVA/alginate gel with presence of solidifying solution should be considered as a potential method for anammox sludge immobilization.

The role of bicarbonate concentration on increase of anammox activity could be explained as follows. The increase of anammox activity was due to suppression of pH increasing in the reactor. It was known that the higher pH strongly decrease the anammox activity[6]. The pH in the anammox reactors is the most important factor in order to attain higher nitrogen removal performance because the pH in the anammox reactor increase by the increase of nitrogen concentration and anammox activity. The pH should therefore be controlled in the reactor. In our study, an anammox reactor was operated without any pH control; therefore; bicarbonate addition in the influent was used as another way to suppress pH increasing in the reactor. The reason why anammox activity was increased by bicarbonate addition

is the buffer effect of pH by high bicarbonate concentration. Hydrochloride acid was added to adjust influent pH when bicarbonate concentration increased, it means that the influent has better buffer effect of pH and is not easily increase and/or decrease the pH. As a result, the variation of effluent pH which reduces anammox activity was suppressed (from 7.3 - 8.6 in phase 1 to 7.9 - 8.2 in phase 2)[21]. In addition, bicarbonate should be considered as the main carbon source for anammox bacteria. The concentration of inorganic carbon resulted from a balance of assimilation, respiration and CO_2 stripping because of N_2 gas generation by anammox reaction. At low bicarbonate concentration, N_2 gas production caused CO_2 stripping and consequently a decrease of the available inorganic carbon occurred[22] leading to the lack of carbon source.

Assessed stoichiometric characteristics of the anammox reaction Fig. 8 indicates the relationship between TN removal, NO_2^- removal and NO_3^- production to NH_4^+ removal during the entire anammox treatment.

The ratios of TN removal, NO_2^- removal and NO_3^- production to NH_4^+ removal were 2.02:1.21:0.19, which was close to the experimental stoichiometry for anammox[6]. However, a relative low NO_3^- of 0.19 could be assumed by heterotrophic denitrification using decayed organisms as the carbon source (endogenous denitrification)[13].

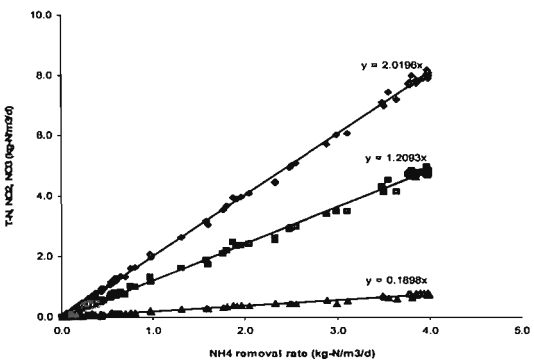

Fig. 8 TN removal, NO_2^- removal and NO_3^- production as functions of NH_4^+ removal during the entire experiment. TN removal (closed diamond); NO_2^- removal (closed square); NO_3^- production (closed triangle)

This study investigated the nitrogen removal performance using PVA/alginate gel entrapped anammox sludge. A high TN removal rate of 8.2 kg-N m^{-3} d^{-1} was obtained at a NLR of 9.9 kg-N m^{-3} d^{-1}. It was also demonstrated that bicarbonate addition could positively affect anammox activity due to better buffer effect of pH. It was confirmed by operation of reactor 2 and during operation phase 2 of reactor 1 that a bicarbonate concentration of 1.0 g l^{-1} as $KHCO_3$ could effectively improve anammox treatment performance. SEM analysis showed that there were many micropores, suitable for the growth of immobilized anammox sludge, in the beads. In addition, anammox sludge existed not only on the surface but also in the center of PVA gel beads. The high and stable anammox treatment performance obtained in this study demonstrates the usefulness of whole cell entrapment using PVA/alginate gel.

ACKNOWLEDGEMENTS

We would like to thank Kuraray Co. Ltd for their PVA material supporting.

REFERENCES

1) **Mulder, A., Van de Graaf, A.A., Robertson, L.A., and Kuenen, J.G.**: Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor, FEMS Microbiol. Ecol., **16**, pp. 177-184 (1995)

2) **Van Dongen, L.G.J.M., Jetten, M.S.M., and Van Loosdrecht, M.C.M.**: The SHARON-Anammox process for the treatment of ammonium rich wastewater, Water Sci. Technol., **44**, pp. 153-160 (2001)

3) **Fuji, T., Sugino, H., Rouse, J.D., and Furukawa, K.**: Characterization of the microbial community in an anaerobic ammonium oxidizing biofilm cultured on a nonwoven biomass carrier, J. Biosci. Bioeng., **94**, pp. 412-418 (2002)

4) **Fux, C., Boehler, M., Huber, P., Brunner, I., and Siegrist, H.**: Biological treatment of ammonium rich wastewater by partial nitritation and subsequent anaerobic ammonium oxidation (anammox) in a pilot plant, Biotechnology, **99**, pp. 295-306 (2002)

5) **Schmidt, I., Sliekers, O., Schmid, M., Bock, E., Fuerst, J., Kuenen, J.G., Jetten, M.S.M., and Strous, M.**: New concept of microbial treatment process for the nitrogen removal in wastewater, FEMS Microbiol. Rev., **27**, pp. 481-492 (2003)

6) **Strous, M., Heijnen, J.J., Kuenen, J.G., and Jetten, M.S.M.**: The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms, Appl. Microbiol. Biotechnol., **50**, pp. 589-596 (1998)

7) **Strous, M., Van Gerven, E., Zheng, P., Gijs Kuenen, J., and Jetten, M.S.M.**: Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (anammox) process in different reactor configurations, Water Res., **31**, pp. 1955-1962 (1997)

8) **Hashimoto, S. and Furukawa, K.**: Immobilization of activated sludge by PVA-Boric acid method, Biotechnol. Bioeng., **30**, pp. 52-59 (1987)

9) **Asano, H., Myoga, H., Asano, M., and Toyao, M.**: A study of nitrification utilizing whole microorganisms immobilized by the PVA-freezing method, Water Sci. Technol., **26**, pp. 1037-1046 (1992)

10) **Sumino, T., Nakamura, H., and Mori, N.**: Immobilization of activated sludge by polyethylene glycol prepolymer, J. Ferment. Bioeng., **73**, pp. 37-42 (1992)

11) **Vogelsang, C., Husby, A., and Osgaard, K.**: Functional stability of temperature-compensated nitrification in domestic wastewater treatment obtained with PVA-SBQ/alginate gel entrapment, Water Res., **31**, pp. 1659-1664 (1997)

12) **Isaka, K., Date, Y., Sumino, T., and Tsuneda, S.**: Ammonium removal performance of anaerobic ammonium oxidizing bacteria immobilized in polyethylene glycol gel carrier, Appl. Microbiol. Biotechnol., **76**, pp. 1457-1465 (2007)

13) **Furukawa, K., Inatomi, Y., Qiao, S., Quan, L., Yamamoto, T., Isaka, K., and Sumino, T.**: Innovative treatment system for digester liquor using anammox process. Bioresource Technol., **100**, pp. 5437-5443 (2009)

14) **Chang, C.C. and Tseng, S.K.**: Immobilization of Alcaligenes eutrophus using PVA crosslinked with sodium nitrate, Biotechnol. Tech., **12**, pp. 865-868 (1998)

15) **Hsia, T.H., Feng, Y.J., Ho, C.M., Chou, W.P., and Tseng, S.K.**: PVA-alginate immobilized cells for anaerobic ammonium oxidation (anammox) process, J. Ind. Microbiol. Biotechnol., **35**, pp. 721-727 (2008)

16) **Furukawa, K., Rouse, J.D., Bhatti, Z., Imajo, U., and Ishida, H.**: Anaerobic oxidation of ammonium confirmed in continuous flow treatment using nonwoven biomass carrier, Japan J. Water Treat. Biol., **38**, pp. 87-94 (2005)

17) **Van de Graaf, A.A., De Bruijn, P., Robertson, L.A., Jetten, M.S.M., and Kuenen, J.G.**: Autotrophic growth of anaerobic ammonium oxidizing microorganisms in a fluidized bed reactor, Microbiology, **142**, pp. 2187-2196 (1996)

18) **APHA, AWWA, WEF**: Standard methods for the examination of water and wastewater, 19th edition, American Public Health Association, Washington, D.C., USA, (1995)

19) **Kanda, J.**: Determination of ammonium in sea water based on the indophenol reaction with o-Phenylphenol (OPP), Water Res., **29**, pp. 2746-2750 (1995)

20) **Tran, T.T.H, Luong, N.K., Liu, Z., Fujii, T., Rouse, J.D., and Furukawa, K.**: Nitrogen removal by immobilized anammox sludge using PVA gel as biocarrier, Japan J. Water Treat. Biol., **42**, pp. 139-149 (2006)

21) **Tang, C., Zheng, P., Mahmood, Q., and Chen, J.**: Start-up and inhibition of the Anammox process seeded with anaerobic granular sludge, J. Ind. Microbiol. Biotechnol., **36**, pp. 1093-1100 (2009)

22) **Wett B. and Rauch W.**: The role of inorganic carbon limitation in biological nitrogen removal of extremely ammonia concentrated wastewater, Water Res, **37**, 1100-1110 (2003)

(Submitted 2010. 4. 12)
(Accepted 2010. 5. 19)