ANAMMOX TREATMENT PERFORMANCE USING MALT CERAMICS AS A BIOMASS CARRIER

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Abstract

This study focuses on anaerobic ammonium oxidation (anammox) treatment performance for nitrogen removal using malt ceramics (MC) as a biomass carrier. A fixed-bed reactor using MC material with 3 to 5 mm diameter pieces (Reactor 1) was operated for 368 days and another using MC material with 10 to 15 mm diameter pieces (Reactor 2) was operated for 280 days. For Reactor 1, with an HRT of 3 h, the influent concentrations of NH_4-N and NO_2-N were both increased stepwise from 150 mg N/L to 225 mg N/L with NH_4-N removal efficiency of about 81% and NO_2-N removal efficiency of 90%. Then, the influent concentrations of NH_4-N and NO_2-N were increased to 250~275 mg N/L, each, and removal efficiencies of NH_4-N and NO_2-N decreased to 75% and 85%, respectively. The T-N removal rates of Reactor 1 increased stepwise from 0.6 to 3.1 kg N/m^3/day from day 187 to day 315. For Reactor 2, with an HRT of 3 h, the influent concentrations of NH_4-N and NO_2-N were both increased stepwise from 100 mg N/L to 225 mg N/L and removal efficiencies of NH_4-N and NO_2-N were high at 80% and 93%, respectively. However, when the influent concentrations of NH_4-N and NO_2-N were increased to 250~275 mg N/L, each, removal efficiencies of NH_4-N and NO_2-N decreased to 72% and 84%, respectively. The T-N removal rates of Reactor 2 were similar to those of Reactor 1 with 0.7 to 3.1 kg N/m^3/day from the day 146 to day 230.

Keywords: anammox, biomass carrier, fixed-bed reactor, malt ceramics, MC, NH_4-N, NO_2-N, T-N

1. Introduction

Traditional biological nitrogen removal processes using nitrification and denitrification for nitrogen removal have been widely studied. However, the newly discovered anammox (Anaerobic Ammonium Oxidation) process for nitrogen removal has only been developed over the last 10 years. This process is based on energy conversion from anaerobic ammonium oxidation using nitrite as the electron acceptor without addition of an organic carbon source[1)]. The main carbon source for the growth of anammox bacteria is carbon dioxide[2)].

The stoichiometry of the anammox reaction was determined based on mass balances using anammox enrichment cultures, as follows[3)]:

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13H^+ \rightarrow$$

$$1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \quad (1)$$

Recently, KSU-1 (AB057453), KU-1 (AB054006) and KU-2 (AB054007) anammox strains were discovered at Kumamoto University, Japan. KSU-1 is a new anammox strain with less sequence similarities to all other reported anammox planctomycetes[4)]. KU-1 and KU-2 strains are similar to

Candidatus Brocadia anammoxidans (AJ131819)[5] and anaerobic ammonium-oxidizing planctomycete KOLL2a (AJ250882)[6], respectively.

The extremely slow growth rate of anammox bacteria with a doubling time of around 11 days[3] requires long period of cultivation for getting sufficient anammox sludge. Consequently, the application of different kinds of biomass carriers for the anammox process is an issue of much attention. Novel carriers such as a non-woven material for use in an upflow column reactor[5, 7, 8] and PVA-gel beads for packed-bed[9] and fluidized-bed reactors[10] have been studied with the anammox process.

Malt ceramics (MC) is considered in this study as a new biomass carrier material. MC is produced from beer barley husk and beer dregs. The raw material of the beer dregs is dried and molded in to cylindrical shapes, which are carbonized and crushed to different sizes. In addition, manufacturing process of MC material does not require chemicals. Therefore, MC material is natural product and low cost which is possible to reduce nitrogen removal cost. In this research, 3 to 5 mm and 10 to 15 mm diameter MC material sizes are used in two fixed-bed reactors of the same configuration. The objectives of this research are to develop a novel anammox process for nitrogen removal using MC as a biomass carrier material, and to compare the nitrogen removal capabilities of the two fixed-bed reactors using 3 to 5 mm and 10 to 15 mm diameter MC material sizes by this newly developed anammox process.

2. Materials and methods

Laboratory scale experimental set-up Two fixed-bed reactors were used for two sizes of MC material, each with a total volume of 1.62 L. The MC material volume of 0.65 L was considered to be the reaction zone volume, which was used for determinations of hydraulic retention time (HRT). The clarification zone (above the reaction zone) was 0.34 L.

Influent wastewater was fed in up-flow mode using peristaltic pumps (Eyela Co., Ltd., Tokyo). Nitrogen gas was collected by using gas sampling bags. Airtight integrity was maintained in the capped reactors using effluent water traps. Reactor temperatures were maintained at 33°C to 35°C by using external ribbon heating elements. A schematic diagram of the fixed-bed reactors is shown in **Fig. 1**.

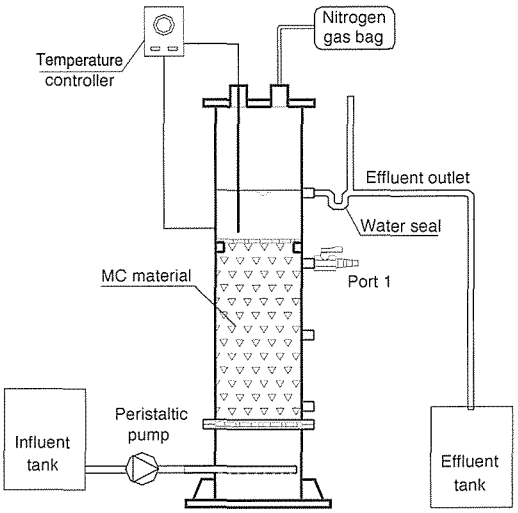

Fig. 1 Schematic diagram of fixed-bed reactor using malt ceramic as a biomass carrier

Biomass carrier materials

Two different sizes of MC material were 3-5 mm and 10-15 mm as shown in **Figs. 2a and 2b** with a specific gravity of 1.96. This material has a porous microstructure with an average porous diameter of 2.17 nm (Asahi Breweries Ltd., Ibaraki, Japan). MC material was mixed with 130 spacer glass segments (20 tubes per 100 ml of MC volume) to distribute wastewater evenly. Each spacer had an inner diameter of 4 mm, outside diameter of 6 mm and length of 25 mm.

Seed sludge

Before start-up, the MC materials were soaked in the effluent of a 50-L upflow anammox column reactor using a nonwoven biomass carrier for 3 weeks. 0.65 g of anammox sludge taken from a 50-L nonwoven anammox reactor was used as seed sludge. This seed sludge was homogenized and mixed with the MC material before start-up of the anammox reactors.

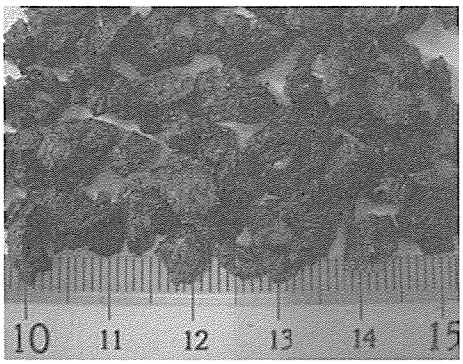

Fig. 2a Malt ceramic with 3 to 5 mm diameter pieces

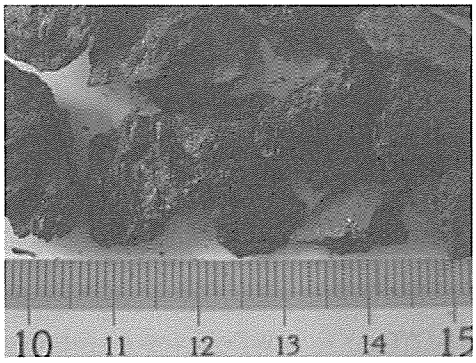

Fig. 2b Malt ceramic with 10 to 15 mm diameter pieces

Synthetic wastewater

Synthetic wastewater was prepared by adding ammonium and nitrite in the forms of $(NH_4)_2SO_4$ and $NaNO_2$, respectively to a mineral medium according to the composition given in **Table 1**. Tap water of groundwater origins was used for the preparation of this synthetic wastewater.

Table 1 Composition of synthetic wastewater

Composition	Concentration
$(NH_4)_2SO_4$ (mg N/L)	25-275
$NaNO_2$ (mg N/L)	25-275
$KHCO_3$ (mg/L)	125.1
KH_2PO_4 (mg/L)	54 .4
$FeSO_4.7H_2O$ (mg/L)	9.0
EDTA (mg/L)	5.0
Tap water (L)	1.0

Analytical methods

Ammonium concentrations were measured by the phenate method using ortho-phenylphenol as a substitute for liquid phenol[11]. In accordance with Standard Methods[12], nitrite concentrations were estimated by the colorimetric method and nitrate by the UV spectrophotometric screening method. 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 corrected by calculation. DO of influent synthetic wastewater was measured by using a DO meter (D-55, Horiba).

3. Results and discussion

Hereafter, the fixed-bed reactor using MC material with 3 to 5 mm diameter pieces is referred to as Reactor 1 and the fixed-bed reactor using MC material with 10 to 15 mm diameter pieces is referred to as Reactor 2.

Nitrogen removal performances for Reactor 1 and Reactor 2

Figs. 3a and 3b show the influent and effluent concentrations of nitrogenous compounds for Reactor 1 and Reactor 2.

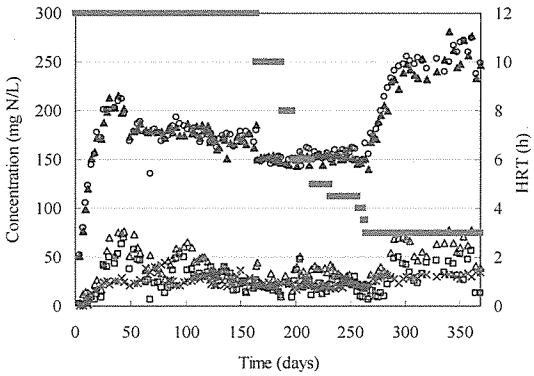

Fig. 3a Time courses of influent and effluent concentrations of nitrogen compounds for Reactor 1 with different HRTs
Symbol: ▲Inf. NH_4-N, △Eff. NH_4-N, ○Inf. NO_2-N, □Eff. NO_2-N, ×Eff. NO_3-N, ■ HRT

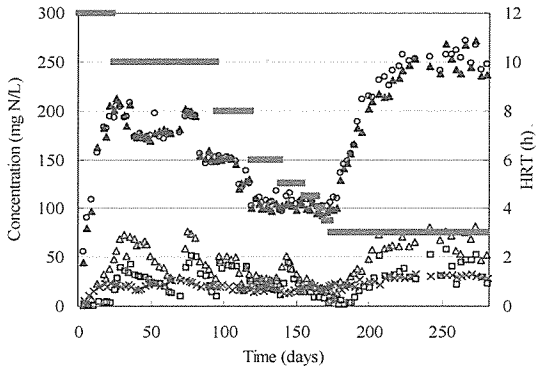

Fig. 3b Time courses of influent and effluent concentrations of nitrogen compounds for Reactor 2 with different HRTs
Symbol: ▲Inf. NH_4-N, △Eff. NH_4-N, ○Inf. NO_2-N, □Eff. NO_2-N, ×Eff. NO_3-N, ■ HRT

NH_4-N and NO_2-N removal efficiencies for Reactor 1

Influent NH_4-N and NO_2-N for Reactor 1 were increased stepwise from 50 to 175 mg N/L during the first 23 days with an HRT of 12 h, and good treatment results were obtained with maximum NH_4-N and NO_2-N removal efficiencies of 90% and 95%, respectively. Subsequently, influent NH_4-N and NO_2-N were increased to 200 mg N/L for the next 20 days (day 24~43) and NH_4-N and NO_2-N removal efficiencies decreased to 62% and 64%, respectively. These results show that the anammox bacteria could not adapt to the high substrate concentration. For this reason, influent NH_4-N and NO_2-N were reduced to 175 mg N/L during the next 120 days (day 44-163) until the NH_4-N and NO_2-N removal efficiencies increased to 80~88% and 85~90%, respectively. Then, influent NH_4-N and NO_2-N were kept at 150 mg N/L and the influent flow rates were increased gradually from 1.3 L/d to 4.5 L/d (HRT reduced from 12 h to 3.5 h) over the next 100 days (164-263) and the removal efficiencies of NH_4-N and NO_2-N were quite high and stable at 80% and 86%, respectively. During the next 35 days (day 264-298), the influent flow rate was kept at 5.2 L/d (HRT of 3 h) and influent NH_4-N and NO_2-N were increased stepwise from 150 mg N/L to 225 mg N/L, respectively, and NH_4-N and NO_2-N removal efficiencies were 81% and 90% during this term. Subsequently, influent NH_4-N and NO_2-N were increased to 250~275 mg N/L, but NH_4-N and NO_2-N removal efficiencies decreased to 75% and 85%, respectively, during the next 69 days due to the high influent concentrations and short HRT of 3 h.

NH_4-N and NO_2-N removal efficiencies for Reactor 2

Influent NH_4-N and NO_2-N for Reactor 2 were increased stepwise from 50 to 200 mg N/L during the first 23 days with an HRT of 12 h, and high NH_4-N and NO_2-N removal efficiencies of 89% and 98%, respectively, were obtained. Over the next 150 days (day 24-173), influent NH_4-N and NO_2-N fluctuated from 200 mg N/L to 100 mg N/L with increasing flow rate from 1.3 L/d to 4.5 L/d (HRT from 12 h to 3.5 h) while NH_4-N and NO_2-N removal efficiencies decreased to 72% and 83%, respectively. For the next 40 days (day174-213), HRT was kept at 3 h and influent NH_4-N and

NO_2-N were increased stepwise from 100 mg N/L to 225 mg N/L and the NH_4-N and NO_2-N removal efficiencies were high at 80% and 93%, respectively. However, the NH_4-N and NO_2-N removal efficiencies decreased to 72% and 84%, respectively, over the next 67 days after increasing influent NH_4-N and NO_2-N concentrations to 250~275 mg N/L. The reason for these decreases in removal efficiencies were the high influent concentrations and short HRT of 3 h.

T-N removal efficiencies for Reactor 1 and Reactor 2

Figs. 4a and 4b show influent and effluent T-N concentrations and T-N removal efficiencies for Reactor 1 and Reactor 2. Influent T-N levels for Reactor 1 and Reactor 2 were changed from 100 mg N/L to 500 mg N/L. With an HRT of 12 h, the T-N removal efficiency of Reactor 1 was 73% during the first 163 days and the T-N removal efficiency of Reactor 2 was higher at 87% only during the first 23 days. With an HRT of 10 h to 3.5 h, T-N removal efficiencies of Reactor 1 and Reactor 2 were 76% (next 100 days) and 74% (next 150 day), respectively. With an HRT of 3 h and influent T-N levels of 200~450 mg N/L, T-N removal efficiencies of Reactor 1 and Reactor 2 were similarly of 78% (35 days) and 79% (40 days). However, with HRT of 3h and influent T-N levels of 500~550 mg N/L, T-N removal efficiencies for both Reactor 1 and Reactor 2 were lower of 74% (69 days) and 73% (67 days) than before. The reason was mentioned in the discussions about the NH_4-N and NO_2-N removal efficiencies for Reactor 1 and Reactor 2.

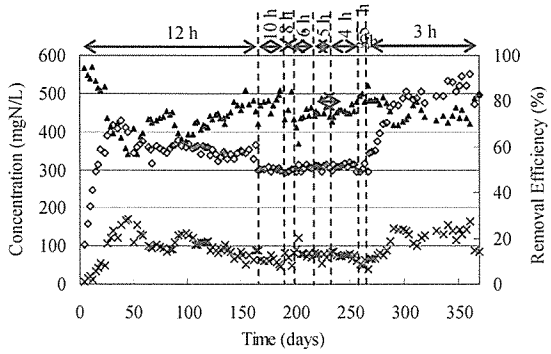

Fig. 4a Time course of T-N concentrations and removal efficiencies for Reactor 1
Symbols: ◇Inf. T-N, ×Eff. T-N, ▲T-N Rem. Eff., ■HRT

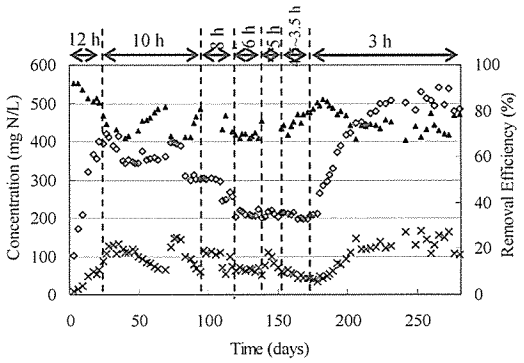

Fig. 4b Time course of T-N concentrations and removal efficiencies for Reactor 2

Symbols: ◇Inf. T-N, ×Eff. T-N, ▲T-N Rem. Eff., ■HRT

T-N removal rates for Reactor 1 and Reactor 2

As shown in **Figs. 5a and 5b**, T-N removal rates for Reactor 1 were about 0.5 – 0.6 kg N/m^3/day during 186 days and T-N removal rates for Reactor 2 were about 0.5 – 0.7 kg N/m^3/day during a shorter time of 145 days. Then, T-N removal rates for Reactor 1 were increased stepwise from 0.6 to 3.1 kg N/m^3/day over the next 129 days. T-N removal rates for Reactor 2 were similar to those for Reactor 1 and increased stepwise from 0.7 to 3.1 kg N/m^3/day over the next 85 days. The high T-N removal rates obtained in this study show that the anammox bacteria grew actively in the anammox reactors using MC as a biomass carrier. However, the T-N removal rates did not increased to more than 3.3 kg N/m^3/day during the next 53 days for Reactor 1 and 3.2 kg N/m^3/day during the next 50 days for Reactor 2. The effluent NH_4-N and NO_2-N concentrations were high as shown in Figs. 3a and 3b when the influent concentrations of these parameters were increased to 250~275 mg N/L with an HRT of 3 h.

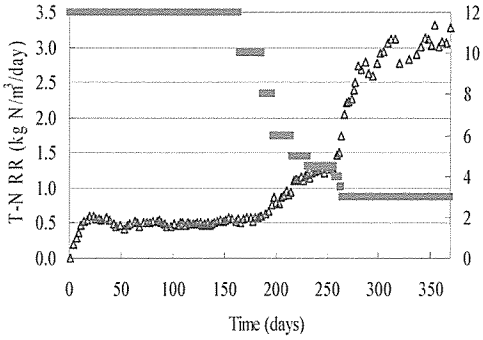

Fig. 5a T-N Removal Rates for Reactor 1
Symbols: △ T-N RR, ■ HRT

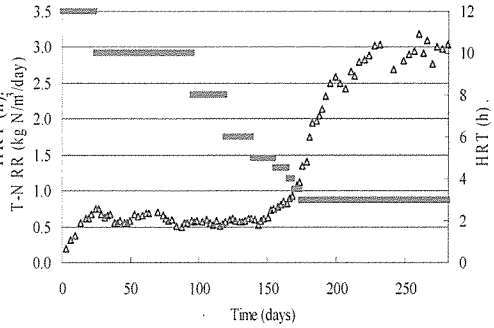

Fig. 5b T-N Removal Rates for Reactor 2
Symbols: △ T-N RR, ■ HRT

These data show that the T-N removal rates for Reactor 1 and Reactor 2 did not differ much. However, the changes in influent concentrations, flow rates and HRTs were not same. T-N loading rate for Reactor 1 could not increase quickly due to biomass clog at bottom part of Reactor 1 without attached much at whole reactor with the smaller spacers between the grains of material in Reactor 1 and high substrate concentration inhibited activity of anammox bacteria which causes reduction of T-N removal efficiency. On the other hand, T-N loading rate for Reactor 2 could increase quickly because biomass could attach more at whole reactor with the larger spacers between the grains of material in Reactor 2 and high substrate concentration did not inhibit much to activity of anammox bacteria. The operating time for Reactor 1 to reach T-N removal rate of 3.0 kg $N/m^3/d$ was 3 months longer than that at Reactor 2. The reason for this result was that the influent wastewater for Reactor 2 was distributed more equally in Reactor 1 owing to the larger gaps between the grains of material.

Observation of the attached biomass

MC materials with attached anammox biomass in Reactor 1 and Reactor 2 are shown in **Fig. 6**. Small amounts of anammox biomass were observed on MC from the upper and middle parts of Reactor 1 **(Fig. 6a)**. Thin brownish attached biomass was observed on MC from the upper and middle parts of Reactor 2 **(Fig. 6c)**. **Figs. 6a and 6c** indicate that the attached biomass at the upper and middle parts of Reactor 2 grew much more than the attached biomass at the upper and middle parts of Reactor 1. This shows that the influent wastewater in Reactor 2 could penetrate to the nooks easier than in Reactor 1 and the larger gaps between the grains in Reactor 2 formed the larger space for the growth of microorganisms in compare with Reactor 1. Therefore, microorganisms can attach and grow to the large size MC material easier than the small size.

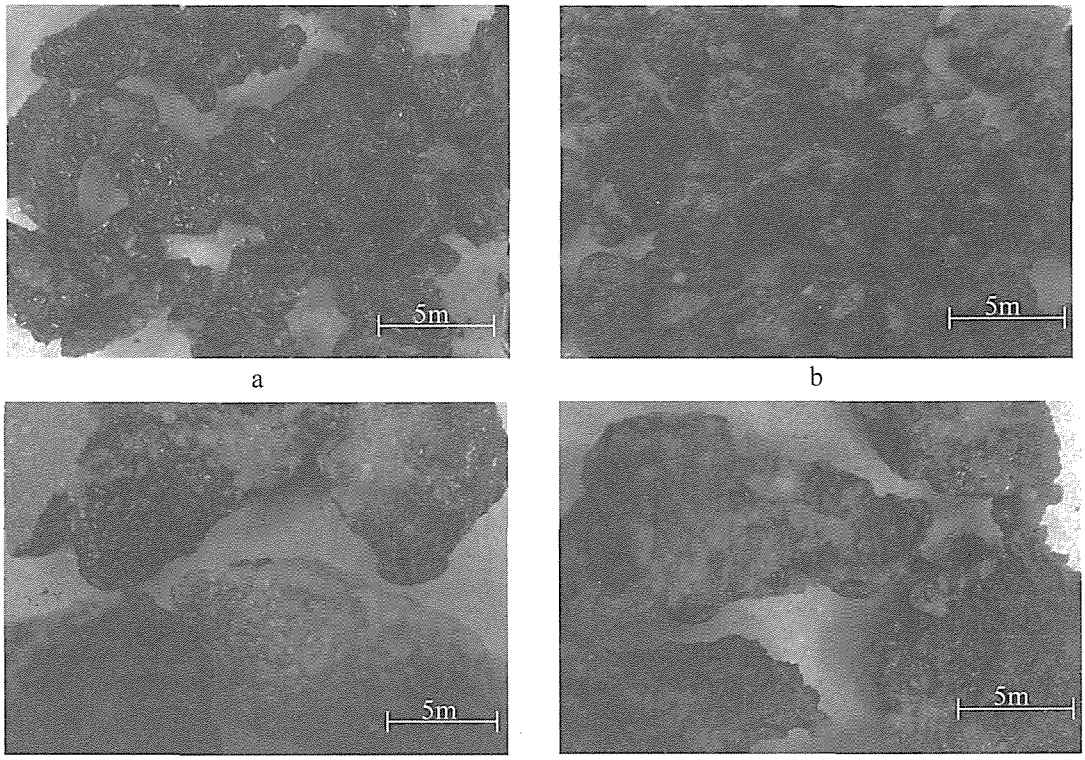

Fig. 6 MC material with attached biomass at the end of the operation phase
(a) MC at upper and middle parts of Reactor 1, (b) MC at bottom part of Reactor 1,
(c) MC at upper and middle parts of Reactor 2, (d) MC at bottom part of Reactor 2

The red biomass grew more densely at the bottom parts of Reactor 1 and Reactor 2 as shown in **Figs. 6b and 6d**. This is typical for fixed bed reactors. The influent wastewater supplied to the bottom parts of the reactors was distributed unequally. The influent concentrations of nitrogen compounds at the bottom parts of reactors were always higher than at the middle and upper parts of the reactors. Consequently, the microorganisms at the bottom parts consumed most of the substrate, thus the microorganisms at the middle and upper parts lacked sufficient nutrients.

Gene analysis by Denaturing Gradient Gel Electrophoresis (DGGE) method

At the end of the operational phase, MC material with attached biomass from Reactor 2 was analyzed by the DGGE method. The seed sludge used for Reactor 1 and Reactor 2 was from a 50-L reactor using a nonwoven biocarrier that was known to have been seeded with the KSU-1 and KU-2 strains. In addition, the synthetic wastewaters of these reactors were the same in composition utilizing the same tap water of groundwater origins drawn at the test site at Kumamoto University, Japan. Consequently, the attached biomass sample from Reactor 2 was analyzed for gene identification. Both KSU-1 and KU-2 strains were detected in this sample; however, the density of bacteria in the bottom part of Reactor 2 was much greater than at middle part as shown in **Fig. 7** as discussed above. Furthermore, the KSU-1 strain was more predominant than the KU-2 strain, which was also know to be the care in the seed sludge of the nonwoven source culture.

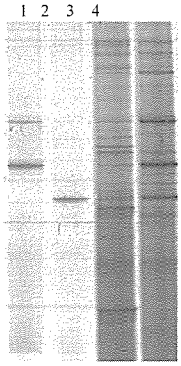

Fig. 7 DGGE of PCR products from anammox sludge of Reactor 2
Lane 1: KSU-1 Marker
Lane 2: KU-2 Marker
Lane 3: anammox sludge at the middle part
Lane 4: anammox sludge at the bottom part

4. Conclusion

With an HRT of 3 h and influent T-N concentrations of 200~450 mg N/L, T-N removal efficiencies for Reactor 1 and Reactor 2 were similarly 78% and 79%. However, with an HRT of 3 h and influent T-N concentrations of 500~550 mg N/L, T-N removal efficiencies for both Reactor 1 and Reactor 2 were lower at 74% and 73%. Thus, HRT should be kept longer than 3 h for influent T-N concentrations of 500-550 mg N/L or higher. The high T-N removal rates for both Reactor 1 and Reactor 2 were obtained with similar values of 3.3 kg N/m^3/day and 3.2 kg N/m^3/day, which is suitable for removal of wastewater containing high NH_4-N concentrations. However, the operating time of Reactor 1 was 3 months longer than Reactor 2 to obtain the similar results. The apparent reason of this result was that influent wastewater in Reactor 2 was more evenly distributed owing to the larger gaps in the material matrix. Consequently, the application of MC material with size of 10 to 15 mm could be better than the smaller size of 3 to 5 mm. Red biomass grew densely at the bottom parts of Reactor 1 and Reactor 2 and only sparingly at the upper and middle parts of the reactors.

By the DGGE method, both KSU-1 and KU-2 strains were detected in the anammox sludge from Reactor 2. However, the density of bacteria at bottom part of Reactor 2 was much greater. Furthermore, the KSU-1 strain was more predominant than KU-2 strain.

5. References

1. **Jetten, M.S.M, Strous, M., van de Pas-Schoonen, K.T., Schalk, J., van Dongen, U.G.J.M., van de Graaf, A.A., Logemann, S., Muyzer, G., van Loosdrecht, M.C.M. and Kuenen, J.G.**: The anaerobic oxidation of ammonium, *FEMS Microbiol. Rev.* 22, 421-37 (1999).
2. **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 micro-organisms in a fluidized bed reactor. *Microbiology* 142, 2187-2196 (1996).
3. **Strous, M., Heijinen, J.J., Knenen, 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, 589 - 596 (1998).
4. **Fujii 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, 412-418 (2002).
5. **Furukawa, K., Rouse, J.D., Imajo, U., Nakamura, K. and Ishida, H.**: Anaerobic oxidation of ammonium confirmed in continuous flow treatment using a novel nonwoven biomass carrier. *Jpn. J. Wat. Treat. Biol.* 38, 87-94 (2002).
6. **Imajo, U., Ishida, H., Fujii, T., Sugino, H., Rouse, J.D. and Furukawa, K.**: Gen bank direct submission. AB054007 (uncultured anoxic sludge bacterium KU2) (2001).

7. **Furukawa, K., Rouse, J.D., Yoshida, N. and Hatanaka, H.**: Mass cultivation of anaerobic ammonium-oxidizing sludge using a novel nonwoven biomass carrier. *J. Chem. Eng. Jpn.* 36, 1163-1169 (2003).
8. **Rouse, J.D., Yoshida, N., Hatanaka, H., Imajo, U. and Furukawa, K.**: Continuous treatment studies of anaerobic oxidation of ammonium using a nonwoven biomass carrier. *Jpn. J. Wat. Treat. Biol.* 39, 33-41 (2003).
9. **Rouse, J.D., Fujii, T., Sugino, H., Tran, H. and Furukawa, K.**: PVA-gel beads as a biomass carrier for anaerobic oxidation of ammonium in a packed-bed reactor, CD-ROM. *In Proceedings of 5th International Exhibition and Conference on Environmental Technology*. Heleco '05 (2005).
10. Tran Thi Hien Hoa, Luong Ngoc Khanh, Liu Zhijun, Takao Fujii, Joseph D. Rouse and Kenji Furukawa: Nitrogen removal by immobilized anammox sludge using PVA gel as biocarrier. *Japanese Journal of Water Treatment Biology,* Vol. 42, No. 3, 139-149, 2006.
11. **Jota, K.**: Determination of ammonium in seawater based on the indophenol reaction with O-phenylphenol (OPP). *Wat. Res.* 29, 2746-2750 (1995).
12. **APHA, AWWA, WPCF**: Standard methods for the examination of water and wastewater, 19th edition. American Public Health Association, Washington, D.C. (1995).