The relationship between the activity and community structure of microbes associated with the oxidation of ammonia in a full-scale rockwool biofilter was examined by kinetic, denaturing gradient gel electrophoresis (DGGE), and sequence analyses. The packing materials were sampled from two different depths at 3 sites. Estimated \( V_{\text{max}} \) values were similar among depths at same sampling sites, while \( V_{\text{max}} \) differed in the mid-point sample. The lower depth of this site had the highest \( V_{\text{max}} \). A correspondence analysis showed the DGGE profile of ammonia-oxidizing bacterial \( \text{amoA} \) of the lower depth of the mid-point sample to be distinguishable from the others. Banding patterns at other sites were similar among depths. Banding patterns of ammonia-oxidizing archaeal \( \text{amoA} \) of the mid-point sample were also similar among depths. The results suggested an association between the ammonia-oxidizing bacterial community’s composition and ammonium oxidation kinetics in samples. Sequence analysis indicated that the ammonia-oxidizing bacterial community mainly belonged to the \textit{Nitrosomonas europaea} lineage and \textit{Nitrosospira} cluster 3. The ammonia-oxidizing archaeal \( \text{amoA} \)-like sequences were related to those belonging to soil and sediment groups, including one with 84% nucleotide similarity with \textit{Nitrospumilus maritimus}.

Key words: rockwool biofilter, ammonia-oxidizing bacteria, ammonia-oxidizing archaea, PCR-DGGE, ammonium oxidation kinetics
within the biofilter was examined by an MPN (Most Probable Number) technique, and a kinetic study of the oxidation process. The community structure of ammonia-oxidizing bacteria and ammonia-oxidizing archaea was examined by DGGE and sequence analyses. The presence of the crenarchaeal amoA gene was confirmed by retrieving sequences and comparing them with those in databases.

Materials and Methods

Biofilter packing material samples

The biofilter packing materials were sampled in June 2006 from a deodorization biofilter (5.8 m x 8.0 m in cross-section and 3 m high (2.5 m for packing materials)) that has been operating at the National Institute of Livestock and Grassland Science since 1998. The sampling site and the main characteristics of the samples were described in detail previously (41). Briefly, wet biofilter packing materials (ca. 230 g) were sampled using a soil sampler (5 cm ID, Daiki Rika Kogyo, Saitama, Japan) from depths of 50 cm and 140 cm at three sites (a, b, c) horizontally. Sites “a” and “c” tended to be exposed to gas emitted from the initial and later stages of the composting process, respectively, and site “b” was in the middle. After sampling, aggregates were broken down by sieving (4.0 mm mesh) and urethane was cut into pieces of ca. 1 cm. The separated particles were then mixed well. The sample for molecular analysis was stored at −20°C until DNA extraction.

Enumeration of ammonia-oxidizers and nitrite-oxidizers by the MPN

The population sizes of ammonia- and nitrite-oxidizers of the biofilter packing materials were determined by the MPN method. The basal medium and procedures were based on a previous study (5) with some modifications. The medium consisted of 0.5 g of [L-2,5-methylammonium bromide], 1.0 g of zirconium beads (0.2 mm Ø, Nippon Gene, Tokyo, Japan), and 180 mL of 10% SDS. The tubes were horizontally agitated at 2.500 rpm for 360 s with a shaker (M-BR-022, Taiotec, Saitama, Japan), then incubated at 65°C for 30 min with shaking every 10 min. The nucleic acids were extracted with a phenol-chloroform-isoamylalcohol solution (25:24:1, v/v/v) and shaken for 10 s. The extraction with buffer and shaking was then repeated three times. The lysate was extracted with a chloroform-isoamylalcohol solution (24:1, v/v). The nucleic acids were precipitated by adding a 0.6 volume of isopropanol, washed with 70% ethanol, and finally dissolved in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) overnight. The contents of the three tubes were then mixed together. Crude DNA was incubated at 37°C for 30 min with RNase A (final concentration, 0.1 g L−1, Wako, Osaka, Japan). DNA was precipitated with the same volume of 2% PEG (20% polyethylene glycol 6000, 0.6 M NaCl) after incubation at 4°C for 2 h. The overall extraction was done twice.

PCR amplification and cloning of amoA of ammonia-oxidizing archaea

The primers, amoA19F (5′-ATG GTC TGG CTW AGA CG-3′) (18) and amo643R (5′-TCC CAC TTW GAC CAR GCG GCC ATC CA-3′) (38) were used to amplify approximately 600 bp of the amoA gene of ammonia-oxidizing archaea. The reaction mixture (25 µL) contained 2.5 µL of 10× PCR Gold Buffer, 0.2 µL of each dNTP, 2.5 mM MgCl2 solution, 1 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA), 0.2% bovine serum albumin (Takara Bio, Otsu, Japan), approximately 5 ng of DNA template, and 0.5 µM of each primer. The amplification was performed using an iCycler (Bio-Rad, Hercules, CA, USA) with initial denaturation at 94°C for 10 min, then 35 cycles of 1 min at 95°C, 1 min at 55°C, and 40 s at 72°C, and a final extension at 72°C for 10 min. The PCR product from sample b-50 cm was purified for cloning using a kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. It was ligated to the pGEM T-easy vector (Promega, Madison, WI, USA) and transformed in JM109 competent cells (Zymo research, Orange, CA, USA). The presence of inserts was checked by direct amplification with the primer set described above. Unique clones were identified by digestion with Rsal (Nippon Gene, Tokyo, Japan) (recognition site, GTAC). Plasmid DNA from transformants that produced unique restriction patterns were purified using a QIAprep Spin Miniprep kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. It was ligated to the pGEM T-easy vector (Promega, Madison, WI, USA) and transformed in Escherichia coli JM109 competent cells (Zymo research, Orange, CA, USA). The presence of inserts was checked by direct amplification with the primer set described above. Unique clones were identified by digestion with Rsal (Nippon Gene, Tokyo, Japan) (recognition site, GTAC). Plasmid DNA from transformants that produced unique restriction patterns were purified using a QIAprep Spin Miniprep kit (Qiagen) and sequenced with a Dye Terminator Cycle Sequencing with Quick Start Kit (Beckman Coulter, Fullerton, CA, USA).

DGGE targeting amoA of ammonia-oxidizing bacteria and ammonia-oxidizing archaea

For ammonia-oxidizing bacteria, an amoA-specific primer set with a GC clamp, amoA-1F-GC (5′-CCG CCC CCG GCG CCG CCG CCC CCC CCC CCC CCG GGT TTC TAC

The Michaelis-Menten equation by non-linear regression with a Levenberg-Marquardt algorithm using KaleidaGraph 4 (Hulinks, Tokyo, Japan). The Michaelis-Menten expression is

\[ v = \frac{V_{max} \cdot s}{K_m + s} \]

Where \( v \) is the NO\textsubscript{3}−/NO\textsubscript{2}− production rate (µmol [g dry sample]\textsuperscript{-1} hr\textsuperscript{-1}); \( s \) is the NH\textsubscript{4}+ concentration (mM); \( K_m \) is the Michaelis-Menten constant (mM); and \( V_{max} \) is the maximum NO\textsubscript{3}−/NO\textsubscript{2}− production rate (µmol [g dry samples]\textsuperscript{-1} hr\textsuperscript{-1}).
TGG TGG T-3', underlined sequence denotes the GC clamp) and amoA1F-GC (5'-GGG CCG GGC GCC GGC CGG CCC GC CGC GTC CCG CCC GCG CCC GAT GGT GTG CTG GCT WAG ACG-3', underlined sequence denotes the GC clamp) and amo247Ry1 (5'-CAA ACC ATG CGC CTC TAT TG CQA CCA CCC A-3', underlined sequence denotes modification in this study), was used (1). The reaction mixture (50 µL) contained 5 µL of 10×PCR Gold Buffer, 0.2 M of each dNTP, 2.5 mM MgCl₂ solution, 1 U of AmpliTaq Gold (Applied Biosystems), 0.2% bovine serum albumin (Takara Bio), 10 ng of DNA template, and 0.5 µM of each primer. The amplification was performed using an iCycler (Bio-Rad) with initial denaturation at 94°C for 5 min, then 40 cycles of 45 s at 94°C, 30 s at 57°C, and 1 min at 72°C, and a final extension at 72°C for 7 min. The products were separated on a 6% polyacrylamide gel along a gradient of 45% to 65% gradient (100% denaturant contained 7 M urea and 40% formamide) in 0.5×TAE at 100 V for 17 h at 60°C using a DCode Universal Mutation Detection System (Bio-Rad). Bands were visualized by UV excitation of SYBR Green I (Molecular Probes, Eugene, OR, USA)-stained gels and photographed.

For ammonia-oxidizing archaea, an amoA-specific primer set with a GC clamp, amoA1F-GC (5'-GGG CCG GGC GCC GGC CGG CCC GC CGC GTC CCG CCC GCG CCC GAT GGT GTG CTG GCT WAG ACG-3', underlined sequence denotes the GC clamp) and amoA247Ry1 (5'-CAA ACC ATG CGC CTC TAT TG CQA CCA CCC A-3', underlined sequence denotes modification in this study), was used (38). The length of the PCR fragments was 223 bp. Amo247Ry1 was modified to a non-degenerate primer based on the sequences of the clones obtained in this study because use of a degenerate primer resulted in two DGGE bands even from one plasmid DNA. The patterns obtained using a non-degenerate primer were similar to those obtained using a degenerate primer but some bands probably caused by the degenerate primer disappeared. PCR conditions were as for amplification of the amoA of ammonia-oxidizing archaea. The products were separated on a 6% polyacrylamide gel along a gradient of 35% to 55% gradient in 0.5×TAE at 120 V for 8 h at 60°C. The running conditions were optimized by using four clones obtained in this study.

Statistical analysis
DGGE bands were classified into four categories based on intensity (0, no band; 1, weak; 2, moderate; 3, high intensity [only for ammonia-oxidizing bacteria]). A correspondence analysis was performed with DGGE bands and samples as descriptors by using the CORRESP procedure of SAS Version 9.1 (SAS Institute, Cary, NC, USA).

Sequencing of DGGE bands and phylogenetic analysis
High intensity DGGE bands were excised and diluted in TE, reamplified with the primer set described above, and electrophoresed again. The purified bands were sequenced with a Dye Terminator Cycle Sequencing with Quick Start Kit (Beckman Coulter). Phylogenetic affiliations were determined using the BLAST program at the DDBJ web site (http://www.ddbj.nig.ac.jp). Multiple alignments and phylogenetic analyses were performed using MEGA version 3.1 (15).

Accession numbers
The nucleotide sequences determined in this study have been deposited in the DDBJ database, under the following accession numbers: DGGE bands excised from the gel of amoA of ammonia-oxidizing bacteria, AB303315–AB303317, AB303324–AB303326 and AB303328–AB303330, DGGE bands excised from the gel of amoA of ammonia-oxidizing archaea, AB525381–AB525386 and clones RW-1 to RW-4 of ammonia-oxidizing archaea, AB525377–AB525380.

Results
Evaluation and kinetics of ammonium oxidation in packing materials
Ammonia-oxidizers numbered 5.01×10⁶–1.11×10⁶, and nitrite-oxidizers, 4.25×10⁵–5.11×10⁶ cells [g dry samples]⁻¹ (Table 1). The population of ammonia-oxidizers was smaller in a-50 cm than b-140 cm and c-140 cm. The population of nitrite-oxidizers was smaller in c-50 cm than c-140 cm. Ammonia-oxidizers were less abundant than nitrite-oxidizers, although all samples contained small amounts of NO²⁻ (0.1–0.2 mg [100 g dry samples]⁻¹) (43).

Responses to NH₄⁺ concentrations differed among sampling positions (Fig. 1). The estimated Kᵣ values were similar to each other at different depths of the same sample sites (Table 2). The maximum NO₂⁻+NO₃⁻ production rate differed between the two depths at site “b”, whereas it was

<table>
<thead>
<tr>
<th>Sample origin (site-depth)</th>
<th>Ammonia-oxidizer (×10⁶)</th>
<th>Nitrite-oxidizer (×10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-50 cm</td>
<td>0.50 (0.16– 2.40)</td>
<td>19.1 ( 2.78– 94.6 )</td>
</tr>
<tr>
<td>a-140 cm</td>
<td>2.75 (0.28–11.6 )</td>
<td>4.28 ( 1.00– 15.9 )</td>
</tr>
<tr>
<td>b-50 cm</td>
<td>2.33 (0.41–11.2 )</td>
<td>10.2 ( 4.68– 26.8 )</td>
</tr>
<tr>
<td>b-140 cm</td>
<td>11.1 (4.20–42.5 )</td>
<td>4.62 ( 2.12– 13.7 )</td>
</tr>
<tr>
<td>c-50 cm</td>
<td>3.25 (1.24– 8.82)</td>
<td>4.25 ( 1.09– 9.10 )</td>
</tr>
<tr>
<td>c-140 cm</td>
<td>11.0 (3.81–28.6 )</td>
<td>51.1 (21.8–141 )</td>
</tr>
</tbody>
</table>

* Values represent means of duplicate samples. Lower and upper 95% confidence limits are shown in parentheses.

Fig. 1. Effects of NH₄⁺-N concentrations on NO₂⁻-N and NO₃⁻-N production rates of biofilter packing materials. A, B, C; sampling sites “a”, “b”, and “c”, respectively. Curves indicate the fitted Michaelis-Menten equation used for the calculation of kinetic parameters.
similar among depths at site “a” as well as site “c”. The \( V_{\text{max}} \) was highest in sample b-140 cm (Table 2).

### Community structure of ammonia-oxidizing bacteria and ammonia-oxidizing archaea determined by PCR-DGGE targeting amoA genes

The community structure of ammonia-oxidizing bacteria and archaea was compared among positions within the full-scale biofilter. Fig. 2 and Fig. 3 show DGGE patterns for amoA of ammonia-oxidizing bacteria and ammonia-oxidizing archaea, respectively. Banding patterns were similar between the duplicate samples in all samples for both microbial communities. The number of bands varied from 2 to 14 for ammonia-oxidizing bacteria among the positions, and overall patterns for ammonia-oxidizing bacteria differed without common bands even within the same biofiltration plant. On the other hand, for ammonia-oxidizing archaea, 2 to 7 bands were visible, and band A6 appeared in every sample. Banding patterns for amoA of ammonia-oxidizing archaea also varied among samples, however, they appeared to be more similar than those for ammonia-oxidizing bacteria.

![Fig. 2. Duplicate DGGE profiles of ammonia-oxidizing bacterial amoA fragments of the biofilter packing materials (a-50 cm, 140 cm, b-50 cm, 140 cm, c-50 cm, 140 cm). Bands indicated by black arrows were excised and sequenced.](image)

### Table 2. Estimated values of kinetic parameters, \( K_m \) and \( V_{\text{max}} \)

<table>
<thead>
<tr>
<th>Sample origin (site-depth)</th>
<th>( K_m ) (mM, NH(_4^+))</th>
<th>( V_{\text{max}} ) (( \mu )mol [g dry samples](^{-1} ) h(^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-50 cm</td>
<td>0.05 (0.01)</td>
<td>0.29 (0.01)</td>
</tr>
<tr>
<td>a-140 cm</td>
<td>0.09 (0.03)</td>
<td>0.25 (0.01)</td>
</tr>
<tr>
<td>b-50 cm</td>
<td>0.32 (0.07)</td>
<td>0.19 (0.01)</td>
</tr>
<tr>
<td>b-140 cm</td>
<td>0.24 (0.16)</td>
<td>0.36 (0.03)</td>
</tr>
<tr>
<td>c-50 cm</td>
<td>0.68 (1.24)</td>
<td>0.22 (0.09)</td>
</tr>
<tr>
<td>c-140 cm</td>
<td>1.05 (0.88)</td>
<td>0.24 (0.05)</td>
</tr>
</tbody>
</table>

Values in parentheses represent standard errors.

To compare banding patterns among samples, a correspondence analysis was performed (Fig. 4). The results showed that the ammonia-oxidizing bacteria of sample b-140 cm had a distinct pattern (Fig. 4a). Banding patterns were similar among depths at sites “a” and “c.” Plots of site “b” for ammonia-oxidizing archaea were also close to each other vertically (Fig. 4b).

### Phylogenetic analysis of DGGE bands and ammonia-oxidizing archaeal amoA clones

The phylogenetic relationships of amoA gene sequences of ammonia-oxidizing bacteria are shown in Fig. 5. The phylogenetic tree revealed that amoA sequences obtained from the DGGE analysis were mainly of the Nitrosomonas europaea lineage (Bands B2 and B3) and Nitrosospira cluster 3 (Band B4, B6–B9). The specific band contributing to the pattern of sample b-140 cm, as revealed by the correspondence analysis, was B6. The sequence of this band was related to the Nitrosospira multiformis ATCC25196 (93% similarity) (21). Band B4, which appeared in most samples except b-140 cm and b-50 cm-1, was related to Nitrosospira sp. Np 39-19 (21). Band B3 appeared at site “c” with high intensity and had 99% sequence similarity with Nitrososponas sp. GH22 (21, 36).

The DGGE band A1 of ammonia-oxidizing archaeal amoA had 82% sequence similarity with Nitrosopumilus maritimus SCM1 isolates from a marine aquarium (12). A1 appeared in samples a-140 cm, b-50 cm, b-140 cm, and c-50 cm. Bands A2, A3, A4, A5 and A6 were all related to the ‘Candidatus Nitrososphaera gargetsis’ clone obtained from an enrichment sample of a hot spring (8), with 86–92% identity at the nucleotide level. Band A6 appeared with high intensity in most of the samples. Twenty-eight clones were obtained from the ammonia-oxidizing archaeal amoA clone library of sample b-50 cm and four unique patterns were identified by digestion with Rsal (clone RW-1, clone RW-2, clone RW-3, and clone RW-4). The number of clones with the same digestion patterns was 1, 3, 23, and 1, respectively. Clone RW-2

![Fig. 3. Duplicate DGGE profiles of ammonia-oxidizing archaeal amoA fragments of the biofilter packing materials (a-50 cm, 140 cm, b-50 cm, 140 cm, c-50 cm, 140 cm). Bands indicated by black arrows were excised and sequenced.](image)
Nitrososphaera gargensis RW-1, RW-3 and RW-4 were affiliated with the ‘Candidatus Nitrosopumilus maritimus’ SCM1. The closest relatives of these clones had 84% DNA similarity and 95% amino acid similarity with clone GA15P03. The sequences obtained from the biofilter packing materials fell into both the soil/sediment cluster and the sediments cluster. Within the sediments cluster, clone RW-2 affiliated with cluster B, which consisted of clones obtained from the activated sludge bioreactors.

Discussion

The ammonia-oxidizing activities and community structure of the microbes responsible for nitrification in a deodorization biofilter have not been fully elucidated in spite of the importance of nitrification to the NH$_3$ gas treatment process. In this study, the microbial community associated with the oxidation of ammonia in a full-scale biofilter with rockwool packing materials was examined by an MPN technique, a kinetic analysis of ammonium oxidation, and PCR-DGGE. The estimated $K_m$ (saturation constant for activity, 0.05–1.05 mM NH$_4^+$) was comparable to the range of values calculated in other investigations; 0.12–14 mM (27), 0.5 mM (6), 0.002–0.04 mM (33). Ammonium oxidizing activity determined by $V_{max}$ (0.19–0.36 μmol [g dry samples]$^{-1}$ h$^{-1}$) was higher than that in sediment (0.32–7.5 nmol g$^{-1}$ h$^{-1}$) and grassland soil (21–96 nmol g$^{-1}$ h$^{-1}$) (3), similar to that in agricultural soil (ca. 0.07–0.71 μmol g$^{-1}$ h$^{-1}$) (1). The values obtained were lower than the nitrification potential of a peat biofilter determined by 48-hour incubation (270–1,557 μmol g$^{-1}$ h$^{-1}$) (37).

The kinetic analysis revealed differences in responses to the different NH$_4^+$ concentrations among positions (Fig. 1). The estimated $K_m$ values were smaller for site “a” than other sites. The concentration of NH$_3$ was lower near site “a” in the two weeks prior to sampling (43). Therefore, the low NH$_3$ concentration could have affected the microbial substrate affinity. The $V_{max}$ value was highest in sample b-140 cm, which also had the highest potential nitrification activity in a previous study (43). To compare the population size of nitrifiers among samples, a MPN count was performed. This method is often used to enumerate nitrifiers in soils (20) and manure composts (5) and can determine the potential activity of a microbial population. It should be noted that the approach depends on a population being able to proliferate under certain conditions. The rate of ammonium oxidation per cell was estimated at 218–5,800 fmol cell$^{-1}$ h$^{-1}$ in this study, considerably higher than that in pure cultures (0.9–23 fmol cell$^{-1}$ h$^{-1}$) (27) and soil (0.2–15.6 fmol cell$^{-1}$ h$^{-1}$) (22). Therefore, the MPN values might be underestimated as pointed out previously (25). The differences in population size could be detected with 7.57 mM (NH$_4^+$) and 50 mM NH$_4^+$, which was the same as in previous studies (5, 30). The population of ammonia-oxidizers was larger in sample b-140 cm than a-140 cm and c-140 cm than a-50 cm, and that of nitrite-oxidizers was larger in c-140 cm than a-140 cm and b-140 cm. The NO$_2^-$ production rate at 5 and 8 mM ammonium, on the other hand, was larger in a-50 cm, a-140 cm, and b-140 cm than in others (Fig. 1). One explanation for the relationship between the population size of ammonia-oxidizing bacteria and ammonia-oxidizing activity is that changes in nitrification reflect phenotypic
changes in ammonia-oxidizing bacteria as reported previously (19). However, the possibility cannot be excluded that the MPN values did not reflect the actual differences in nitrifier population size because the ammonia-oxidizing bacterial growth kinetics differed among species (27) and each sample had a different ammonia-oxidizing bacterial community. A knowledge of the target community’s composition is also required for the correct interpretation of results of real-time PCR assays because amplification efficiency differs among ammonia-oxidizing bacterial species according to a recent study (32). Therefore, quantitative techniques and experimental conditions need to be used in accordance with the community’s composition.

DGGE of amoA showed heterogeneity in the community
structure of the ammonia-oxidizing bacteria, indicating a different composition at different positions within the biofilter (Fig. 2). The correspondence analysis showed that the banding pattern of sample b-140 cm was distinguishable from that of the other samples, and vertically similar at sites “a” and “c”, respectively (Fig. 4a). The kinetics of ammonium oxidation were also vertically similar at sites “a” and “c”, while $V_{\text{max}}$ values varied between the two depths of site “b”. The specific band for b-140 cm (Band B6) was related to *Nitrososphaera multiformis*, whereas the bands which appeared intensively in sample “a” and b-50 cm (Band B7–9) were grouped together with *Nitrososphaera briensis* within *Nitrososphaera* cluster 3. Although both *N. briensis* and *N. multiformis* are grouped within *Nitrososphaera* cluster 3, their cell activities are reportedly different and the activity is higher for *N. multiformis* (0.023 pmol cell$^{-1}$ h$^{-1}$) than *N. briensis* (0.004 pmol cell$^{-1}$ h$^{-1}$) (2). There is still a possibility that the highest $V_{\text{max}}$ in sample b-140 cm resulted from the greater population size of the microbes due to estimation bias; however, each component of the ammonia-oxidizing bacterial community could play a role in determining the activity. Substrate affinity tended to be higher for sample “a” than sample “c” (Fig. 1). Sample “c” had DGGE Band 3 with high intensity, and the sequence of this band was closely related to *Nitrosomonas* sp. GH22, which had a high $K_s$ (saturation constant for growth) and was identified as an (NH$_4$)$_2$SO$_4$-insensitive
strain (35). On the other hand, the DGGE bands related to 
*Nitrosomonas* sp. were weak in sample “a”. The $K_\text{m} (\text{NH}_3)$ is 
reportedly higher for *Nitrosomonas europaea* and its rela-
tives (30–61 μM) (13) than for *Nitrosospira* sp. (6–11 μM) (9).
Therefore, the substrate affinity in the samples can be
attributed to the specific species composing the community.

As we reported previously (43), the inlet NH$_3$ concentra-
tion fluctuated during the composting period and differed
among inlet ports. The NH$_3$ load was assumed to be high at
site “b”. In addition, the NH$_3$ concentration varied with the
turning of manure composts. These differences in substrate
concentration both in space and in time probably affected
the structure of the ammonia-oxidizing bacterial community,
and various species with different kinetics of ammonium oxida-
tion were selected. The concentration of NH$_3$ is one of the
factors affecting the structure of the ammonia-oxidizing bac-
terial community also in agricultural soils (1, 14). The pH of
the biofilter packing materials was similarly neutral (6.8–
7.4), and the effects of other environmental factors such as
O$_2$ concentration were not examined in this study. However,
pH, O$_2$, and salinity are important to ammonia-oxidizing bac-
teria in gaining ecological niches and affect the abundance
and community structure of ammonia-oxidizing bacteria as
well as NH$_4^+$ concentration (23, 26, 31, 34). The factors that
affect changes in community structure, population size, and
nitrification activity at different times in the deodorization
biofilter need to be carefully analyzed in order to achieve
constant oxidation of NH$_3$ in the apparatus.

The DGGE patterns of ammonia-oxidizing archaeal amoA
were more similar than those of ammonia-oxidizing bacterial
amoA among samples (Fig. 3) and no clear association with
ammonium oxidation kinetics was observed. Actually, both
the MPN and kinetics methods used in this study were not
optimized for ammonia-oxidizing archaea. Therefore, it is
possible that the ammonia-oxidizing archaean may require dif-
f erent environmental conditions. The sequences retrieved
from the samples fell into both the soil/sediment cluster and
sediment cluster (Fig. 6). The ammonia-oxidizing archaeal
amoA clone RW-2 was affiliated with cluster B which con-
sisted of clones obtained from activated sludge bioreactors
(24). Conditions in the biofilter may be relatively similar to
those in activated sludge bioreactors, for example, electronic
conductivity (ca. 3–4 dS m$^{-1}$; values of samples from different
sampling times), compared to seawater samples with high
salinity levels. The closest relative of the other three clones
was the moderately thermophilic ‘Candidatus *Nitro-
sosphaera gorgensis*’ clone GA15P03, obtained from an en-
richment sample from a hot spring (8). A preliminary DGGE
analysis indicated that some ammonia-oxidizing bacteria
appeared in both the manure compost and the biofilter pack-
ing materials. The possibility cannot be excluded that some
ammonia-oxidizing archaea are derived from the composting
process with exhaust air. The physiology of ammonia-oxi-
dizing archaea and its contribution to nitrification in deodor-
ization biofilters should be elucidated in a future study.

In conclusion, the microbial community associated with
ammonia oxidation in a full-scale biofilter with rockwool
packing materials was examined by an MPN technique, a
kinetic analysis of ammonium oxidation, and PCR-DGGE.
The kinetic analysis showed differences in responses to the
different NH$_4^+$ concentrations among sampling positions.
The differences among samples both in ammonia oxidation
kinetics and in DGGE banding profiles of ammonia-oxidizing
bacterial amoA suggested an association between the
community composition of the ammonia-oxidizing bacteria
and the ammonium oxidation kinetics in the samples.
Sequence analysis indicated that the ammonia-oxidizing
bacterial community mainly belonged to the *Nitrosomonas
europaee* lineage and *Nitrosospira* cluster 3. The ammonia-
oxidizing archaeal amoA-like sequences were related to
those which belonged to soil and sediment groups including
one with which had 84% and 95% similarity at the nucle-
o tide and amino acid level with *Nitrosopumilus maritimus*
SCM1, respectively. Further study is warranted to elucidate
the function and contribution of ammonia-oxidizing archaea
to nitrification in ammonia deodorization biofilters.

Acknowledgements

The authors wish to thank all members of the Waste Recycling
Research Team, NILGS, Japan, for their help with the experiments,
and Mrs. N. Akasaka and Mrs. K. Sumiya for assistance.

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