Development of a super high-rate Anammox reactor and \textit{in situ} analysis of biofilm structure and function

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Abstract
The anaerobic ammonium oxidation (Anammox) process is a new efficient and cost effective method of ammonium removal from wastewater. Under strictly anoxic condition, ammonium is directly oxidised with nitrite as electron acceptor to dinitrogen gas. However, it is extremely difficult to cultivate Anammox bacteria due to their low growth rate. This suggests that a rapid and efficient start-up of Anammox process is the key to practical applications. To screen appropriate seeding sludge with high Anammox potential, a real-time quantitative PCR assay with newly designed primers has been developed. Thereafter, the seeding sludge with high abundance of Anammox bacteria (1.7 $\times$ 10$^8$ copies/mg-dry weight) was selected and inoculated into an upflow anaerobic biofilters (UABs). The UABs were operated for more than 1 year and the highest nitrogen removal rate of 24.0 kg-N m$^{-3}$ day$^{-1}$ was attained. In addition, the ecophysiology of Anammox bacteria (spatial distribution and \textit{in situ} activity) in biofilms was analysed by combining a full-cycle 16S rRNA approach and microelectrodes. The microelectrode measurement clearly revealed that a successive vertical zonation of the partial nitrification (NH$_4^+$ to NO$_2^-$), Anammox reaction and denitrification was developed in the biofilm in the UAB. This result agreed with the spatial distribution of corresponding bacterial populations in the biofilm. We linked the micro-scale information (i.e. single cell and/or biofilm levels) with the macro-scale information (i.e. the reactor level) to understand the details of Anammox reaction occurring in the UABs.

Keywords
Anaerobic ammonium oxidation (Anammox); biofilm structure and function; upflow anaerobic biofilter

Introduction
The anaerobic ammonium oxidation (Anammox) process is a new efficient and cost effective method of ammonium removal from wastewater. Anammox reaction converts ammonium and nitrite to dinitrogen gas directly under anaerobic conditions. This process has many advantages over conventional nitrification-denitrification processes as it requires no aeration and external organic carbon source and produces less excess sludge. To date, a high nitrogen removal rate of 8.9 kg-N m$^{-3}$ day$^{-1}$ was achieved by the Anammox process using a gas-lift reactor (Sliekers \textit{et al.}, 2003), which could make the reactor footprint smaller than that of conventional systems. However, establishment of Anammox bioreactors is still difficult, mainly due to their extremely low growth rate (the doubling time was reported to be approximately 11 days) (Strous \textit{et al.}, 1998). Although the first full scale SHARON-Anammox reactor was built for treatment of the rejection water from the anaerobic digestion plant in Rotterdam, Netherlands (Abma \textit{et al.}, 2005), it took quite a long while to start-up this plant. This indicates that the rapid and efficient start-up of the Anammox process is apparently the key to practical applications. However, there is still little information on efficient screening methods of appropriate seeding sludges for the Anammox process.
Therefore, to screen the appropriate seeding sludge for rapid start-up of the Anammox process, we developed a real-time quantitative PCR (RTQ-PCR) assay with newly designed primers for quantification of the Anammox bacteria in the sludge. Consequently, the seeding sludge with high abundance of Anammox bacteria was inoculated into an upflow anaerobic biofilter (UAB), and the UABs were operated over 1 year. Thereafter, the spatial distribution and in situ activity of Anammox bacterial community in the biofilm were analysed by combining 16S rRNA gene-based molecular biological techniques and microelectrodes.

**Material and methods**

**Real-time quantitative PCR for screening sludge**

In this study, copy numbers of 16S rRNA gene of Anammox bacteria in three sludge samples taken from domestic wastewater treatment plants (WWTPs) were quantified with RTQ-PCR assay to select seeding sludge with high abundance of Anammox bacteria. An SYBR Green RTQ-PCR assay was conducted using the specific RTQ-PCR primer sets AMX809F and AMX1066R (Tsushima et al., in preparation). Each PCR mixture (25 μL) was composed of 12.5 μL of 1 × SYBR Green PCR, master mix (Applied Biosystems), 300 nM of each forward and reverse primers, 100 μg/mL of BSA (Sigma) and either 2.5 μL of template DNA or 10 to 10⁸ copies per well of the standard vector plasmid carrying ca. 1,500 bp of 16S rRNA gene of the clone related to Candidatus Brocadia anammoxidans (AF375994) in the previous study.

Carbon to nitrogen ratio (C/N) of influent wastewater was also measured because organic compounds in wastewater affect the activity of Anammox bacteria (van de Graaf et al., 1996).

**Anammox activity test**

The sludges taken from WWTPs were used as inoculum to evaluate the Anammox activity. Each sludge was diluted to 20 mg-MLSS/L with the Anammox enrichment medium and transferred into 100-mL serum bottles. Oxygen was removed from the mixed liquor by purging with N₂ gas (99.9%) for 10 min. The Anammox enrichment medium consisted of (NH₄)₂SO₄ (1.0–6.0 mM), Na₂NO₂ (1.0–6.0), KHCO₃ (5.0), KH₂PO₄ (0.2), MgSO₄·H₂O (1.2), CaCl₂·2H₂O (1.4), 1 mL of trace element solution I (EDTA (13.4), FeSO₄ (32.9)) and trace element solution II (EDTA (40.3), ZnSO₄·7H₂O (1.5), CoCl₂·4H₂O (1.0), MnCl₂·4H₂O (5.0), CuSO₄·5H₂O (1.0), Na₂MoO₄·2H₂O (0.9), NiCl₂·6H₂O (0.8), Na₂SeO₄·10H₂O (0.6) and H₃BO₃ (0.02)) (van de Graaf et al., 1996) and pH was controlled at 7.5 with sulphuric acid (97%). One-cm³ polyester porous nonwoven carriers were added into the serum bottles (Japan Vilene Co., Ltd., Tokyo, Japan) as biomass carriers, and the serum bottles were sealed tightly with rubber caps. Each sludge was incubated for 70–140 d at 37 °C in the dark and the concentrations of NH₄⁺, NO₂⁻ and NO₃⁻ were measured. The medium in the reactor was replaced several times when the Anammox activity deteriorated.

**Operation of upflow anaerobic biofilters**

Three UABs made up of cylindrical glass were used in this study. UAB-A and UAB-B had an inner diameter of 50 mm and height of 500 mm. The liquid volume was 0.8 L. UAB-A and UAB-B consisted of 12 vertically suspended polyester porous nonwoven strips (210 × 20 × 8.0 mm) with a total surface area of 1,000 cm². Pre-incubated sludge for 70–140 d after Anammox activity was detected was inoculated into each UAB. UAB-C had an inner diameter of 30 mm and height of 400 mm. The liquid volume was 0.2 L. The UAB-C consisted of high-density polyethylene (350 × 25 × 4.0 mm). Influent
medium for each UAB was same as the one used for Anammox activity test described above and continuously fed in up-flow mode using a peristaltic pump. The concentrations of \((\text{NH}_4\text{)}_2\text{SO}_4\) and \(\text{NaNO}_2\) in the medium were gradually increased to 6.2–24.6 mM and 3.7–22.8 mM, respectively. The medium was continuously purged with \(\text{N}_2\) gas (99.9%) to maintain dissolved oxygen concentration less than 0.5 mg/L. All UABs were operated at 37°C in the dark.

**Phylogenetic analysis**

The 16S rRNA gene clone libraries were constructed from the biofilms in UAB-B and UAB-C to analyse microbial community structure. Total DNA was extracted from sludges (approximately 0.2 mL) with Fast DNA spin kit (Bio 101, Qbiogene Inc., CA, USA) as described in the manufacturer’s instructions. The 16S rRNA genes were amplified by PCR with the primer set of Bac11f and 1492r as described by Weisburg et al. (1991). Cloning and sequencing were performed as described previously by Okabe et al. (2003). In total, 61 clones were randomly selected from clone libraries, and the partial sequences of approximately 900 bp of 16S rRNA genes were analysed. Clustal W (Thompson et al., 1994) was used for sequence alignment. The phylogenetic tree was constructed using the neighbour-joining method (Saito and Nei, 1987) with Tree Explore. Bootstrap resampling analysis for 100 replicates was performed to estimate the confidence of tree topologies.

**Microelectrodes**

To measure concentration microprofiles, Anammox biofilms taken from UAB-C were incubated in a reactor equipped with measurement ports through which a microelectrode was inserted into the biofilms at 37°C in the dark. The reactor had an inner diameter of 26 mm and height of 280 mm. The liquid volume was 0.15 L. The reactor was filled with a polyester porous nonwoven strip (250 × 20 × 8.0 mm) with a total surface area of 100 cm². The medium used for microprofile measurements was same as the one used for the operation of UABs.

Clark-type O₂ microelectrodes (Revsbech, 1989) and liquid ion-exchanging membrane (LIX) microelectrodes for \(\text{NH}_4^+\), \(\text{NO}_2^-\) and \(\text{NO}_3^-\) (de Beer et al., 1997) were prepared and calibrated as described previously. All measurements were performed in the reactor of the synthetic medium at 37°C as previously described by Satoh et al. (2003). Net volumetric \(\text{NH}_4^+\), \(\text{NO}_2^-\) and \(\text{NO}_3^-\) consumption rates (\(C(\text{NH}_4^+), C(\text{NO}_2^-)\) and \(C(\text{NO}_3^-); \mu\text{mol/cm}^3/\text{h}\)) in the biofilm were calculated from the average steady-state concentration profiles using Fick’s second law of diffusion. The details of this method were described previously by Lorenzen et al. (1998). The effective diffusion coefficients for \(\text{NH}_4^+\), \(\text{NO}_2^-\) and \(\text{NO}_3^-\) in the biofilm were calculated from the free solution molecular diffusion coefficients for \(\text{NH}_4^+\), \(\text{NO}_2^-\) and \(\text{NO}_3^-\), respectively. Diffusion coefficients of \(1.38 \times 10^{-5} \text{cm}^2\text{s}^{-1}\) for \(\text{NH}_4^+\), \(1.23 \times 10^{-5}\text{cm}^2\text{s}^{-1}\) for \(\text{NO}_2^-\), and \(1.23 \times 10^{-5}\text{cm}^2\text{s}^{-1}\) for \(\text{NO}_3^-\) at 20°C were used for the calculation of volumetric consumption rates in the biofilm.

**Fluorescence in situ hybridisation**

The biofilm samples were fixed in 4% paraformaldehyde solution and embedded in Tissue-Tek OCT compound (Miles, Elkhart, Ind.). Vertical thin sections (20-μm-thick) of the fixed biofilm were prepared by using a cryostat (Reichert-Jung Cryocut 1800, Leica). All in situ hybridisations were performed according to the procedure described by Amann (1995) and Okabe et al. (1999). In situ hybridisation of biofilm sections was performed with the previously reported 16S rRNA-targeted oligonucleotide probes. The FISH probes used in this study were AMX820 for Anammox bacteria, NSO190 and Nse1472 for ammonium-oxidising
bacteria (AOB) and EUB338mix (EUB338 + EUB338II + EUB338III) for mostly eubacteria (Table 1). The probes were labelled with fluorescein isothiocyanate (FITC) or tetramethylrhodamine 5-isothiocyanate (TRITC) at the 5' end. A model LSM510 confocal laser scanning microscope (CLSM, Carl Zeiss) equipped with an Ar ion laser (488 nm) and HeNe laser (543 nm) was used. All images were combined, processed and analysed with the standard software package provided with the LSM510.

Analytical measurements
Concentrations of \( \text{NH}_4^+ \), \( \text{NO}_2^- \), and \( \text{NO}_3^- \) were determined by an ion-exchange chromatography (DX-100, Nippon Dionex) with an IonPac CS3 cation column and IonPac AS9 anion column after filtration with 0.2-μm pore size membranes (Dismic-13CP, Advantec). The C/N ratio was determined based on the influent concentration of dissolved organic carbon (DOC) or biochemical oxygen demand (BOD). DOC concentration was measured by a TOC-analysers (TOC-5000A; Shimadzu, Kyoto, Japan) after filtration with 0.7-μm pore size glass fibre filters (Dismic-13CP, Advantec). BOD concentration was measured with azide modification of the Winkler Method (APHA/AWWA/WEF, 1992). T-N concentration was measured using the UV spectrometric method after oxidation with potassium persulphate (Valderrama, 1981).

Results and discussion
Screening of seeding sludge with real-time quantitative PCR
RTQ-PCR assay was conducted to quantify the copy number of 16S rRNA gene of Anammox bacteria. Table 2 shows sludge-C taken from a denitrification reactor in a domestic WWTP had the highest copy number of 16S rRNA gene of Anammox bacteria (1.7 × 10^8 copies/mg-dry weight). The other sludge had one order of magnitude lower copy numbers 1.3–1.9 × 10^7 copies/mg-dry weight. The influent C/N ratios of sludge-A and C were relatively low (0.9).

These sludges were diluted to 20 mg-MLSS/L with the medium and inoculated into the 100-mL serum bottles. These sludges were incubated for 70–140 d to determine Anammox activity (Figure1). In Anammox activity tests, sludge-A showed simultaneous decrease in nitrite and nitrate and no decrease in ammonium, indicating that denitrification was taking place. For sludge-B, Anammox reaction was not observed for the initial 107 d. However, after day 107, the repetitive significant Anammox reaction was observed and the nitrogen removal rate reached 0.03 kg-N m^-3 d^-1 after 130 d. For sludge-C, Anammox reaction was observed from the beginning of experiment. Thereafter, the nitrogen

### Table 1 A list of 16S rRNA targeted-oligonucleotide probes used in this study

<table>
<thead>
<tr>
<th>Probe</th>
<th>Probe sequence (5' to 3')</th>
<th>FA (%)</th>
<th>Specificity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUB338</td>
<td>GCTGCCCTCCCGTAGGAGT</td>
<td>-b</td>
<td>Most Bacteria</td>
<td>Amann et al., 1990</td>
</tr>
<tr>
<td>EUB338 II</td>
<td>GCAGCACCACCAGTGAGTGT</td>
<td>-b</td>
<td>Planctomycetales</td>
<td>Daims et al., 1999</td>
</tr>
<tr>
<td>EUB338 III</td>
<td>GCTGCCACCCGTAGGTGT</td>
<td>-b</td>
<td>Verrucomicrobiales</td>
<td>Daims et al., 1999</td>
</tr>
<tr>
<td>AMX820</td>
<td>AAAACCCTCTCTACTTTAGTGGCC</td>
<td>40</td>
<td>Candidatus Brocadia anammoxidans and Kuenenia stuttgartiensis</td>
<td>Schmid et al., 2001</td>
</tr>
<tr>
<td>NSO190</td>
<td>CGATCCCCCTGCTTTTCTCCC</td>
<td>55</td>
<td>β-Proteobacterial ammonia-oxidizing bacteria</td>
<td>Mobarry et al., 1996</td>
</tr>
<tr>
<td>Nse1472</td>
<td>ACCCCAGTCATGACCCCC</td>
<td>50</td>
<td>Nitrosomonas eutropha, N. Europaea and N. halophila</td>
<td>Juretschko et al., 1998</td>
</tr>
</tbody>
</table>

*a*Formamide concentration in the washing buffer  
*b*Usable at any formamide concentrations
removal rate increased to 0.06 kg-N m$^{-3}$ d$^{-1}$ after day 60. Thus, sludge-C had the highest Anammox potential for a rapid and efficient start-up of ANAMMOX reactor. This is because the sludge-C had the highest 16S rRNA copy number and lower C/N ratio, as shown in Table 1.

### Start-up and operation of up-flow anaerobic biofilter

Sludge-A, sludge-B and sludge-C were incubated for 150, 141 and 66 d in the batch cultures, and were then inoculated into UAB-A, UAB-B and UAB-C reactors, respectively. In UAB-A, Anammox reaction was not observed during the initial 70 days, even though the influent loading rate was increased. In UAB-B, an apparent Anammox reaction was observed after day 7 and the nitrogen removal rate reached 1.2 kg-N m$^{-3}$ d$^{-1}$ on day 75. However the nitrogen removal rate remained within the range 1.2–1.8 kg-N m$^{-3}$ d$^{-1}$. In UAB-C, apparent Anammox reaction was observed from the beginning and the nitrogen removal rate increased with increasing influent nitrogen load (Figure 2). The colour of the biomass in the reactor changed from dark grey to bright pink until day 50 and to dark brown until day 90 when the nitrogen removal rate was 14.0 kg-N m$^{-3}$ d$^{-1}$. The nitrogen stoichiometric ratio was 1:1.25:0.17 for the conversion of NH$_4^+$ and NO$_2^-$ to the production of NO$_3^-$ between days 0 and 115. This stoichiometric ratio was similar to the previously reported ratio of 1:1.31:0.22 (Strous et al., 1998). The nitrogen removal rate finally reached 24.0 kg-N m$^{-3}$ d$^{-1}$ on day 115 when the nitrogen loading rate was 36.2 kg-N m$^{-3}$ d$^{-1}$. This nitrogen removal rate was approximately 2.5 times higher than the value that was previously reported in on other study with a 1.8 L gas-lift reactor fed synthetic wastewater (Sliekers et al., 2003). Therefore, we could successfully develop a super high-rate Anammox reactor within only 120 d by inoculating pre-cultured sludge-C which had the high potential of Anammox reaction. This study clearly demonstrated that inoculating the sludge that had high copy number of 16S rRNA gene of Anammox bacteria and low C/N ratio resulted in a rapid and efficient start-up of the Anammox reactor and consequently attached the highest nitrogen removal rate that have ever been reported.

The copy numbers of 16S rRNA gene of Anammox bacteria was quantified by RTQ-PCR in the biofilm in UAB-C on days 0 and 55 were $1.3 \times 10^9$ and $1.4 \times 10^{12}$ copies/mL-reactor (data not shown). Unfortunately, the number of 16S rRNA gene copies

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**Table 2** Quantification of copy number of 16S rRNA gene of ANAMMOX bacteria and influent C/N ratio

<table>
<thead>
<tr>
<th>Sludge Treating method Type of WWTP</th>
<th>Concentration (copies/mg-dw)</th>
<th>C/N ratio (mg-C/mg-N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sludge-A MBR Domestic</td>
<td>$1.3E + 07$</td>
<td>0.9</td>
</tr>
<tr>
<td>Sludge-B Anaerobic digestion Domestic</td>
<td>$1.9E + 07$</td>
<td>1.5</td>
</tr>
<tr>
<td>Sludge-C Denitrification Domestic</td>
<td>$1.7E + 08$</td>
<td>0.9</td>
</tr>
</tbody>
</table>

WWTP, wastewater treatment plant; MBR, membrane bioreactor

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**Figure 1** Concentration profiles during Anammox activity tests. Closed circle, NH$_4^+$; open square, NO$_2^-$; open triangle, NO$_3^-$.
per cell of the Anammox bacteria has not been determined so far. If it was assumed that an Anammox bacterial cell contains one copy of 16S rRNA gene, the populations of Anammox bacteria in the reactor would be $1.3 \times 10^9$ and $1.4 \times 10^{12}$ cells/mL. The number of Anammox bacteria increased approximately $1.1 \times 10^3$ times for 53 d, suggesting that the specific growth rate was $0.0055 \text{ h}^{-1}$ (the doubling time of 5.2 d). This calculation indicated that the Anammox bacteria in our UAB reactor have higher growth rates than the value ($0.0027 \text{ h}^{-1}$) reported by Strous et al. (1998), but lower than the value ($0.0163 \text{ h}^{-1}$) reported by Isaka et al. (2006). Additionally, the specific nitrogen removal rate was calculated to be $1.55 \text{ g-N g-biomass}^{-1} \text{ d}^{-1}$, which was higher than $1.30 \text{ g-N g-biomass}^{-1} \text{ d}^{-1}$ (Jetten et al., 1998).

Phylogenetic analysis

The 16S rRNA gene clone libraries were constructed to analyse community structures of Anammox bacteria and other bacteria in UAB-B and UAB-C. In total, approximately 900 bp of 16S rRNA genes of 61 clones (20 clones from UAB-B and 41 clones from UAB-C) were analysed and grouped into seven operational taxonomic units (OTUs) based on 97% sequence similarity among the OTU. Their representative sequences were used for the following phylogenetic analysis. The clones related to Anammox bacteria accounted for 60 and 70% of total clones analysed in UAB-B and UAB-C, respectively. The clones grouped into OTU1 (from UAB-B), OTU2 (from UAB-B) and OTU3 (from UAB-C) were related to the Candidatus *Brocadia anammoxidans* with 95, 92 and 91% sequence similarity, respectively (Figure 3). All clones related to Anammox bacteria detected from UAB-C were grouped into OTU3. Thus, it was speculated that the difference in the performance between UAB-B and UAB-C was due to the different species. Anammox bacteria represented by OTU3 would have high nitrogen removal capacity of $1.55 \text{ g-N g-biomass}^{-1} \text{ d}^{-1}$ ($2.9 \times 10^{-15} \text{ g-N copy}^{-1} \text{ d}^{-1}$).

The remaining four OTUs (OTU4-OTU7) were affiliated with the *Betaproteobacteria* with 91–98% sequence similarity to such as *Thauera* sp., *Methyloversatilis universalis* strain, *Denitratisoma oestradiolicum* strain and *Acidovorax* sp. (data not shown). These bacteria might denitrify with using organic matter derived from Anammox bacteria.

Microelectrode

Anammox biofilms taken from UAB-C was incubated in a reactor equipped with measurement ports. The concentration profiles of $O_2$, $\text{NH}_4^+$, $\text{NO}_2^-$, and $\text{NO}_3^-$ in the biofilm were directly measured with microelectrodes. The concentration profiles of $\text{NH}_4^+$ and $\text{NO}_2^-$ revealed that $\text{NH}_4^+$ dramatically decreased in the surface biofilm (0–1,100 µm) with the volumetric consumption rates of $23.5 \mu\text{mol NH}_4^+ \text{ N cm}^{-3} \text{ h}^{-1}$ and a part of the consumed $\text{NH}_4^+$ was converted to $\text{NO}_2^-$ in the outermost biofilm with a $\text{NO}_2^-$ peak of

![Figure 2](https://iwaponline.com/wst/article-pdf/55/8-9/9/4397239/9.pdf)

**Figure 2** Time course of nitrogen load (closed square) and removal rate (open square) during operation period in UAB.
800 $\mu$M at 200–300 $\mu$m from the surface. The NO$_3^-$ concentration gradually increased toward the depth of the biofilm and decreased in the deeper part of the biofilm (below 1,100 $\mu$m from the surface). These concentration profiles indicated that the partial nitrification (oxidation of NH$_4^+$ to NO$_2^-$) occurred in the outermost biofilm, a significant Ana-
mnox reaction took place in the middle part of the biofilm, and ordinal denitrification (NO$_3^-$ consumption) occurred in the deeper part of the biofilm.

**Fluorescence in situ hybridisation**

To relate with the microelectrode measurements, the spatial distribution of ammonia-oxidising bacteria (AOB), Anammox bacteria, and heterotrophic bacteria in the biofilm was analysed with FISH using AMX820, Nso190, Nse1472 and EUB338mix probes. The high abundance of AOB hybridised with NSO190 was mainly detected in the outermost biofilm. In addition, these NSO190-hybridised AOB were also hybridised with Nse1472, indicating that they were most likely *Nitrosomonas europa*, *N. europaea* or *N. halophi-
lia* (Figure 4). This is probably because a trace amount of oxygen (<0.5 mg/L) was present in this reactor. The Anammox bacteria hybridised with AMX820 accounted for approximately 90% of total bacteria detected with EUB338mix probe in the middle part of the biofilm, which was just below the AOB layer. ANAMMox bacteria gradually decreased toward the depth and accounted for approximately 50% of EUB338mix in the deeper biofilm. In contrast, the presence of the unidentified *Eubacteria* hybridised with EUB338mix (except AOB) became evident in the deeper part of the biofilm. These
unidentified bacteria might denitrify with using organic matter derived from Anammox bacteria. The microbial spatial distribution by FISH and the microelectrode measurement clearly revealed that a successive vertical zonation of the partial nitrification (NH$_4^+$ to NO$_2^-$), Anammox reaction, and denitrification was developed in the biofilm in the UAB. The coexistence of AOB, Anammox bacteria and unidentified Eubacteria gives mutual advantages, such as that AOB and Eubacteria give the Anammox bacteria an advantage by consuming dissolved oxygen and organic matter derived from Anammox reaction.

Conclusions
We succeeded in a rapid and efficient start-up of the Anammox biofilm reactor with screened high potential seeding sludge by RTQ-PCR and subsequently attained the nitrogen removal rate of 24.0 kg-N m$^{-3}$ d$^{-1}$. Additionally, this study provides the first experimental evidence of in situ analysis of ANAMMOX biofilm community structure and function by combining a full-cycle of 16S rRNA approach and microelectrodes.

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