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Enrichment of anammox bacteria from three sludge sources for the startup of monosodium glutamate industrial wastewater treatment system

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ABSTRACT

Three activated sludges from a landfill leachate treatment plant (S1), a municipal sewage treatment plant (S2) and a monosodium glutamate (MSG) wastewater treatment plant (S3) were used as inocula to enrich anaerobic ammonium oxidation (anammox) bacteria for the startup of MSG industrial wastewater treatment system. After 360 days of cultivation using MSG wastewater, obvious anammox activity was observed in all three cultures. The maximum specific anammox activities of cultures S1, S2 and S3 were 0.11 kg N kg^{-1} VSS day^{-1}, 0.09 kg N kg^{-1} VSS day^{-1} and 0.16 kg N kg^{-1} VSS day^{-1}, respectively. Brownish-red anammox granules having diameters in the range of 0.2–1.0 mm were visible in cultures S1 and S2, and large red granules having diameters in the range of 0.5–2.5 mm were formed in culture S3 after 420 days of cultivation. Phylogenetic analysis of 16S rRNA genes showed that *Kuenenia* organisms were the dominant anammox species in all three cultures. The copy numbers of 16S rRNA genes of anammox bacteria in cultures S1, S2 and S3 were 6.8×10^7 copies mL^{-1}, 9.4×10^7 copies mL^{-1} and 7.5×10^8 copies mL^{-1}, respectively. The results of this study demonstrated that anammox cultivation from conventional activated sludges was highly possible using MSG wastewater. Thus the anammox process has possibility of applying to the nitrogen removal from MSG wastewater.

1. Introduction

Monosodium glutamate (MSG) is used extensively as a flavor enhancer in food products in Southeast Asia and elsewhere. The MSG industry is one of major fermentation industries in China accounting for roughly half of the entire world's production [1]. The effluent from MSG manufacturing plants is very hard to treat. It has high concentrations of chemical oxygen demand (COD, 10,000–30,000 mg L^{-1}), ammonium (15,000–25,000 mg L^{-1}), sulfate (15,000–30,000 mg L^{-1}) and a very low pH (approximately 2) [2]. Anaerobic and aerobic biological treatments have been used to treat MSG industrial wastewater [2,3], but the conventional technologies only removed COD and had a negligible impact on nitrogen removal. An air-stripping process is commonly used to remove high concentrations of ammonium from MSG wastewater [2], but the application of this physicochemical method is significantly limited due to its high operational cost and low removal efficiency. Furthermore, after the removal of organic matter, MSG wastewater is characterized by low COD values and high ammonium concentrations (low C/N ratio), making it infeasible to remove nitrogen with conventional biological nitrification/denitrification process. Therefore, new biotechnologies are urgently needed to remove nitrogen from MSG wastewater.

Anaerobic ammonium oxidation (anammox) is a promising biological nitrogen removal technology developed in the Netherlands in 1995 [4]. Since no external carbon source is required, the anammox process is considered to be a very attractive alternative for nitrogen removal from wastewater with a low C/N ratio. Compared to conventional nitrogen removal processes, the anammox process can save up to 90% of operating costs and produce a small amount of excess sludge and almost no undesirable byproducts such as greenhouse gases [5,6]. Several full-scale anammox plants have been constructed to remove nitrogen from ammonium-rich wastewaters, such as sludge digester liquor and potato-processing effluent [7,8]. The feasibility of the anammox process for nitrogen removal from other industrial wastewaters needs to be demonstrated. Until now, nitrogen removal from MSG wastewater has mostly been treated by physicochemical and conventional biological methods. There was only one study investigated the treatment of MSG wastewater using the anammox process. Chen et al. [9] found that the anammox culture in which anammox bacteria had already been highly enriched using synthetic wastewater could be

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used to treat MSG wastewater after removal of the most organic matter. But the feasibility of enriching anammox bacteria directly using MSG wastewater for its treatment needs to be shown.

A current limitation to the widespread application of anammox is the very low growth rate of anammox bacteria. The initial doubling time of anammox bacteria was reported to be approximately 11 days [10]. Recently, the anammox bacterial doubling time has been reported to be less than 6 days by selecting an adequate reactor configuration or an appropriate seeding sludge [11,12]. Different reactor configurations have been designed and applied for the anammox process in both laboratory and full-scale reactor systems [13–16]. The SBR was well accepted for anammox enrichment because of its efficient biomass retention, homogeneity of substrates in the reactor, stability and reliability over a long period of operation [9]. Various studies have shown that anammox cultivation from different conventional sludges is possible [17,18]. Chamchoi and Nitisoravut [17] found that different sludges, including sludges from upflow anaerobic sludge blanket (UASB), activated sludge and anaerobic digestion processes, could be used as seeding sludge for anammox enrichment using synthetic wastewater. But the selection of a proper seeding sludge is pivotal to the faster startup of anammox bioreactors [11]. Due to the complexity and biotoxicity of MSG wastewater [3], biological nitrogen removal is very difficult. Application of the anammox process for treatment of MSG wastewater may also be hindered by the low growth rate of anammox bacteria. The feasibility of anammox enrichment from different conventional sludges also needs to be demonstrated.

Therefore, the objective of this study was to enrich anammox bacteria using MSG wastewater in three parallel SBRs from three different sludge sources, including the sludges from a landfill leachate treatment plant, a municipal sewage treatment plant and an MSG wastewater treatment plant, for the startup of MSG wastewater treatment system.

2. Materials and methods

2.1. Seeding sludge

Seeding sludges included sludge (S1) from a landfill leachate treatment plant designed to treat landfill leachate with high concentration of ammonium, sludge (S2) from a municipal sewage wastewater treatment plant, which has been used extensively to enrich anammox bacteria [14,16,17], and sludge (S3) from an MSG wastewater treatment plant. The sludges were washed three times with physiological saline prior to inoculation. Their characteristics are shown in Table 1.

2.2. The characteristics of MSG industrial wastewater

The MSG wastewater was collected from an MSG wastewater treatment plant located in Zhejiang Province, China. The MSG wastewater contained 2000–2500 mg L^{-1} of COD, 1500–2000 mg L^{-1} of NH_4^+-N, 5000–5500 mg L^{-1} of SO_4^{2-}-S, and a pH of 5.5–6.5. The MSG wastewater contained a high concentration of NH_4^+-N. Consequently, nitrogen removal is required before discharging the MSG wastewater into natural water bodies.

2.3. Wastewater treatment system

The combined partial nitrification/anammox process is a promising new method for the treatment of MSG wastewater (Fig. 1). In partial nitrification stage, around 50% of ammonium could be converted to nitrite. Subsequently, ammonium could be oxidized to nitrogen gas using the produced nitrite as the electron acceptor. In this way, a completely autotrophic nitrogen removal from MSG wastewater could be achieved in this combined process. This study mainly focused on the startup of the anaerobic part of the MSG wastewater treatment system (anammox process). The cultivation of anammox bacteria was conducted in three parallel SBRs. Each reactor had a working volume of 2.2 L, a total volume of 2.5 L and a black cloth covering to avoid light inhibition.

2.4. Experimental setup

The three similar SBR reactors (R1, R2 and R3) were seeded with sludges S1, S2 and S3. The anaerobic environment of the SBRs was maintained by flushing with 95% Ar and 5% CO_2 during the experiment. The concentration of CO_2 in this gas was sufficient to maintain the pH between 7.5 and 8.0 in the reactors. The reactors were operated under similar conditions. During the enrichment period, the SBRs worked in 12-h cycles. Each SBR cycle consisted of 11 h and 40 min of filling, 10 min of biomass settling and 10 min of drawing out the liquid. The reactors were operated at 30 ± 1 °C. The hydraulic retention time of the reactors was controlled at 3 days, and the average solid retention time was around 100 days due to the low growth rate of anammox bacteria. The volume exchange ratio of the reactors was set at 30%. In order to prevent substrate inhibition, the MSG wastewater diluted 20–30 times (contained approximately 70 mg L^{-1} of NH_4^+-N) supplemented with 58 mg L^{-1} of NO_2^--N, was used as the medium for anammox enrichment.

2.5. Anammox activity assays

The anammox activity of the three cultures was determined by batch tests. Sealed 65-mL serum bottles containing 40 mL of liquid were used to perform the batch tests. Cultures were rinsed five times with phosphate buffer (0.14 g L^{-1} of KH_2PO_4 and 0.75 g L^{-1} of K_2HPO_4). After washing, 5 mL of culture was added to a serum bottle. The initial concentrations of NH_4^+-N and NO_2^--N were both 40 mg L^{-1}. The original pH was 8.0. Oxygen was removed from the liquid by purging with a mixture of 95% Ar and 5% CO_2 for 30 min. The mineral medium without biomass was incubated as a control. Tests were performed in triplicate at 30 °C in the dark, and the concentrations of NH_4^+-N and NO_2^--N were periodically monitored during incubation.

2.6. Analytical methods

Measurements of NO_3^--N, NO_2^--N, NH_4^+-N, pH, DO, COD, TN and TP were performed according to standard methods [19]. TSS and VSS were measured by the weighing method after drying at 105 °C and burning to ash at 550 °C. The settling ability of the granules (of 50–100 granules) was measured in a 1-L graduated cylinder. The 30-min sludge volume index (SVI_{30}) was determined after 30 min of settling by measuring the volume occupied by the mass of sludge in the 1-L graduated cylinder. The size of the granules was measured by an image analysis system (QCOLite, Germany) with a Leica DM2LB microscope and a digital camera (Canon S30, Japan).

2.7. DNA extraction and PCR amplification

DNA was extracted from the three anammox cultures using the Power Soil DNA Kit (Mo Bio Laboratories, Carlsbad, CA, USA). The extracted DNA was examined in 1.0% agarose gels by electrophoresis.

A primer combination of Amx368f and Amx820r was used for the amplification of anammox bacterial 16S rRNA genes (Table 2). The PCR reaction mixture (25 μL) contained 2.5 μL of 10× PCR buffer (containing 2 mM of $MgCl_2$), 20 mM of deoxyribonucleoside triphosphate, 1 mM of each primer, 1 U of *Taq* polymerase and 1 μL of DNA template (1–10 ng). The thermal cycle program included an initial 3-min denaturation step at 95 °C, 30 cycles of denaturation

Table 1
Basic characteristics of the three seeding sludges.

Seeding sludge	TSS ($g\,L^{-1}$)	VSS ($g\,L^{-1}$)	VSS/TSS	TP ($mg\,L^{-1}$)	TN ($mg\,L^{-1}$)	pH
S1	57.3	17.4	0.30	360	983	7.94
S2	28.3	12.3	0.44	458	881	7.81
S3	39.1	17.8	0.45	290	1592	7.46

Table 2
PCR primers used in this study.

Primers	Sequence 5′–3′	Specificity	*Escherichia coli* position number	Reference
Amx368f	TTCGCAATGCCCGAAAGG	Anammox bacteria	368–385	[20]
Amx820r	AAAACCCCTCTACTTAGTGCCC	*Brocadia*/*Kuenenia*	820–841	[15]
Amx694f	GGGGAGAGTGGAACTTCGG	Anammox bacteria	694–713	[21]
Amx960r	GCTCGCACAAGCGGTGGAGC	Anammox bacteria	960–979	[21]

at 95 °C for 1 min, annealing at 52 °C for 1 min, elongation at 72 °C for 1 min, and a final 10-min extension step at 72 °C. The amplified products were examined by electrophoresis in a 1.0% agarose gel.

2.8. Clone library and phylogenetic tree

The PCR products were cloned using the pMD19-T vector (TaKaRa Bio Inc., Shiga, Japan) according to the manufacturer's instructions. Plasmid DNA was isolated using the Gene JET™ Plasmid Miniprep kit (Fermentas Life Sciences, Germany) and was digested with 5 U of EcoRI enzyme in EcoRI buffer for 1.5 h at 37 °C. The digestion products were examined for an insert of the expected size using agarose (1.0%) gel electrophoresis. At least 50 inserts from each sample were subjected to sequence analysis using an ABI3100 automated sequencer (Applied Biosystems, California, USA).

Phylogenetic analyses of the sequences were conducted using ARB software as previously described [20]. Phylogenetic analyses were performed using the maximum likelihood, neighbour-joining and maximum parsimony methods with 50% sequence conservation filters for *Planctomycetes*. Bootstrap values of 100% were obtained for each branch within the anammox bacteria cluster.

2.9. Nucleotide sequence accession numbers

The sequences obtained in this study are available in GenBank under accession numbers JF271887 to JF271915.

2.10. Real-time quantitative PCR (qPCR)

A primer set (Amx694f–Amx960r) (Table 2) targeting 16S rRNA genes was used to quantify the anammox bacteria. The qPCR was performed with an iCycler iQ5 thermocycler and a real-time detection system (Bio-Rad, California, USA) according to the method described by Ni et al. [21]. The standard curves for anammox bacteria were constructed from a series of 10-fold dilutions of a plasmid DNA with an insert of 16S rRNA genes of *Candidatus Kuenenia stuttgartiensis*.

3. Results

3.1. Enrichment of anammox bacteria

For the first 100 days of enrichment in each reactor, the concentration of NH_4^+-N in the effluent was always higher than that in the influent (Fig. 2). The average effluent NH_4^+-N concentrations of R1, R2 and R3 were 85.6 $mg\,L^{-1}$, 88.8 $mg\,L^{-1}$ and 98.7 $mg\,L^{-1}$, respectively, when the influent NH_4^+-N concentration was 70 $mg\,L^{-1}$. A significant decrease of NO_2^--N concentration was observed in the effluent (Fig. 2). The average effluent NO_2^--N concentrations of R1, R2 and R3 were 44.0 $mg\,L^{-1}$, 46.5 $mg\,L^{-1}$ and 15.6 $mg\,L^{-1}$, respectively, when the influent NO_2^--N concentration was 58 $mg\,L^{-1}$. These observations suggested that denitrification was the dominant reaction in each reactor during this period.

During the 101–360 days, no obvious anammox activity could be observed (Fig. 2). The removal efficiencies of NH_4^+-N and NO_2^--N of the three reactors were not stable (Fig. 2). The average effluent NH_4^+-N concentrations of R1, R2 and R3 decreased to 64.20 $mg\,L^{-1}$,

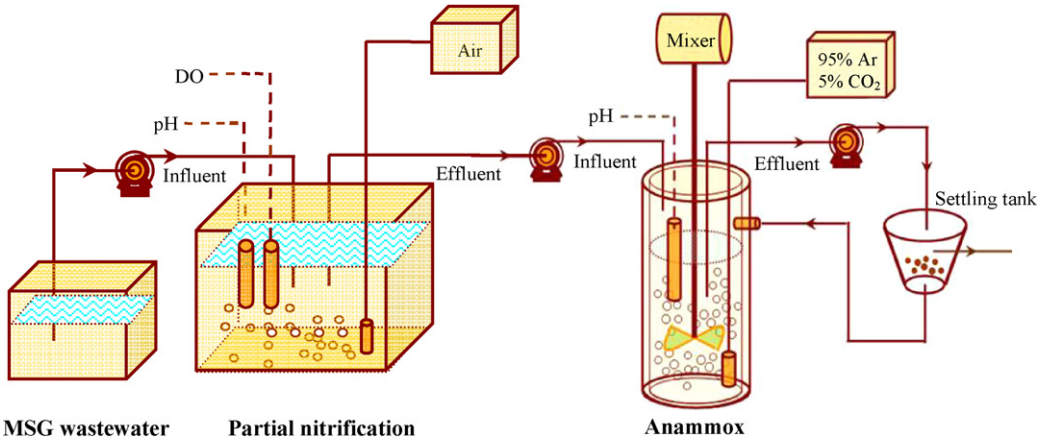

Fig. 1. A schematic diagram of MSG wastewater treatment system.

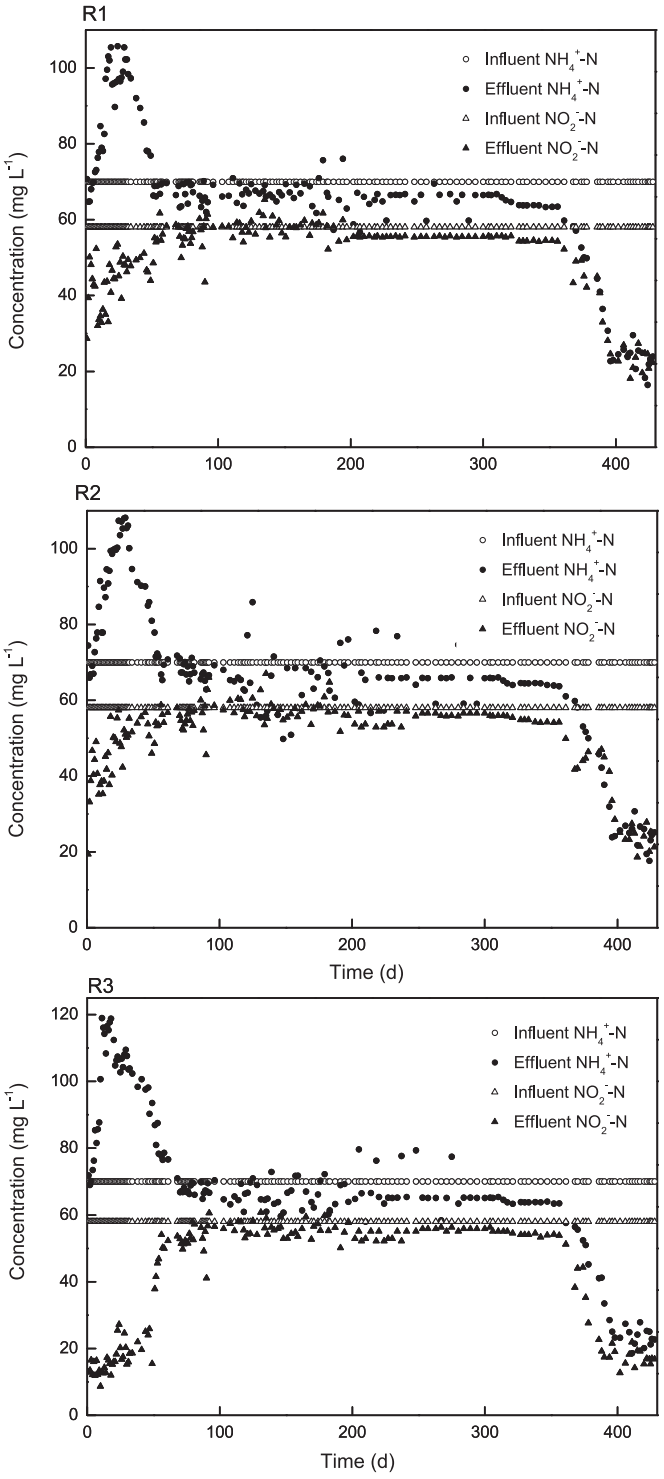

Fig. 2. Performance of the three reactors during the experimental period.

63.42 mg L^{-1} and 65.26 mg L^{-1}, respectively, when the influent NH_4^+-N concentration was 70 mg L^{-1}. The average effluent NO_2^--N concentrations of R1, R2 and R3 were 56.79 mg L^{-1}, 56.62 mg L^{-1} and 51.69 mg L^{-1}, respectively, when the influent NO_2^--N concentration was 58 mg L^{-1}.

A significant removal of ammonium and nitrite was observed after 360 days of cultivation (Fig. 2). The NH_4^+-N removal efficiencies of R1, R2 and R3 were 69.2%, 70.5% and 74.1%, respectively, after 420 days of cultivation. The NO_2^--N removal efficiencies of R1, R2 and R3 were 70.6%, 72.4% and 77.6%, respectively. The

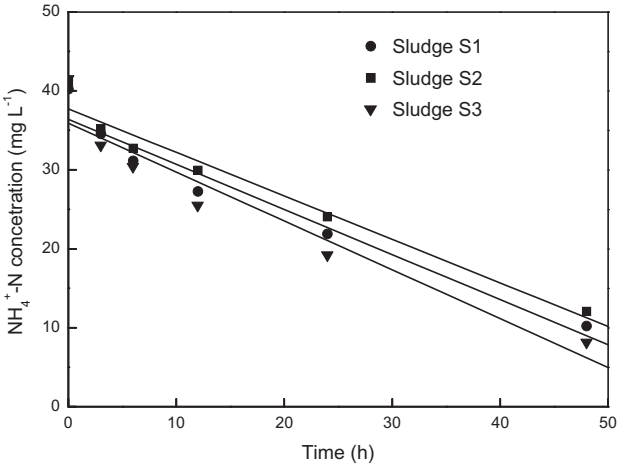

Fig. 3. The consumption of NH_4^+-N by anammox bacteria during activity test.

stoichiometric ratios of NH_4^+-N and NO_2^--N conversion in the three reactors were 1:1.02, 1:1.03 and 1:1.05, respectively. These values were close to the reported value for anammox process [9]. These observations suggested that the anammox reaction prevailed in all three reactors in this enrichment stage.

3.2. Activity of anammox culture

Batch tests were conducted to determine the specific anammox activities of the three cultures after 420 days of cultivation. The results showed that all three cultures could utilize NH_4^+-N and NO_2^--N simultaneously. The stoichiometric ratios of NH_4^+-N and NO_2^--N conversion for cultures S1, S2 and S3 were 1:1.01, 1:1.21 and 1:1.10, respectively, which are similar to the values obtained from the three cultures (1:1.02, 1:1.03 and 1:1.05 in cultures S1, S2 and S3, respectively). The maximum specific anammox activities were obtained with linear curve fitting (Fig. 3). The maximum specific anammox activities of cultures S1, S2 and S3 were 0.11 kg N kg^{-1} VSS day^{-1}, 0.09 kg N kg^{-1} VSS day^{-1} and 0.16 kg N kg^{-1} VSS day^{-1}, respectively. Therefore, culture S3 showed the highest anammox activity among the three cultures.

3.3. Biomass concentration and specific growth rate of anammox bacteria in anammox culture

The biomass concentration and specific growth rate of anammox bacteria in three anammox cultures were determined after 420 days of cultivation. As shown in Table 3, both TSS and VSS clearly increased after enrichment in all three cultures, compared to the corresponding seeding sludges. The VSS/TSS ratio increased from 0.44 to 0.50 in culture S2 and from 0.45 to 0.53 in culture S3, respectively, while no significant change was observed in culture S1. Therefore, culture S3 had the largest increase in biomass, as determined by the VSS/TSS values. The specific growth rates of anammox bacteria in cultures S1, S2 and S3 were 0.0019 h^{-1}, 0.0021 h^{-1} and 0.0024 h^{-1}, respectively. These results indicated that anammox bacteria in culture S3 had a much faster growth rate than those in cultures S1 and S2 under the given conditions.

3.4. Sludge characteristics of anammox culture

Granular sludge was cultivated in the three cultures in this study. Brownish-red anammox granules having diameters in the range of 0.2–1.0 mm were visible in cultures S1 and S2 after 420 days of cultivation. The SVI_{30} values of cultures S1 and S2 were 18.8 mL g^{-1} VSS and 16.6 mL g^{-1} VSS, respectively. The settling

Table 3
Biomass of three anammox cultures.

Anammox culture	Before enrichment			After enrichment		
	TSS ($g L^{-1}$)	VSS ($g L^{-1}$)	VSS/TSS	TSS ($g L^{-1}$)	VSS ($g L^{-1}$)	VSS/TSS
S1	57.3	17.4	0.30	74.6	22.5	0.30
S2	28.3	12.3	0.44	49.0	24.4	0.50
S3	39.1	17.8	0.45	40.6	21.5	0.53

velocities of cultures S1 and S2 were 95.5 $m h^{-1}$ and 97.6 $m h^{-1}$, respectively. Whereas large red granules having diameters in the range of 0.5–2.5 mm were formed in culture S3 after 420 days of cultivation, which had a lower SVI_{30} value (12.7 $mL g^{-1}$ VSS) and a higher settling velocity (106.4 $m h^{-1}$) than cultures S1 and S2. Thus the formed granules in culture S3 showed better settling properties than those in cultures S1 and S2.

3.5. Phylogenetic analysis and quantification of anammox bacteria

The anammox bacterial community composition of the three cultures was determined by clone libraries after 420 days of cultivation. Phylogenetic analysis of 16S rRNA genes showed that three anammox bacterial genera, including *Kuenenia*, *Brocadia* and *Anammoxoglobus*, were found in the cultures (Fig. 4). The sequence of *Kuenenia* organisms showed a high similarity to *Candidatus Kuenenia stuttgartiensis* (98.3–99.2%). The sequence of *Brocadia* organisms was 95.0–98.1% identical to that of *Candidatus Brocadia fulgida*. The sequence of *Anammoxoglobus* organisms was 97.9% identical to that of *Candidatus Anammoxoglobus propionicus*. The anammox bacterial community in culture S1 was composed of *Kuenenia* and *Brocadia* organisms. In culture S2, *Kuenenia*, *Brocadia* and *Anammoxoglobus* genera were found simultaneously. In culture S3, only *Kuenenia* organisms were detected. *Kuenenia* organisms were the dominant anammox species in all cultures (Fig. 4).

The abundances of anammox bacteria in the three cultures were quantified by qPCR, as previously described by Ni et al. [21]. The results showed that culture S3 had the highest number of anammox cells (7.5×10^8 copies mL^{-1}) of the three cultures. The numbers of anammox cells in cultures S1 and S2 were 6.8×10^7 copies mL^{-1} and 9.4×10^7 copies mL^{-1}, respectively.

4. Discussion

For the first 100 days of cultivation, denitrification was the dominant process in each reactor. The denitrifiers used the organic matter present in the MSG industrial wastewater or produced by endogenous respiration of the seeding sludge as the electron donor, and used the NO_2^--N as the electron acceptor. Since Gibbs free energies for anammox and denitrification are −335 kJ mol^{-1} and −427 kJ mol^{-1}, respectively [22,23], it was evident that denitrification is thermodynamically more feasible as compared to anammox. Moreover, anammox bacteria possess a very low growth rate and low cellular synthesis (yield coefficient $Y = 0.066 \pm 0.01$ g VSS g^{-1} NH_4^+-N) [9] when compared to heterotrophic denitrifiers ($Y = 0.3$ g VSS g^{-1} NH_4^+-N) [24]. Therefore the autotrophic anammox bacteria were less competitive for the electron acceptor (NO_2^--N) in comparison to denitrifiers under the coexistence of NO_2^--N, NH_4^+-N and organic matter in the MSG wastewater in the initial enrichment stage. During the 101–360 days, the denitrifying activity significantly decreased. The reason for the decrease of denitrifying activity was probably that most organic matter from the breakdown of the inoculated sludge was exhausted. In the mean time, anammox bacteria exhibited weak anammox activity for the low substrate (NO_2^--N) pressure during this enrichment stage. After 360 days of cultivation, three active anammox cultures capable of using NO_2^--N as the electron acceptor to remove NH_4^+-N in the absence of oxygen were successfully enriched from the three activated sludges. In this enrichment stage, the organic matter from the breakdown of the inoculated sludge was completely exhausted. Because the MSG wastewater used in this study had been treated by anaerobic fermentation, the majority of organic matter in this wastewater was non-biodegradable or slowly biodegradable organic matter. Therefore, the denitrifiers could not compete for NO_2^--N with anammox bacteria for the shortage of the electron donor (biodegradable organic matter). In contrast, anammox bacteria could use NH_4^+-N and NO_2^--N simultaneously under conditions with low biodegradable organic content and anammox gradually became the dominant reaction. The successful anammox enrichment from different seeding sludges indicates that conventional activated sludge could be used as anammox seeding source for the startup of MSG industrial wastewater treatment system. Compared to other anammox enrichment studies using similar reactors and seeding sludges, the time required for enrichment in this study was relatively longer [17,18]. This may be caused by the presence of large amounts of biodegradable organic matter in the three reactors. The biodegradable organic matter had an adverse impact on the growth of anammox bacteria because of its contribution to denitrification during the initial enrichment stage. Synthetic wastewater was used to enrich anammox bacteria in the most reported anammox enrichment studies. In the present study, real wastewater (MSG wastewater) was used to enrich anammox bacteria. The complex nature of MSG wastewater, which contains uncertain components, may also inhibit the growth of anammox bacteria.

Activity tests showed that the culture S3 had the highest specific anammox activity among the three cultures. The culture S3 also had the highest specific growth rate and the largest increase in biomass. Furthermore, large red granules which showed better settling properties than those in cultures S1 and S2 were formed in culture S3. Ni et al. [21] reported a high settling velocity (41–79 $m h^{-1}$) of the anammox granules in an UASB reactor. Tang et al. [12] also reported a high settling velocity of 73–88 $m h^{-1}$ and SVI_5 of 24–25 $mL g^{-1}$ VSS in high-loaded anammox UASB reactors containing granular sludges. By comparison with these values, the anammox granules formed in the three cultures in this study have better settling properties (the settling velocities and SVI_{30} ranged from 95.5 $m h^{-1}$ to 106.4 $m h^{-1}$ and 12.7 $mL g^{-1}$ VSS to 18.8 $mL g^{-1}$ VSS in the three cultures). Granulation plays an important role in biological wastewater treatment systems. Researchers have reported that the formation of granules could benefit both the anammox bacteria and the effluent quality [12,13,21]. Granulation significantly enhances the settling ability of anammox biomass, leading to effective bacterial retention in the reactor. In the present study, the formation of granules with good settling properties resulted in high biomass concentrations in the three cultures. The higher specific anammox activity, specific growth rate of anammox bacteria and biomass increment found in culture S3 could be attributed to the formation of bigger granules. Considering the higher anammox activity and better settling properties of culture S3, sludge S3 which taken from an MSG wastewater treatment plant was identified as the best seeding source for anammox enrichment for MSG wastewater treatment. Sludges S1 and

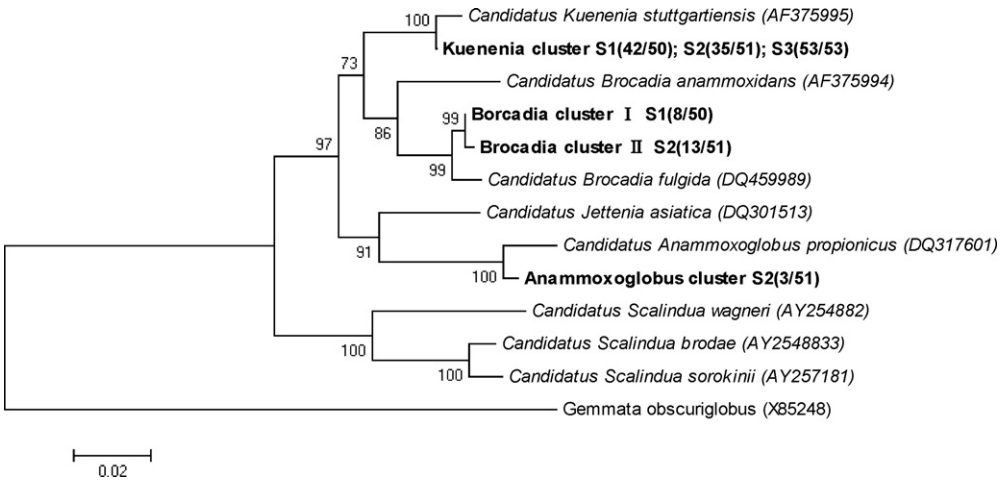

Fig. 4. Phylogenetic tree of anammox bacteria in three anammox cultures.

S2 could also be used to industrialize the anammox process for industrial wastewater treatment, which requires large volumes of the seeding source. Biological treatment of MSG wastewater is very difficult because of the strong biotoxicity of MSG wastewater [3]. Since sludge S3 was collected from an MSG wastewater treatment plant, the microorganisms in the sludge had already been exposed to the MSG wastewater, potentially increasing their resistance to the complex components in MSG wastewater. Sludges S1 and S2, collected from a landfill leachate treatment plant and a municipal sewage wastewater treatment plant, respectively, were not exposed to MSG wastewater prior to enrichment. Hence, the microorganisms in sludges S1 and S2 required more time to adapt to the new environment (MSG wastewater) than those in sludge S3. This could be the main reason why sludge S3 was the best anammox seeding source for treatment of MSG wastewater among the three sludges.

In all three cultures, only one dominant anammox species (*Candidatus Kuenenia stuttgartiensis*) could be detected under the applied growth conditions. This is consistent with other reports [18,25,26]. *Kuenenia* organisms, first discovered in a wastewater treatment plant [15], are ubiquitous in different nitrogen removal systems [25,27]. The anammox species detected in the three cultures further proved that *Kuenenia* organisms are broadly distributed in wastewater treatment systems. The domination of *Kuenenia* organisms in all three cultures indicated that *Kuenenia* organisms better adapted to the MSG wastewater. In addition to *Kuenenia* organisms, *Brocadia* and *Anammoxoglobus* organisms were also detected in the cultures. *Candidatus Brocadia fulgida* was the dominant anammox species in the reactor which was amended with acetate [14], and *Candidatus Anammoxoglobus propionicus* was the dominant anammox species in the reactor which was fed with propionate [26]. It could be speculated that the presence of constituents such as organic acids in MSG wastewater could be responsible for the presence of anammox bacteria related to *Candidatus Brocadia fulgida* and *Candidatus Anammoxoglobus propionicus*. The abundance of anammox bacteria was 10^7–10^8 copies mL^{-1} in the cultures, similar to previously reported values (10^7–10^9 copies mL^{-1}) [21,25]. Different anammox bacterial abundances were detected in the three cultures, and culture S3 had the highest number of anammox cells. Although there is no direct relationship between the number of anammox cells and anammox activity, various studies showed strong positive correlations between the anammox bacterial abundance and activity [11,25]. Therefore, the higher anammox activity in culture S3 may also be related to the higher number of anammox cells.

5. Conclusion

Anammox biomass was successfully enriched from three activated sludges taken from a landfill leachate treatment plant (S1), a municipal sewage treatment plant (S2) and an MSG wastewater treatment plant (S3) using MSG wastewater, which suggested that the anammox process was suitable for nitrogen removal from MSG industrial wastewater and conventional activated sludge could be used as seeding source. Sludge S3, which showed higher anammox activity and better settling properties after enrichment, was recognized as the best seeding sludge. Sludges S1 and S2 could also be used to industrialize the anammox process for industrial wastewater treatment, which requires large volumes of the seeding source.

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