

High rate nitrogen removal by ANAMMOX internal circulation reactor (IC) for old landfill leachate treatment

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Abstract

This study aimed to evaluate the performance of a high rate nitrogen removal lab-scale ANAMMOX reactor, namely Internal Circulation (IC) reactor, for old landfill leachate treatment. The reactor was operated with pre-treated leachate from a pilot Partial Nitrification Reactor (PNR) using a high nitrogen loading rate ranging from 2 to 10 kg N m⁻³ d⁻¹. High rate removal of nitrogen (9.52 ± 1.11 kg N m⁻³ d⁻¹) was observed at an influent nitrogen concentration of 1,500 mg N L⁻¹. The specific ANAMMOX activity was found to be 0.598 ± 0.026 gN₂-N gVSS⁻¹ d⁻¹. Analysis of ANAMMOX granules suggested that 0.5 – 1.0 mm size granular sludge was the dominant group. The results of DNA analysis revealed that *Candidatus Kueneniastuttgartiensis* was the dominant species (37.45%) in the IC reactor, whereas other species like uncultured *Bacteroidetes* bacterium only constituted 5.37% in the system, but they were still responsible for removing recalcitrant organic matter.

Keywords: Leachate, IC; ANAMMOX, Nitrogen removal and granular sludge

1. Introduction

Old landfill leachates are referred to as wastewater and their treatment is very complicated and costly due to high loads of refractory organic compounds, low BOD₅/COD ratio, high salinity and ammonia. The concentration of ammonia in leachate increases gradually according to the age of landfill leachate, which can turn into a serious environmental problem and make waste management difficult (Renou et al., 2008; Sri Shalini and Joseph, 2012).

Partial nitrification (PN) followed by the ANAMMOX process may represent a good alternative to the conventional nitrification–denitrification process for nitrogen removal (van Dongen et al., 2001; Fux et al., 2002; Schmidt et al., 2003). The advantages of this process are that it consumes 50% less oxygen, requires no external organic carbon, has less sludge and results in lower carbon dioxide emissions (Reginato et al., 2005). The PN and ANAMMOX processes were successfully conducted in single reactors or in two consecutive reactors (Jetten et al., 1998; van Dongen et al., 2001). In the case of high influent TKN concentration, the separated two-step process is preferred because it promotes ANAMMOX bacterial activity and suppresses heterotrophic bacterium growth in the reactor (Van Hulle et al., 2007). This combined PN-ANAMMOX process was investigated in previous studies at low nitrogen loading rates, which ranged from 0.17 to 0.96 kg N m⁻³ d⁻¹ (Liang and Liu, 2008; Liu et al., 2010; Rusalleda et al., 2010; Wang et al., 2010; Xu et al., 2010). A more recent analysis indicated that an ANAMMOX reactor operated for a long period of time was only stably run under nitrogen loading rate of 1 kg N m⁻³ d⁻¹ and 85 ± 1% of nitrogen removal. In this study *Kuenenia Stuttgariensis* affiliates served as the dominated species (Li et al., 2014).

In fact, to date ANAMMOX granules have not been widely studied but there is some evidence that removing 50% of nitrogen can be achieved at extremely high nitrogen loading rate of 50 – 60 kg N m⁻³ d⁻¹ in a high rate ANAMMOX UASB reactor (Tang et al., 2014).

Moreover, a few studies have reported that concentrations of Ca(II) and Fe(II) in feeding

wastewater can have an impact on intracellular chemical composition as well as bacterial activity (Liu et al., 2013; Qiao et al., 2013). Based on the settling properties of ANAMMOX granular sludge as documented in other studies, granules with a diameter of 1.75 – 2.20 mm were ideal for the ANAMMOX process because they avoided floatation (Lu et al., 2012 & 2013).

A few years ago, we developed a model called the hybrid ANAMMOX reactor (HAR) which removed nitrogen from leachate. However, operating this reactor presented some disadvantages: a) the small formation of granular sludge which entailed the highest influent nitrogen loading rate of $8 \text{ kgN m}^{-3} \text{ d}^{-1}$; and b) the appearance of sludge clogging up the effluent when the reactor operated for a long period of time. To overcome the above drawbacks, a novel reactor known as ANAMMOX internal circulation reactor (IC) was introduced in this study and the performance of this high rate nitrogen removal IC reactor for old landfill leachate treatment was evaluated.

Due to the slow growth rate of the Anammox bacteria, the reactors are usually operated at an extremely long sludge retention time in order to cultivate the bacteria successfully. In the free cell or flocculent sludge Anammox systems, the cultured biomass is easily to be washed out. In addition, Anammox bacteria have a trend to grow in aggregates, high-rate granule system was considered to be one of the suitable and promising methods for Anammox process (Tang et al., 2014). Previous study postulated that IC-granules are larger than UASB-granules grown on similar wastewater, while the strength of IC-granules is lower as a result of the higher sludge loading rate. The IC reactor is an anaerobic sludge bed reactor constructed in the form of tall cylindrical vessel. It uses an internal circulation based on airlift principle, generated by the biogas that is produced inside the reactor. Optimal biomass retention is provided by a two-stage separator. The first stage separates the majority of the biogas from the water, so that the second stage mainly separates the active biomass from the treated effluent. Internal Circulation (IC) reactors can be operated at higher volume loading rates than UASB reactors.

The two-stage design of the IC-reactor allows 3-6 times higher applied loading rate. Although wash-out is slightly enhanced in IC-reactors, the conditions in the second stage are tranquil enough to ensure adequate biomass retention in IC-reactors. This internal circulation ensures dilution of the raw influent and ensures optimal mixing conditions for the anaerobic granular sludge and the wastewater. Furthermore, the liquid upflow velocity is 8-20 times higher and the average shear rate in IC reactors is approximately twice as high compared to UASB reactors, still granules develop successfully in IC-reactors. That is the main things that makes the anammox granular sludge improve good mixing conditions and reduce the sludge clogging in the IC (Pereboom and Vereijken, 1994). The performance of the Anammox process is enhanced by the use of appropriate effluent recirculation with other positive operation conditions. The characteristics of recycled effluent were close to the interior water conditions of the Anammox system, which improved the nitrogen elimination efficiency and process stability. The appropriate rate of circulation of 1.0-1.7 helped maintain circulation velocity in the IC that improve anammox granular formation (Jin et al., 2012).”

2. Materials and Methods

2.1. Internal Circulation Reactor

The Internal Circulation (IC) reactor was made of acrylic tubes with a total volume of 13.8 litres and working volume of 10 litres. The reactor was 1,450 mm in height and 100 mm in diameter. The granules were mixed with feed leachate by flowing from a downpipe that circulated flow in the lower compartment. The leachate moved from the lower compartment to the upper compartment. This resulted in stratification of granular sludge in the upper compartment. Adequate up-flow velocity and shear stress were maintained in favour of granule formation, reduction of sludge washout and negative effects of high nitrite concentration on ANAMMOX bacteria growth. A three-phase funnel separator at the top of

the reactor served to collect the resultant nitrogen gas. The schematic diagram of IC is presented in Figure 1.

Insert Figure 1

2.2. Leachate and ANAMMOX sludge

Landfill leachate used in this study was collected from Go Cat municipal solid waste landfill which is located in Ho Chi Minh City, Vietnam. The landfilling for this landfill was completed in 2007. The feed leachate was pre-treated using a pilot Partial Nitritation Reactor (PNR), which was run at the same time as the ANAMMOX reactor. The PNR was run at nitrogen loading rates (NLR) of 0.8-0.9 kg N m⁻³ d⁻¹ and initial ammonia concentration of 2,830-3,096 mg N L⁻¹. The effluent of the PNR obtained ammonia:nitrite ratios ranging from 0.9 to 1.35 and contained COD of 1,565 ± 102 mg L⁻¹. The feed leachate was PNR effluent diluted with tap water to obtain the influent ammonia and nitrite concentration varying between 235-655 mg N L⁻¹ and 261–858 mg N L⁻¹, respectively. The PNR effluent did not reach the ammonia:nitrite ratio of 1.00–1.35, so NH₄Cl and NaNO₂ salts were added.

The feed sludge was taken from the hybrid ANNAMOX reactor used in a previous study (Nhat et al. 2014). 65 g VS of the feed sludge, equivalent to 6,500 mg VSS L⁻¹ and 13,000 mg SS L⁻¹ was seeded into the IC.

2.3. Operating conditions

The IC experiment lasted 280 days at an ambient temperature ranging from 25 to 30⁰C. The experiment comprised 5 phases, corresponding to different NLR ranging from 2 to 10 kg N m⁻³ d⁻¹. The operating conditions are shown in Table 1.

Insert Table 1

During the operation phase, pH of the feed leachate was manually adjusted in the 6.8 – 7.2 range using 10% HCl solution. The experiment was conducted under dark conditions using a black plastic sheet fully enclosing the reactor body to prevent algae growth. Na₂SO₃ salt was added into the PN-SBR effluent tank to reduce DO to less than 0.5 mg L⁻¹.

2.4. Specific ANAMMOX Activity

The specific ANAMMOX activity (SAA) was determined by a manometric method adapted from another research analysis (Dapena-Mora et al., 2007). Batch assays were performed in 120 mL glass vials (57 mL of actual volume) sealed with a rubber septum. The experiment lasted 4 – 4.5 hours, with mixing at 150 rpm and DO < 0.1 mg L⁻¹. A differential pressure sensor (Omron, Japan) was used to measure pressure; the average error in pressure measurement was less than 5%. The granular sludge collected from the IC reactor and used for batch tests was previously washed and re-suspended in mineral solutions according to the research of Dapena-Mora et al. (2007).

2.5. ANAMMOX sludge characteristics

Granular ANAMMOX sludge was characterized by assessment of trace metals, phosphorous content, settling velocity, specific gravity versus the size of granules and granule size distribution. Three 100 mL samples of ANAMMOX granular sludge that were taken at different heights of the IC reactor were mixed together. The granules obtained were then sieved through four sieves with different meshes (0.5 mm, 1 mm, 2 mm and 3 mm). Trace metals including Ca, Mg and Fe and phosphorous in the destroyed sludge were determined

according to standard methods (APHA et al., 1999). The sludge destruction procedure was based on EPA Method 200.7 (1994). The physical characteristics of the ANAMMOX sludge consisting of specific gravity and settling velocity, were measured according the method employed in a previous study (Zheng et al., 2005). The granule size distribution was determined by a Laser Scattering Particle Size Distribution Analyser LA-950 (HORIBA, Japan). This method measures sludge samples smaller than 3.0 mm in size. To remove granules larger than 3.0 mm, the sludge sample was sieved using a 3.0 mm mesh sieve.

2.6. DNA extraction and PCR amplification

The granular sludge sample originated from the IC reactor. Metagenomic DNA was extracted from the granular biomass using FasDNA Spin kit for Soil (MP Biomedicals, OH, USA), according to the instruction manual. The amplification of 16S rRNA gene was done at the Macrogen Company laboratory using V34 primer variable region forward primer:

5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG

(ref.) and reverse primer

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC

-3'. Polymerase chain reaction (PCR) was carried out at the Macrogen Co. The set-up the reaction of DNA includes 2.5 μl of Microbial DNA 5 $\text{ng } \mu\text{l}^{-1}$, 5 μl of Amplion PCR Forward Primer 1 μM , 5 μl of Amplion PCR Reverse Primer 1 μM and 12.5 μl of 2X KAPA HiFi HotStart ReadyMix PCR kit. Total volume was 25 μl . PCR was executed in a thermal cycler using the following program: 3 minutes for initial denaturation at 95°C, 25 cycles of 30s at 95°C, 30s at 55°C, 30s at 72°C, and then 5 minutes at final elongation at 72°C. The amplicon DNAs were performed using PCR clean-up, 2nd stage PCR, PCR Clean-Up 2 and finally Library Quantification and Normalization as described in the 16S Library Preparation Workflow (Figure 2). Raw sequences were then filtered, aligned and clustered at 0.03

distances using the furthest algorithm to generate operational taxonomic units (OTUs). OTUs were then classified using the same database from Mothur software package Version 1.34.3). OTUs of dominant groups were then classified again using the Blast (online database) for finding the closest matches.

Insert Figure 2

2.7. Analytical methods

pH and DO were measured using a pH meter (HI 8314, Hanna) and DO meter (InoLab 740, WTW, Germany), respectively. Determination of nitrogen species including ammonia, nitrite and nitrate was conducted according to standard methods (APHA et al., 1999).

3. Results and discussion

3.1. Performance of IC reactor

Concentrations of nitrogen species in the influent and effluent with time is presented in Figure 3.

Insert Figure 3

During phase I (day 1 – 28), the nitrite removal efficiency (NRE) progressively increased from 46% on day 1 to 96% on day 18, reaching a peak of 100% on day 25. The average total nitrogen removal rate (NRR) was $1.70 \pm 0.44 \text{ kg N m}^{-3} \text{ d}^{-1}$. During this phase, the influent nitrate fluctuated between 30 and 57 mg N L^{-1} , corresponding to 5.9% and 8.9% of influent total nitrogen, respectively. Despite the rise of NLR to $4.0 \pm 0.04 \text{ kg N m}^{-3} \text{ d}^{-1}$, the NRE of 80% was observed on the first day of phase II (day 29 – 45). The high removal efficiency indicates that the ANAMMOX bacteria had the ability to adapt to and resist the loading shock

at higher NLR, with considerable NRR of $3.83 \pm 0.20 \text{ kg N m}^{-3} \text{ d}^{-1}$. The effluent concentrations of ammonia and nitrite nitrogen were always under detection limits at the end of this phase. Insignificant differences between phase I and phase II with reference to influent nitrate were found and nitrate nitrogen was in the range of $31\text{--}55 \text{ mg N L}^{-1}$. During phase III (day 46 – 70), the total influent nitrogen concentration increased from $528 \pm 19 \text{ mg N L}^{-1}$ to $809 \pm 12 \text{ mg N L}^{-1}$ while NLR increased from $4.0 \pm 0.04 \text{ kg N m}^{-3} \text{ d}^{-1}$ to $6.0 \pm 0.09 \text{ kg N m}^{-3} \text{ d}^{-1}$, which is equivalent to NRR of $5.17 \pm 0.48 \text{ kg N m}^{-3} \text{ d}^{-1}$ after 25 days of operation. Furthermore, the effluent nitrate concentration (39 mg N/L) was less than the theoretical value (90 mgN/L). This may be due to the coexistence of denitrification and the ANAMMOX process. Phase IV (day 71 – 87) started at slightly lower NRE (76%) due to a sudden increase in influent total nitrogen concentration to $1,290 \pm 9 \text{ mg N L}^{-1}$, corresponding to NLR to $8.0 \pm 0.06 \text{ kg N m}^{-3} \text{ d}^{-1}$. However, a rapid increase of NRE to 93% was observed on day 9 of phase IV and NRE of 100% after day 13. Thus, the IC performed excellently with average NRR of approximately $7.00 \pm 0.86 \text{ kg N m}^{-3} \text{ d}^{-1}$.

Long-term steady operation of IC with very high NRR of $9.52 \pm 1.11 \text{ kg N m}^{-3} \text{ d}^{-1}$ was obtained at NLR of $10.0 \pm 0.04 \text{ kg N m}^{-3} \text{ d}^{-1}$ in Phase V (day 87 – 280), whereas other studies only ran at NLR under $1 \text{ kg N m}^{-3} \text{ d}^{-1}$ (Liang and Liu, 2008; Liu et al., 2010; Rusalleda et al., 2010; Wang et al., 2010; Xu et al., 2010). In addition, a recent study concerning old leachate treatment showed that HAR achieved NRR of $6.7 \text{ kg N m}^{-3} \text{ d}^{-1}$ (Nhat et al., 2014). However, at high NLR, the HAR encountered clogging by the sludge which limited substrate diffusion and caused the ANAMMOX biomass to decay. Nearly complete removal of nitrogen (ammonia and nitrite) was found after 28 days of this phase. On day 118, a significant decline in nitrogen removal was observed due to high influent pH (8.2), which was caused by no pH adjustment. In order to recover the ANAMMOX activity, the PN-treated leachate was diluted with tap water in order to gradually increase nitrogen concentration (from $200\text{--}1,500 \text{ mg N L}^{-1}$). It took a recovery process lasting 22 days for it to reach a level of stable performance

(NRE of 99%) at NLR of $10.0 \pm 0.04 \text{ N m}^{-3} \text{ d}^{-1}$. Another problem occurred on day 178, that the faulty effluent recirculating pump created a high nitrite concentration in the bottom compartment, causing inhibition of ANAMMOX bacteria. Troubleshooting of this problem was similar to that on day 118. After 5 days, the IC was completely recovered.

3.2. Specific ANAMMOX activity

Insert Table 2

Ammonium and nitrite are the main substrates for Anammox bacteria so the increase in availability of these substrates in the reactor should increase the activity of bacteria because of enhancing the diffusion both into the depth of biofilm and through cell membrane of bacteria. Specific Anammox activity (SAA) is defined as transformation of nitrogen from different forms into nitrogen gas. The granular ANAMMOX sludge characterized by high activity plays a pivotal role in the performance of high-rate bioreactors. When the maximum specific activity was exceeded, the system became unstable and biomass started to float (Dapena-Mora et al., 2007).

In addition, the common methodological approaches adopted to detect, identify and confirm ANAMMOX bacteria or their activity includes (a) Chemical analyses of the nitrogen compounds to detect the change in the concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ owing to ANAMMOX activity (showed in section 3.1 in this study); (b) Application of molecular techniques such as Polymerase Chain Reaction (PCR), which are based on the nucleic acid analysis for identification. A number of specific sequences and primers were developed to amplify the 16S rRNA from the environmental and enrichment samples using the PCR based approach (showed section 3.4 in this study).

Table 2 shows the SAA value of the study was higher than those of other studies. This proves that ANAMMOX bacteria have the ability to adapt, grow and thrive in the IC reactor with old leachate feeding.”

3.3. ANAMMOX sludge characteristics

The sludge profile is presented in Figure 4. The initial sludge concentration of 13,000 mg SS L⁻¹ was seeded at the beginning. On day 280, sludge concentrations at the bottom, middle and top of IC were 66,530 ± 1,960 mg MLSS L⁻¹, 60,499 ± 5,793 mg MLSS L⁻¹ and 59,016 ± 2,732 mg MLSS L⁻¹, respectively (Figure 4). It was found that the MLVSS:MLSS ratio and SVI of the reactor were 0.57 ± 0.01 and 9.92 ± 0.93 ml g⁻¹, respectively. Table 3 shows characteristics of ANAMMOX sludge.

Insert Figure 4

Insert Table 3

The granule distribution values were calculated based on the results from the laser scattering particle size distribution analyser shown in Figure 5. It emerged that the diameter of the dominant ANAMMOX granules in IC reactor ranged from 0.5 to 1 mm, which accounted for 58% of the total number of particles and 35% of the total mass. The settling velocity of the dominated granules (2.03 cm s⁻¹) was in the range of the efficient settling velocity of ANAMMOX sludge (2.0 and 2.4 cm s⁻¹) in terms of bacteria growth and activity promotion (Tang et al., 2011). Moreover, the VSS:SS ratio of 0.63 and the Fe content of 11.7 mg Fe gVSS⁻¹ were the highest values compared to the other granule sizes. In other research, Huang et al. (2014) reported that ferrous was the determining factor in nitrogen removal efficiency and granules of 0.5–1.0 mm size could be maintained for maximum performance. Calcium

concentration, magnesium concentration and density of these ANAMMOX granules, were 180 mgCa gVSS⁻¹, 9.5 mgMg gVSS⁻¹ and 1.05–1.06 g cm⁻³, respectively.

Insert Figure 5

3.4. DNA Analysis

Insert Table 4

The purpose of analysing the microbial community by 16S rRNA using 16S V3 and V4 regions were to investigate the microbial community structure of IC granular sludge. 35409 sequences were obtained and these were grouped into 156 representative OTUs.

Results shows that the most dominant population was closely related to *Candidatus Kueneniastuttgartiensis* (37.45%), which has also been reported in previous studies (Furukawa et al., 2002; Li et al., 2014). Apart from this, although uncultured *Bacteroidetes* bacterium had a very low relative abundance (RA) of 5.37%, this population played a key role in degrading refractory organic matter, particularly decaying aquatic humic substances (AHS) and removing volatile fatty acids (VFAs) (Liang and Liu, 2008). Indeed, this result was identical to the results reported in Nhat et al. (2014).

The ANAMMOX bacteria outnumbered other bacteria in the reactor can be explained as follows. The feed leachate into the IC was pre-treated using a pilot Partial Nitritation Reactor (PNR). The influent has the ratios of NO₂-N/NH₄-N = 1.0-1.3 that suitable for the growth of anammox bacteria under anaerobic condition. Moreover, old leachate after the partial nitritation process contains very low biodegradable COD. Under these conditions, denitrifiers

grow very limited due to lack of rapid biodegradable COD. The most dominant population was closely related to *Candidatus Kueneniastuttgartiensis* (37.45%, in this study). In addition, ANAMMOX population was related to *Kueneniastuttgartiensis* and *Brocadia anammoxidans*. Besides some other bacteria were also identified *Cytophaga*, *Flavobacterium*, *bacteroides*, etc. (Schmid et al., (2001).

4. Conclusions

The results of this study demonstrated that the use of an IC reactor running at very high NLR of $10 \text{ kg N m}^{-3} \text{ d}^{-1}$ proved to be a feasible method for removing nitrogen from old landfill leachate. The ANAMMOX granules with a diameter ranging from 0.5–1.0 mm demonstrated to be the best performing and dominating group. Furthermore, the PCR data revealed that the ANAMMOX bacteria outnumbered other bacteria in the reactor with relative abundance (RA) of about 37.45%.

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ACCEPTED MANUSCRIPT

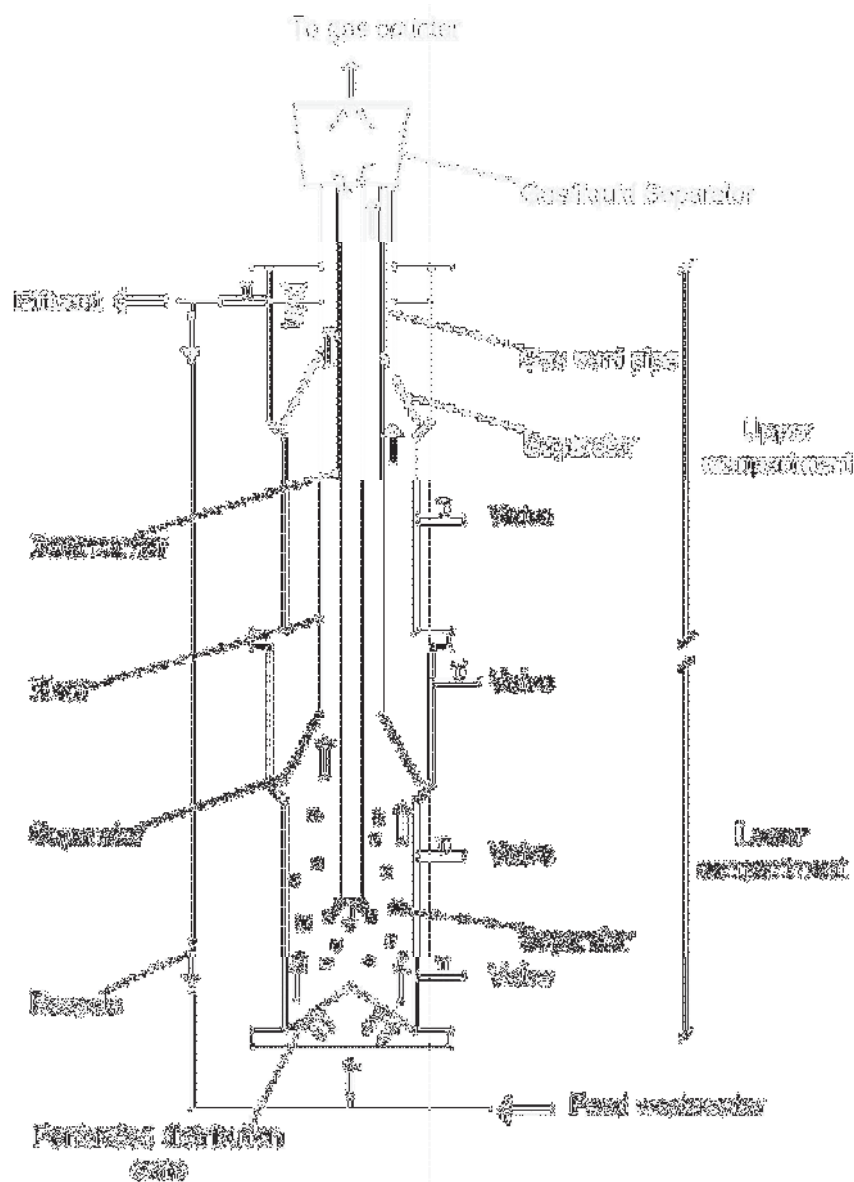


Figure 1. Schematic diagram of ANAMMOX IC reactor

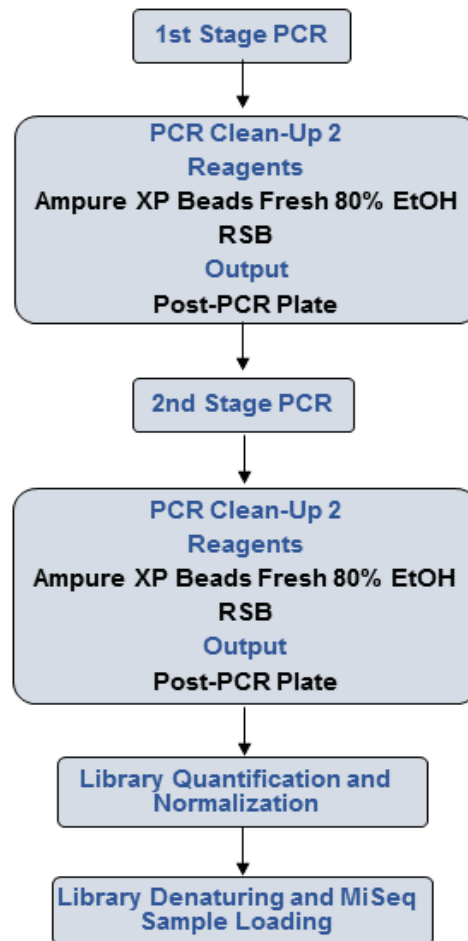


Figure 2. 16S Library preparation workflow

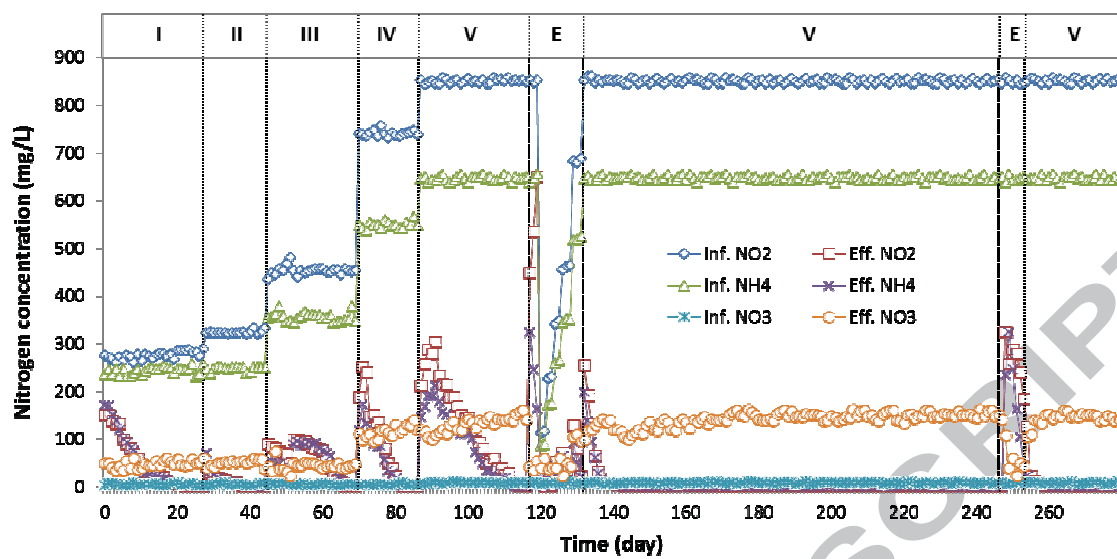


Figure 3. Time course of concentration of nitrogen species in the experiment

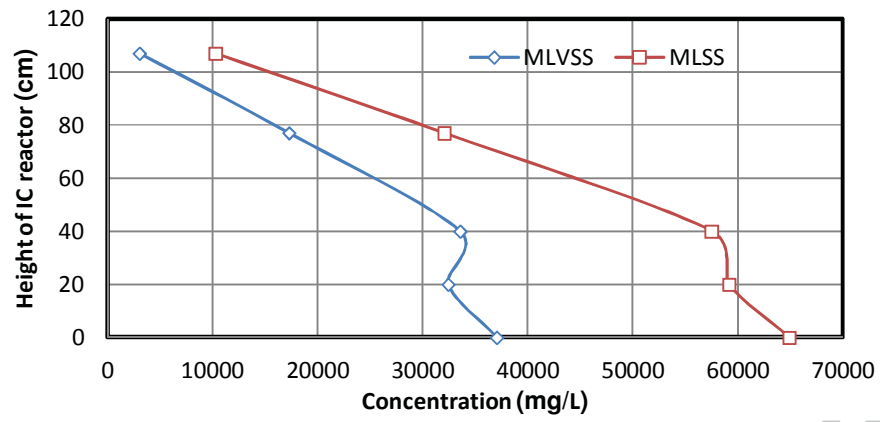


Figure 4. ANAMMOX sludge profile on day 280

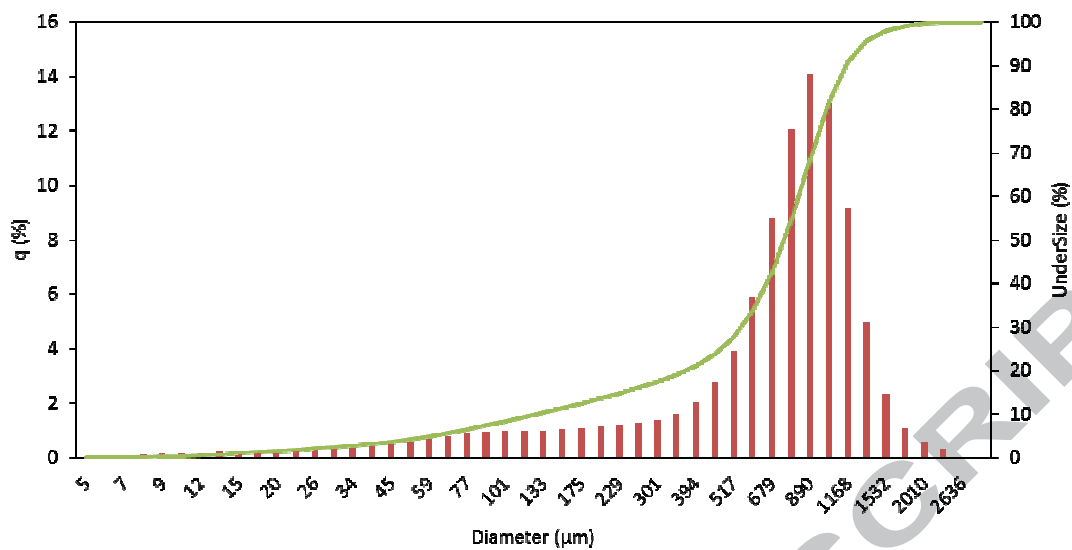


Figure 5. Granule size distribution of ANAMMOX sludge in IC reactor

Table 1. Operating conditions of IC reactor

	Phase I	Phase II	Phase III	Phase IV	Phase V
Influent $\text{NH}_4^+\text{-N}$, mg N L^{-1}	231 - 257	238 - 251	344 - 379	538 - 566	638 - 655
Influent $\text{NO}_2^-\text{-N}$, mg N L^{-1}	261 - 290	321 - 323	435 - 481	732 - 757	845 - 858
TN loading rate, mg N L^{-1}	2.1 ± 0.05	4.0 ± 0.04	6.0 ± 0.09	8.0 ± 0.6	10.0 ± 0.4
Influent flow rate, l day^{-1}	40	70	74	62	67
Circulation rate, l day^{-1}	67	67	67	67	67
Up-flow velocity, m h^{-1}	0.57	0.73	0.75	0.68	0.71
HRT, h	6.00	3.42	3.24	3.87	3.63
Operating time, d	1 - 28	29 - 45	46 - 70	71 - 87	88 - 280

Table 2. Result of SAA batch assays compared to other studies

SAA (g N ₂ -N gVSS ⁻¹ d ⁻¹)	References	ANAMMOX reactor	Nitrogen concentration (mg N L ⁻¹)
0.598 ± 0.026	This study	IC with landfill leachate	NH ₄ -N = NO ₂ -N = 70
0.43 ± 0.11	Scaglione et al., 2012	SBR with synthetic wastewater	NH ₄ -N = NO ₂ -N = 70
0.32 ± 0.13	Scaglione et al., 2012	SBR with landfill leachate	NH ₄ -N = NO ₂ -N = 70
0.28 ± 0.02	Dapena-Mora et al., 2007	SBR with synthetic wastewater	NH ₄ -N = NO ₂ -N = 70
0.384 ± 0.125	Lotti et al., 2012	IC full-scale with digested sludge wastewater	NH ₄ -N = NO ₂ -N = 50
0.54 ± 0.10	Ruscalleda, 2011	SBR with landfill leachate	NH ₄ -N = NO ₂ -N = 70

Table 3. Characteristics of ANAMMOX sludge in the study

Diameter (mm)	0.5 – 1	1 – 2	2 – 3	> 3
Settling velocity (cm s ⁻¹)	2.03 ± 0.12	3.9 ± 0.61	7.96 ± 0.67	9.86 ± 0.81
Granule density (g cm ⁻³)	1.05 - 1.06	1.06 - 1.07	1.08 - 1.09	1.09 - 1.10
Calcium (mgCa gVSS ⁻¹)	180 ± 3.97	211 ± 2.83	277 ± 8.30	373 ± 1.48
Magnesium (mgMg gVSS ⁻¹)	9.50 ± 1.81	24.50 ± 1.70	41.00 ± 4.97	58.90 ± 0.89
Iron (mgFe gVSS ⁻¹)	11.71 ± 0.62	7.12 ± 0.58	4.28 ± 0.46	3.82 ± 0.11
Phosphorus (mgP gVSS ⁻¹)	155 ± 0.70	164 ± 0.25	157 ± 0.27	163 ± 0.34
Granule distribution by particles (%)	57.59 ± 3.86	9.48 ± 3.04	2.23 ± 0.92	0.84 ± 0.16
Granule distribution by mass (%)	35.18	16.30	22.57	25.94
VSS:SS ratio	0.63	0.61	0.61	0.51

Table 4. Dominant microbial members of granular sludge from IC reactor

OTU#	Taxonomy	# of sequence	RA (%)	Accession	similarity	References
1	<i>Candidatus Kueneniastuttgartiensis</i>	13261	37.45	AF375995	98%	Schmid et al., 2001
2	Uncultured bacterium (Found from Anammox reactor)	3125	8.83	EF584533	98%	Bae et al., 2010
3	Uncultured bacterium	1587	4.48	KP372914	97%	Sun et al., 2015
4	Uncultured <i>planctomycete</i> clone 5GA_Pla_HKP_17	2800	7.91	GQ356164	99%	Park et al., 2010
5	Uncultured <i>Bacteroidetes</i> bacterium	1900	5.37	EF074300	90%	Unpublished
6	Uncultured <i>Desulfuromonadales</i> bacterium	1583	4.47	KJ611805	98%	Pajares et al., 2015

Remark: RA: relative abundance

ANAMMOX International Recirculation (IC) reactor for leachate treatment:

- High applied nitrogen loading rate of $10 \text{ kg N/m}^3 \cdot \text{d}$
- ANAMMOX granules of 0.5–1.0 mm;
- ANAMMOX bacteria outnumber with relative abundance of 37.45 %.



ANAMMOX granules

Highlights

- An Internal Circulation (IC) ANAMMOX reactor was used for leachate treatment
- The IC reactor achieved high nitrogen removal rate of $9.52 \text{ kg N m}^{-3} \text{ d}^{-1}$
- The specific ANAMMOX activity was found to be $0.598 \text{ gN}_2\text{-N gVSS}^{-1} \text{ d}^{-1}$
- ANAMMOX granule size of 0.5 – 1.0 mm was the dominant in the IC reactor
- *Candidatus Kueneniastuttgartiensis* was the major species (37.45%)