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Start-up of Anaerobic Ammonium Oxidation (Anammox) from Conventional Return Activated Sludge in Up-flow Anaerobic Sludge Blanket (UASB) Reactor for Autotrophic Nitrogen Removal from Wastewater

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ABSTRACT

A bench-scale up-flow anaerobic sludge blanket (UASB) reactor was applied to start-up the anaerobic ammonium oxidation (Anammox) process from conventional return activated sludge. The reactor was operated for approximately 170 days and was fed synthetic wastewater containing mainly nitrite (NO_2^-) and ammonium (NH_4^+) as main substrates. After 106 days of operation, the first evidence of Anammox activity was detected in the reactor by simultaneous consumption of NO_2^- and NH_4^+ coupled to the production of nitrate (NO_3^-). The start-up period of the Anammox UASB reactor was within 4.5 months. During the course of the start-up period, three distinct stages were distinguished: cell lysis, Anammox cultivation and finally the appearance and enrichment of Anammox bacteria. On the basis of the results, it seems that the successful cultivation of Anammox bacteria in this study was probably due to the capacity of UASB reactor in retaining Anammox biomass and the inherent properties of the activated sludge.

KEYWORDS: Anammox, Start-up, Up-flow anaerobic sludge blanket (UASB), Endogenous denitrification, Biological nutrient removal, Return activated sludge.

INTRODUCTION

Ammonium nitrogen is a common pollutant in a variety of wastewaters. Some of the problems caused by inappropriate discharge of such pollutant include eutrophication and oxygen depletion in receiving watercourses (Klees and Silverstein, 1992). Therefore, biological nutrient nitrogen removal (BNNR), being most cost-effective, is an integral part of wastewater treatment. The overwhelming majority of nutrient nitrogen removal options are based on the sequential microbial processes of nitrification and denitrification (Ekama and Wentzel, 2008). However, these combined processes require high input of energy for aeration required for the nitrification step and supplementation of readily biodegradable organic carbon source for the subsequent denitrification step. The newly discovered microbial process of anaerobic ammonium oxidation (Anammox) is a sustainable and cost-effective alternative to the conventional nitrification-denitrification processes for nitrogen removal; particularly, for wastewater with deficiency in organic matter (Mulder er al., 1995; van der Graaf et al., 1995). The Anammox process involves the oxidation of NH_4^+ with NO_2^- as electron acceptor to produce dinitrogen gas

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under anoxic conditions (Eq. (1)) (Van de Graaf et al., 1995, 1996).

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \quad (1)$$

The Anammox process offers several advantages over the conventional BNNR. The process requires less oxygen demand because only about a half of the NH_4^+ needs to be oxidized to NO_2^-. Operational costs are also significantly reduced, since no external carbon source is required. Moreover, the low yield of the Anammox bacteria (0.066 ± 0.01 mol C mol^{-1} NH_4^+-N) results in low sludge production (Liao et al., 2008). Anammox bacteria have a slow growth rate with reported doubling time around 10 to 12 days (Van der Star et al., 2007). However, their specific growth rate is higher than 1 g N g^{-1} biomass dry weight d^{-1} (Strous et al., 1998). Thus, nitrogen removal rates higher than conventional BNNR can be achieved. Nitrogen removal rate up to 26 kg m^{-3} d^{-1} has been reported by (Tsushima et al., 2007).

It is well documented that Anammox is catalyzed by chemolithoautotrophic bacteria belonging to the phylum Planctomycetes (Strous et al., 1999a). These bacteria are grouped into four *Candidatus genera*: *Brocadia* including *B. anammoxidans* (Kuenen and Jetten, 2001; Schmid et al., 2000) and *Brocadia fulgida* (Kartal et al., 2005), *Kuenenia* including *K. stuttgartiensis* (Schmid et al., 2003), *Scalindua* including *S. wagneri*, *S. brodae* and *S. sorokinii* (Jetten et al., 2005; Kuypers et al., 2003; Schmid et al., 2003) and *Anammoxoglobus* including *Anammoxoglobus propionicus* (Kartal et al., 2007). Different Anammox microorganisms have been detected by molecular probing techniques such as PCR, phylogenetic analysis or fluorescent *in situ* hybridization (FISH) in both natural and engineered systems all over the world (Dalsgaard et al., 2005; Kuypers et al., 2003; Third et al., 2005; van de Graaf et al., 1995). These microorganisms have been detected in several wastewater treatment plants in Netherlands, Switzerland, UK, Germany, Australia and Japan (Jetten et al., 1998).

The practical application of the Anammox technology is limited by its long start-up period of the Anammox bioreactor. The start-up of Anammox process is generally time-consuming, lasting months to years (Trigo et al., 2006). For example, the start-up of a full scale application of Anammox process in Rotterdam wastewater treatment to treat sludge digester effluent was approximately two years (Kuenen, 2008). To shorten the start-up process, the availability of appropriate seed containing Anammox activity and the choice of the Anammox bioreactor type are very important. The bioreactor should have efficient biomass retention in order to maximize the biomass concentration. Various bioreactors have been used for the enrichment of the Anammox bacteria including gas lift reactor (Sliekers et al., 2003), sequencing batch reactor (Strous et al., 1998), fluidized bed reactor (van de Graaf et al., 1996) and other types. Among these, upflow anaerobic sludge blanket (UASB) reactors have been proven to be a suitable technology in developing and retaining high concentrations of slow growing anaerobic microorganisms under high contaminant and hydraulic loading conditions. The aim of this study was to apply the UASB reactor for the start-up of Anammox process from local activated sludge.

MATERIALS AND METHODS

Experimental Set-up

A continuous flow experiment was performed in a bench scale UASB reactor as depicted in Figure 1. The reactor was fabricated from glass and had a working volume of 3 L. The UASB reactor was fed with a synthetic wastewater mainly containing ammonium and nitrite for the support of Anammox activity according to the medium described in Table 1. The initial average hydraulic retention time (HRT) of the UASB reactor was around 28 hours, corresponding to an average nitrogen loading rate (NLR) of 0.09 g N $L^{-1}_{reactor}$ d^{-1}. The UASB reactor was covered with aluminum foil to avoid the light inhibition and operated at an average room temperature of 24°C.

Table 1. Composition of synthetic wastewater fed to UASB reactor

Component	Concentration ($g\ L^{-1}$)
NH_4HCO_3	0.22
$NaNO_2$	0.24
$NaH_2PO_4.H_2O$	0.06
$CaCl_2.2H_2O$	0.1
$MgSO_4.7H_2O$	0.20
$NaHCO_3$	1.05
Trace element 1*	1.00 mL
Trace element 2**	1.00 mL

* Trace element solution 1 contained (in $g\ L^{-1}$): $FeSO_4$ (5.00) and Ethylenediamine-tetraacetic acid (EDTA) (5.00).

** Trace element solution 2 contained (in $g\ L^{-1}$): EDTA (15.00); $ZnSO_4 \cdot 7H_2O$ (0.43); $CoCl_2 \cdot 6H_2O$ (0.24); $MnCl_2$ (0.63); $CuSO_4 \cdot 5H_2O$ (0.25); $Na_2MoO_4 \cdot 2H_2O$ (0.22); $NiCl_2 \cdot 6H20$ (0.19); $Na_2SeO_4 \cdot 10H_2O$ (0.21); H_3BO_3 (0.01); $NaWO_4 \cdot 2H_2O$ (0.05).

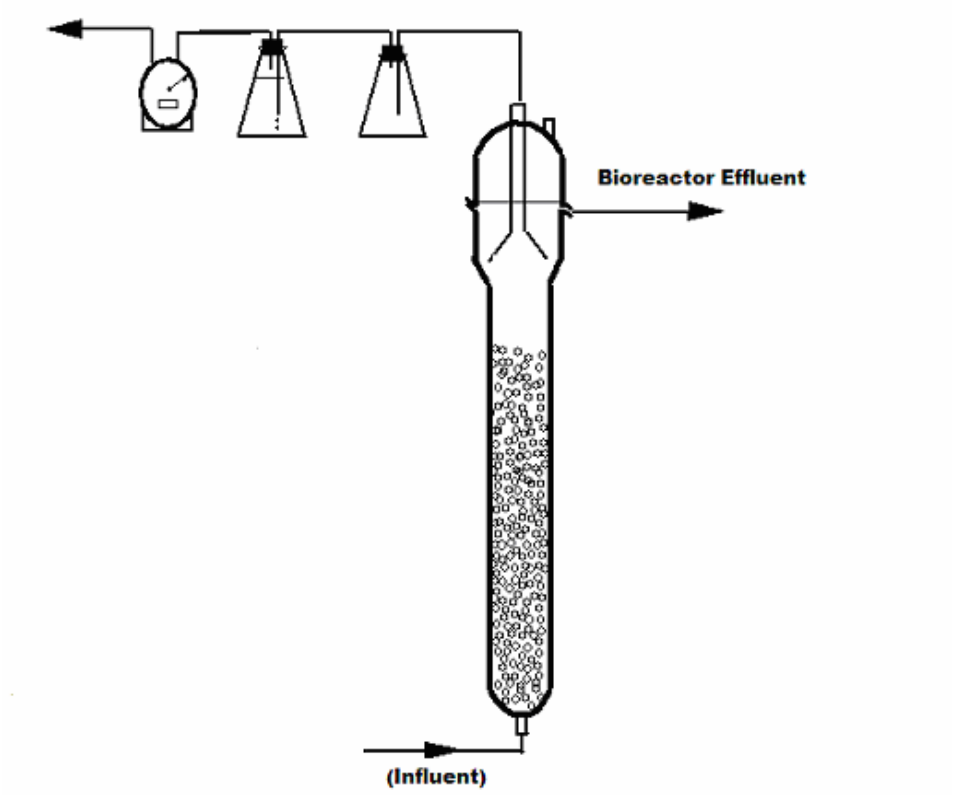
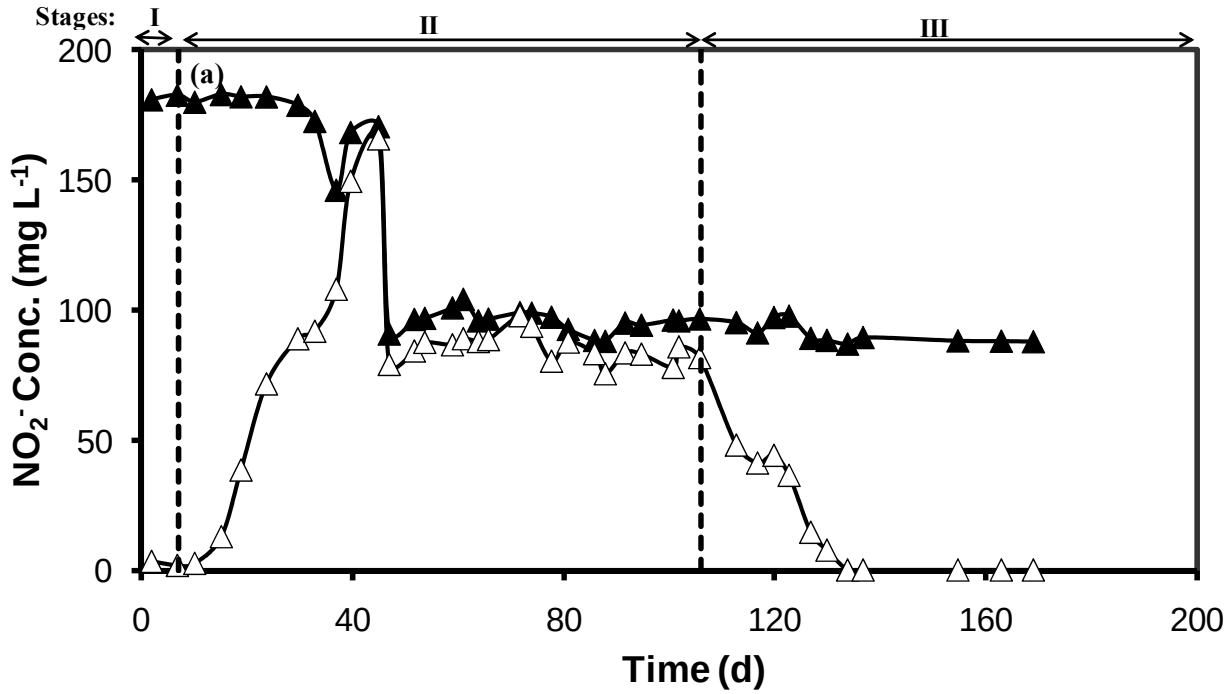
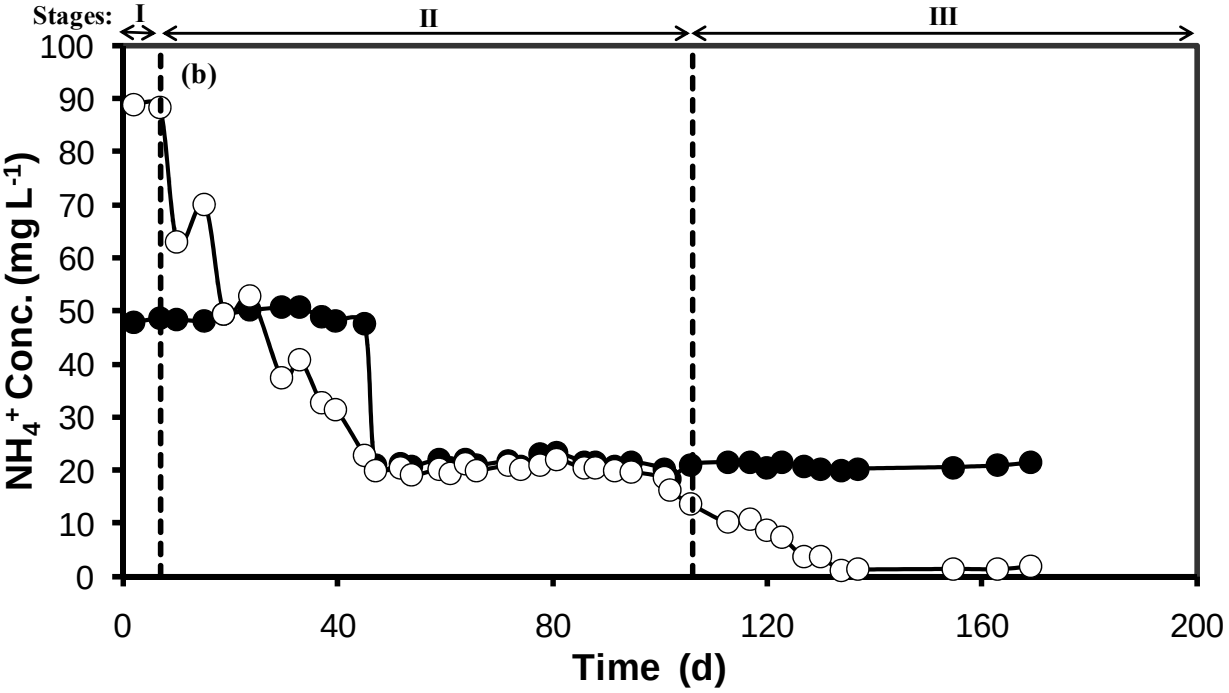


Figure 1: Schematic representation of the of Anammox UASB bioreactor


Stages:
I
II
III
(a)
NO_2^- Conc. (mg L^{-1})
200
150
100
50
0
0
40
80
120
160
200
Time (d)


Stages:
I
II
III
(b)
NH_4^+ Conc. (mg L^{-1})
100
90
80
70
60
50
40
30
20
10
0
0
40
80
120
160
200
Time (d)

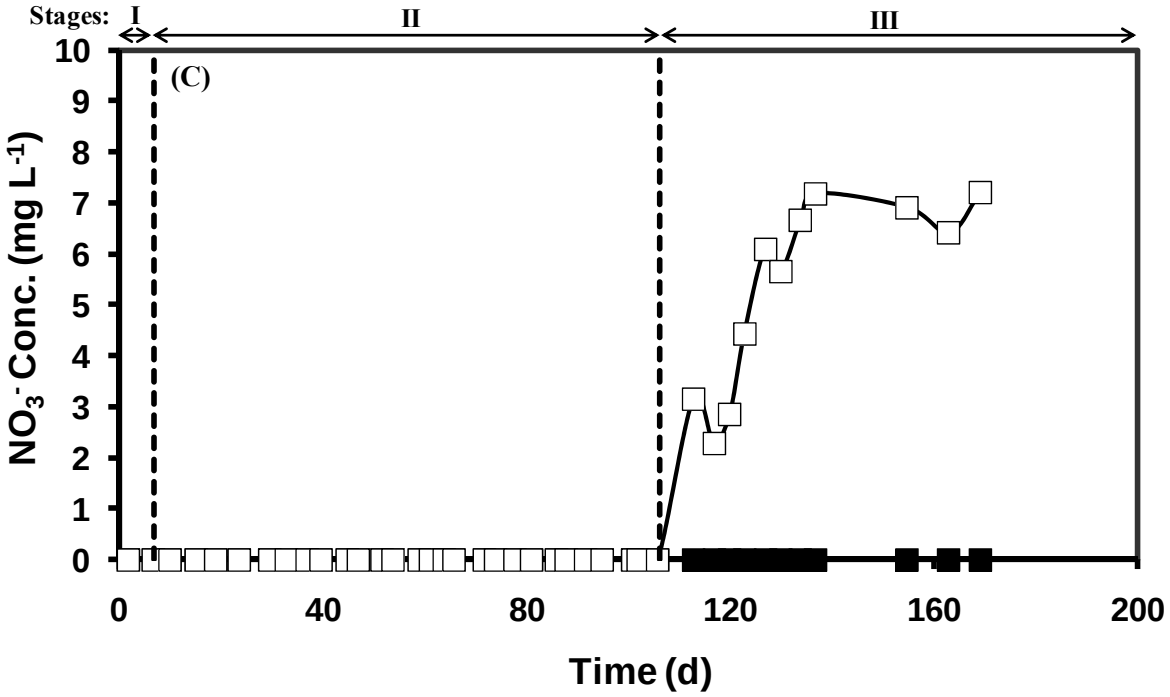


Figure 2: Nitrogen removal profile during 170 days of operating UASB reactor seeded with return activated sludge: panel (a) NO_2^-; panel (b) NH_4^+; panel (c) NO_3^-. Legends: (▲) Influent NO_2^-; (Δ) Effluent NO_2^-; (●) Influent NH_4^+; (○) Effluent NH_4^+; (▄) Influent NO_3^-; (□) Effluent NO_3^-

The biogas from the UASB reactor was collected and passed through two 2-liter Erlenmeyer flasks designed to remove CO_2 from the gas stream. The first flask was empty, preventing any backflow of sodium hydroxide (NaOH) into the UASB reactor, while the second flask was filled with 3.0% (m/v) NaOH, which scrubs CO_2. Following the scrubbing of the biogas, the remaining N_2 gas flow was passed through a wet-type precision gas meter manufactured by Schlumberger Industries.

Seeding Sludge

The UASB reactor was seeded with return activated sludge (RAS) collected from Ina Road municipal wastewater treatment plant (Tucson, AZ, USA). The mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentrations were 11476 and 4895 mg L^{-1}, respectively. RAS was allowed to settle in a 4-L Erlenmeyer flask; then, the reactor was seeded with two thirds of its working volume (2 L of the settled RAS).

Analytical Methods

Ammonium was determined using Mettler Toledo SevenMulti ion selective meter with Mettler Toledo selective ammonium electrode. Nitrite and nitrate were analyzed by suppressed conductivity ion chromatography (IC) using a Dionex 3000 system (Sunnyvale, CA, USA) fitted with a Dionex IonPac AS18 analytical column (4 x 250 mm) and an AG18 guard column (4 x 40 mm). The column was maintained at a temperature of 35ºC. The eluent used was 10.0 mM KOH at a flow rate of 1.0 ml min^{-1}. The injection volume was 25 µl. Before measurement, all samples were passed through a membrane filter (0.45 µm). Other analytical determinations (e.g., pH, TSS, VSS,… etc.) were conducted according to Standard Methods (APHA, 2005).

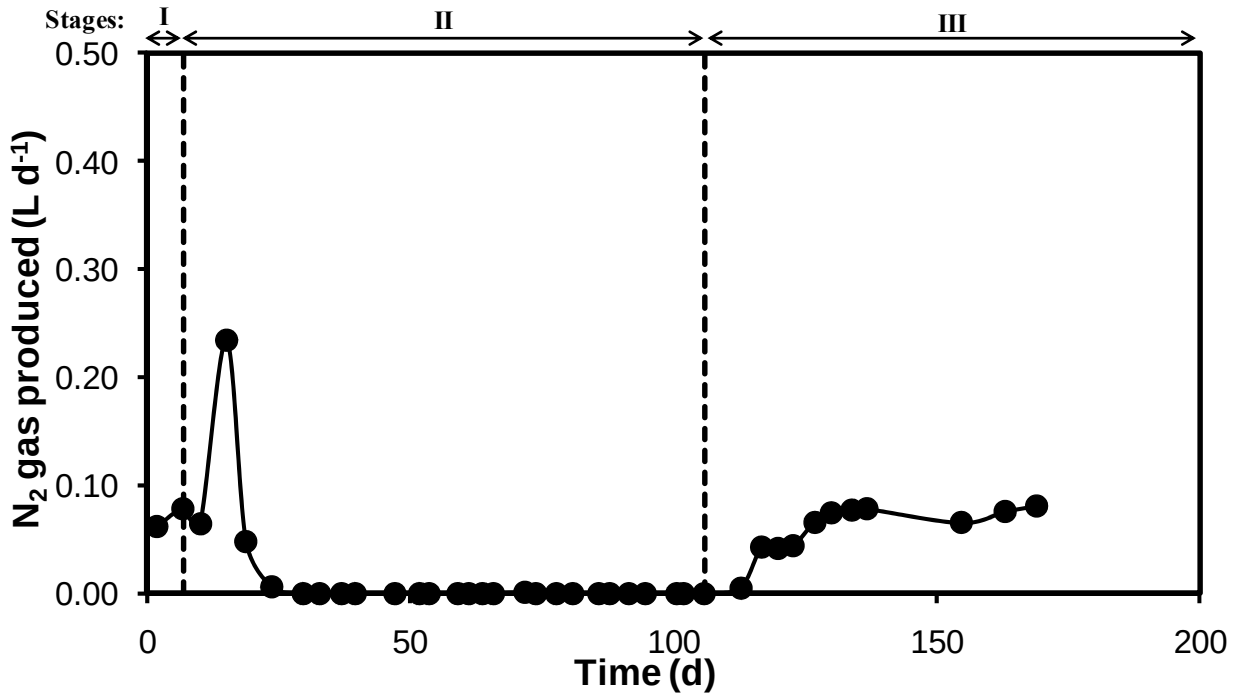


Figure 3: Time course of N_2 gas production during 170 days of operating UASB reactor seeded with return activated sludge

RESULTS AND DISCUSSION

The performance of the bench scale UASB reactor during the start-up period is illustrated in Figure 2. The occurrence of the Anammox activity was demonstrated through a simultaneous depletion of NO_2^- and NH_4^+ coupled to a small production of NO_3^-. Based on the patterns of NO_2^- and NH_4^+ consumption observed in Figure 2 (panels a and b), the start-up period could be divided into three distinct stages: stage I (days 1-7), stage II (days 7-45) and stage III (days 45-193).

Stage I

During this stage, the denitrifying activity was the dominant process and no Anammox activity appeared. The UASB reactor was operated with average influent NO_2^- and NH_4^+ concentrations of 176 and 49 mg L^{-1}, respectively. The NO_2^- was almost completely removed in the initial 10 days (Fig. 2a). Such removal was caused mainly by denitrification which must have been driven by endogenous substrate in the inoculum. In fact, the NH_4^+ concentrations increased due to mineralization of the organic nitrogen during biomass decay (Fig. 2b). The maximum effluent ammonium concentration reached was 89 mg L^{-1} which was much higher than the influent NH_4^+ concentration set at 49 mg L^{-1}. Endogenous denitrification phenomenon was also reported in literature (Jung et al., 2007; Tang et al., 2009; Toh et al., 2002). According to Chamchoi and Nitisoravut (2007), the change in environment of the seed sludge might cause the turnover of the bacteria and thus the former dormant bacteria might be killed. This results in cell lysis and breakdown of organic nitrogen to NH_4^+ nitrogen. The experiments conducted by Imajo et al. (2001) showed that ammonium was produced during the adaptation due to cell decay and starvation.

Also, the dead bacteria release organic matter which can be used by heterotrophic denitrifyers as an organic carbon source and electron donor to support their denitrification activity (Dapena-Mora et al., 2004). In one study conducted to enrich Anammox bacteria from activated sludge in membrane bioreactor (MBR), the chemical oxygen demand (COD) released from the cell lysis ranged from 87 to 191 mg L^{-1} during the first week of operation despite the fact that no organic matter was added to the mineral medium (Wang et al., 2009). This view was supported by the fact that dinitrogen gas (N_2) was produced during this stage (Fig. 3); while no NO_3^- was detected in the effluent (Fig. 2C).

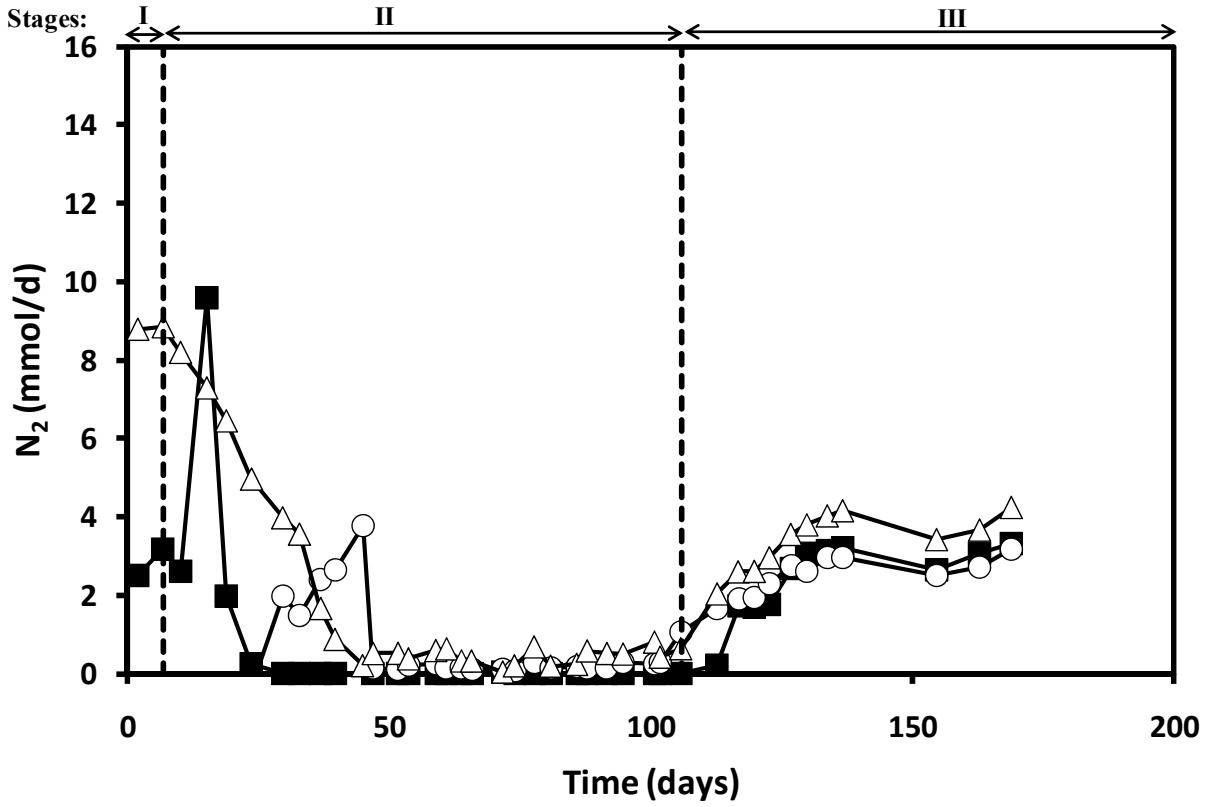


Figure 4: Time course of measured N_2 produced and theoretical N_2 produced based on NO_2^- and NH_4^+ removed. Legends: (▄) Actual N_2 produced; (○) Theoretical N_2 produced based on NH_4^+ removed; (Δ) Theoretical N_2 produced based on NO_2^- removed

Stage II

In this stage, NO_2^- was still removed by endogenous denitrification. However, the activity of the denitrifying bacteria was gradually decreased and finally stopped. This was evident by the accumulation of NO_2^- in the reactor. The maximum effluent NO_2^- concentration reached was 166 mg L^{-1} on day 45 which was very close to the influent concentration. A plausible explanation could be that the organic substrate released from the breakdown of the inoculated sludge started to deplete. This postulation was supported by the steadily gradual decline of effluent NH_4^+ concentration. It is well documented that Anammox is sensitive to substrate inhibition by NO_2^- (Strous et al., 1999b). Therefore, it is essential to keep the NO_2^- concentration below inhibitory levels (70 mg NO_2^--N L^{-1}) for a successful

start-up (Tran et al., 2006). The concentration of influent NO_2^- was reduced by a half on day 45. Likewise, the influent concentration of NH_4^+ was lowered to 25 mg L^{-1} to maintain the same stoichiometric ratio with NO_2^-. Afterwards, there was almost no removal of NO_2^- and NH_4^+. As such, the competition with heterotrophic denitrifyers decreased and NO^-_2 became more available to Anammox bacteria to perform the Anammox reaction with NH_4^+. Thuan et al. (2004) reported that incomplete denitrification due to low carbon to nitrogen ratio led to the availability of excess nitrite in the UASB reactor that could be used by Anammox bacteria. This phenomenon seemed to be necessary for the initiation of the Anammox activity (Chamchoi and Nitisoravut, 2007). The production of both N_2 gas and NO_3^- was not observed during this stage.

Stage III

The final stage is the first evidence of the Anammox reaction and continuous enrichment of Anammox activity. This was illustrated based on three lines of convincing evidences. NO_2^- consumption was occurring simultaneously with NH_4^+ depletion. At the same time, NO_3^- started to accumulate in the effluent of the reactor and reached 7 mg L^{-1}. Finally, N_2 gas production was resumed again. The average N_2 gas produced at the end of the start-up was 0.08 L d^{-1}. From day 137 onwards, the effluent concentrations of NO_2^- and NH_4^+ were almost completely removed. Moreover, Fig. 4 clearly shows that during this stage the theoretical N_2 produced based on NH_4^+ removed (according to stoichiometric ratio in Eq. 1) is very close to actual measured N_2 produced by gas meter. The average theoretical and measured N_2 produced were 2.8 and 3.1 mmol d^{-1}, respectively. However, the theoretical N_2 produced based on the NO_2^- removed was higher than the actual measured N_2. The average theoretical and measured N_2 produced were 3.8 and 3.1 mmol d^{-1}, respectively. This overestimation of theoretical N_2 produced indicates that the molar ratio of N_2 produced to NO_2^- depleted is less than the proposed value of 0.77 in Eq. 1. In fact, the molar ratio of N_2 produced to NO_2^- consumed averaged 0.61 ± 0.021. Also, the stoichiometric molar ratio of NO_2^- removal to NH_4^+ depletion averaged 1.79 ± 0.033 which is higher than the proposed value of 1.32 in Anammox reaction in Eq. 1. Minor variations of molar ratios of $NO_2^-{}_{removed}$/ $NH_4^+{}_{depleted}$ have been reported in literature using various reactor types. In one study with Anammox gas lift reactor, the NO_2^- to NH_4^+ molar ratio was found to be 1.28 (Dapena-Mora et al., 2004). Strous et al. (1997) reported that the molar ratio of NO_2^- to NH_4^+ was 1.40-1.50 and 1.00-1.18 in fluidized bed and fixed bed reactors, respectively. Hsia et al. (2008) found that the ratio of NO_2^- removal to NH_4^+ depletion was 1.53-1.88 for Anammox bacteria immobilized in polyvinyl alcohol (PVA) gel beads.

In this study, The Anammox process was successfully started-up from conventional activated sludge within 4.5 months. This start-up period is in agreement with previously reported values in literature of two to 15 months (Wang et al., 2009; Chamchoi and Nitisoravut, 2007; Third et al., 2005; Toh et al., 2002). The relatively short start-up period in this study may be attributed to the capacity of UASB reactor in retaining Anammox biomass. Moreover, the success of cultivating Anammox bacteria is probably due to the presence of low levels of intrinsic Anammox bacterial in the seeding material. Typically, Anammox occurs in oxygen poor or anaerobic environment, where NO_2^- and NH_4^+ coexist together (Kartal et al., 2010). Such conditions exist in many treatment units operated at municipal wastewater treatment plants (WWTPs). Partial nitrification (Yang et al., 2003) or partial denitrification (Kalyuzhnyi et al., 2006) occurring in biological treatment units can result in the accumulation of NO_2^-. Therefore, it is conceivable that sludge from different biological treatment unit operations at WWTPs contains low levels of intrinsic Anammox bacteria. This would make the application of Anammox process more feasible, especially in situations where the Anammox biomass is not directly accessible.

CONCLUSIONS

In this study, the start-up of Anammox process for autotrophic nitrogen removal from conventional activated sludge in a bench-scale UASB reactor was investigated. On the basis of the results, the following conclusions could be drawn:

1. Anammox process can be readily started up from conventional activated sludge using UASB reactor within 4.5 months.
2. The course of the start-up period of Anammox bioreactor can be divided into three distinct stages: cell lysis; cultivation of Anammox culture; and finally the appearance of Anammox activity.
3. The exhaustion of organic substrate in stage II seems to be necessary for the enhancement of Anammox culture, thereby initiating Anammox activity.
4. The successful cultivation of Anammox is probably due to the capacity of UASB reactor in retaining Anammox biomass and the inherent properties of the return activated sludge.

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