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The Nitrogen Conversion Characteristics of ANAMMOX Sludge After Long-Term Preservation [1]

Yuan Yi[1-2], Huang Yong[2]*, Li Xiang[2], Deng Huiping[1], Zhen Yuhui[2] and Pan Yang[2]

[1]School of Environmental Science and Engineering, Tongji University, Shanghai - 200 092, China.
[2]School of Environmental Science and Engineering, Suzhou university of Science and Technology, Suzhou - 215 009, China.

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The start-up time of an ANAMMOX reactor can be shortened when it is inoculated with ANAMMOX sludge, so it is necessary to develop a cost-effective preservation technique for the sludge. The flocculent ANAMMOX sludge was preserved at 30°C, natural environment, 5°C and -20°C for 15d, 30d, 45d, 150d and 365d, respectively. It was shown that all the preserved ANAMMOX sludge could convert nitrogen, but the conversion ratios of the substrate were not a typical stoichiometry of ANAMMOX reactions. Through the formulas of different metabolic pathways, the methods of nitrogen removal were established. The preserved ANAMMOX sludge all had different levels of nitrification through materials balance. Freezing (-20°C) led to a loss of ANAMMOX activity. The ANAMMOX activity of the sludge preserved at 5°C was higher than preserved at 30°C. But the highest ANAMMOX activity resulted in being kept in a natural environment, and it had a positive linear correlation with the relative amount of substrate. The sludge activity remained at 80% when the relative amount of substrate was above seven. So short-term anoxic (≤ 45d) preserved in a dark natural environment with ambient temperature between 0 to 15°C is a more economical and effective way.

Key words: ANAMMOX sludge; Preservation temperature; Preservation time; Conversion characteristics.

Anaerobic ammonia oxidation (ANAMMOX, see Reaction Formula 1) is the biological oxidation of ammonium (NH_4^+) under anoxic conditions using nitrite (NO_2^-) as the electron acceptor, resulting in the production of dinitrogen gas (N_2). This technology has many advantages, such as not needing to add extra carbon resources, a high volumetric efficiency with the highest nitrogen removal load of up to 61.4 $kgN \cdot m^{-3} \cdot d^{-1}$ [1], less sludge production, and lower operation costs (0.75 ∈ ·$(kgN)^{-1}$ compared with 2~5 ∈ · $(kgN)^{-1}$ of traditional biological nitrogen removal processes[2]. It has been recognized as one of the most sustainable technologies to remove nitrogen from wastewater that is highly concentrated in NH_4^+-N.

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

ANAMMOX has been difficult to integrate into common wastewater treatment practices, mainly due to the growth rate of ANAMMOX bacteria, with doubling times ranging from 1.8 to 11 days[3], resulting in slow start-up times for the treatment processes[4,5]. However, inoculation of ANAMMOX sludge can decrease the start-up time of reactors[6]. With the promotion of

* To whom all correspondence should be addressed.
Huang Yong, professor/ PhD.
Tel.: +86-51269379006;
E-mail: yhuang@mail.usts.edu.cn;
yiyuansuzhou@163.com

ANAMMOX, the demand for high-quality ANAMMOX sludge would increase. So the method to effectively store ANAMMOX sludge needs to be focused on. In wastewater treatment, sludge is usually stored in a starved state. Vlaeminck *et al* had preserved the starved ANAMMOX sludge mixture at -20°C-4°C and 20°C with or without nitrate, respectively[6]. They found the highest ANAMMOX activity was obtained when sludge was preserved at 4°C without nitrate, and the frozen culture did not show any ANAMMOX characteristics[6]. Nevertheless, Rothrock *et al* found that frozen ANAMMOX sludge preserved with skim milk could still show some ANAMMOX activity[7]. Despite refrigeration, the ANAMMOX sludge could maintain high activity, but it still needed higher energy. The goal of this study was to attempt to find a simple and cost effective method to preserve ANAMMOX sludge, which in turn would contribute to the preservation and application of ANAMMOX sludge.

MATERIALS AND METHODS

Synthetic Medium

A synthetic medium was used. The medium contained (per liter): NH_4Cl, 382mg; $NaNO_2$, 641mg; $KHCO_3$, 1000mg; KH_2PO_4, 27mg; $MgCl_2$, 200 mg; $CaCl_2 \cdot 2H_2O$, 136mg, trace element solution I (including $5g \cdot L^{-1}$ EDTA and $5g \cdot L^{-1}$ $FeSO_4$), 1mL and trace element solution II, 1.25mL[8]. pH was adjusted to 7.2 ± 0.2 with HCl.

Inoculums Sludge

The activated culture used in this study was obtained from ANAMMOX reactors. The ANAMMOX granular sludge was ground and washed three times with deionized water. Then 5.1mL-5.3mL flocculent sludge was drawn into a 50mL conical flask. The MLSS/MLVSS ratio of the sludge was 0.465.

Preservative Test

A measured amount of 50mL synthetic medium was drawn into each flask filled with sludge.

Table 1. Preservation temperature and time for ANAMMOX sludge

Time/d	Medium temperature /30°C	Natural environment/0-33°C	Refrigeration /5°C	Freezing/-20°C
15	A1	B1[a]	C1	D1
30	A2	B2[a]	C2	D2
45	A3	B3[b]	C3	D3
150	A4	B4[c]	C4	D4
365	A5	B5[d]	C5	D5

a. Ambient temperature from 15 °C down to 5 °C
b. Ambient temperature from 15 °C down to 0 °C
c. Ambient temperature from 15 °C down to 0 °C, and gradually heated to 33 °C
d. Ambient temperature from 15 °C down to 0 °C, and gradually heated to 33 °C, but then decreased to 5 °C.

The flasks were sparged with dinitrogen gas for 0.5h, sealed up with aluminum foil, and then preserved in the dark at 30°C, a natural environment, at 5°C and -20°C for 15d, 30d, 45d, 150d and 365d, respectively, which are listed in Table 1. All experiments were set with parallel samples.

Determination of the Nitrogen Removal Rate

To determine the nitrogen removal rate, 100mL of synthetic medium was added to the ANAMMOX sludge, it was sparged with dinitrogen gas for 5min, then sealed in the bottle and oscillated at the speed of $110r \cdot min^{-1}$ in a (32±11.4°C) incubator covered with black cloth. The nitrogen removal rate of ANAMMOX sludge was measured after 24 hours. The total nitrogen removal rate of sludge before storage was measured to be $0.107kgN \cdot m^{-3} \cdot d^{-1}$.

Chemical Analysis

The concentrations of nitrogen compounds were measured according to standard methods, as set out by the American Published Health Association[9]. NH_4^+-N, NO_2^--N were measured colorimetrically, NO_3^--N was measured spectrophotometrically. The pH measurement was done using a digital, portable pH meter.

Calculation Formula

Metabolic types of stored sludge can be ANAMMOX, partial nitrification, nitrification, endogenous denitrification and/or endogenous

digestion. The half equations of endogenous denitrification and endogenous digestion are shown as Equations (2) - (4)[10].

$$\frac{1}{2}NO_3^- + \frac{1}{2}H^+ + e^- \rightarrow \frac{1}{2}NO_2^- + \frac{1}{2}OH^-,$$

$$\Delta G^\theta = -41.45KJ \cdot mol^{-1} \quad ...(2)$$

$$\frac{1}{3}NO_2^- + \frac{2}{3}H^+ + e^- \rightarrow \frac{1}{6}N_2 + \frac{2}{3}OH^-,$$

$$\Delta G^\theta = -30.75KJ \cdot mol^{-1} \quad ...(3)$$

$$\frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O \rightarrow \frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^-, \quad ..(4)$$

In these equations ΔG^θ is the free energy released at the standard state and pH = 7.

Equation (5) was established according to the coefficient of the material balance shown in Table 2.

Table 2. Coefficients by the material balance (Unit: mg·L^{-1})

	Converted via ANAMMOX	Converted via partial nitrification	Converted via nitrification	Converted via endogenous denitrification for NO_3^--N	Converted via endogenous denitrification for NO_2^--N	Converted via endogenous digestion
NH_4^+-N	a	b	0	-0.1d	-0.15f	-0.05e
NO_2^--N	1.32a	-b	c	-d	f	0
NO_3^--N	-0.26a	0	-c	d	0	0
ΔN	2.04a	0	0	-0.1d	0.85f	-0.05e

The amount reduced is +, the amount increased is -.

$$a + b - 0.1d - 0.05e - 0.15f = C_{NH_4^+\text{-}N_{in}} - C_{NH_4^+\text{-}N_{eff}}$$

$$1.32a - b + c - d + f = C_{NO_2^-\text{-}N_{in}} - C_{NO_2^-\text{-}N_{eff}}$$

$$0.26a + c - d = C_{NO_3^-\text{-}N_{eff}} - C_{NO_3^-\text{-}N_{in}}, (5)$$

$$2.04a - 0.1d - 0.05e + 0.85f = C_{NH_4^+\text{-}N_{in}} + C_{NO_2^-\text{-}N_{in}} + C_{NO_3^-\text{-}Nin} - C_{NH_4^+\text{-}N_{eff}} - C_{NO_2^-\text{-}N_{eff}} - C_{NO_3^-\text{-}N_{eff}}$$

$$\text{the relative amount of substrate} = \frac{C_{gasserve} \times V_{gasserve}}{M_s \times NRR_S \times PT \times 1000} = \frac{C_{gasserve} \times V_{gasserve}}{M_s \times \frac{C_{activity}}{HRT \times M_s} \times PT \times 1000} = \frac{C_{gasserve} \times V_{gasserve}}{NRRv \times PT \times 1000}, (6)$$

From Equations (2) and (3), nitrate reduction can release more free energy than that of nitrite reduction for each 1mol electronic. Under the same conditions, a nitrate reduction process is easier to accomplish. Besides, similar studies suggested that *Paracocus denitrificans* and *Psuedomonas fluorescens* had the priority to use nitrate as the electron acceptor when the electron donor was limited[11, 12]. There were limited organic carbon sources in the reaction system, so nitrate reduction (Equation 2) was the preferential endogenous denitrification process. Nitrogen loss in the system may only be caused by the ANAMMOX process or endogenous denitrification for nitrite.

If ammonia and nitrite were degraded synchronously, and meanwhile nitrate was produced, nitrogen loss could be due to ANAMMOX process without nitrite-denitrification (i.e. f=0) and endogenous digestion (i.e. e=0).

If ammonia and nitrite were degraded synchronously, but no nitrate was produced, nitrogen loss could be due to ANAMMOX process and endogenous denitrification, without partial nitrification (i.e. b=0), nitrification endogenous denitrification (i.e. c=0) and endogenous digestion (i.e. f=0).

If there was nitrogen loss in the system, but ammonia and nitrite nitrogen were not degraded synchronously, and the concentration of the effluent ammonia nitrogen was higher than that of raw water, no ANAMMOX, partial nitrification or nitrate reduction (i.e. a=0,b=0,d=0) could happen. If there was no nitrogen loss in the system, then there would be no ANAMMOX reaction and no

endogenous denitrification (i.e. a=0, d=0, f=0).

The amount relative of substrate can be calculated by equation (6).

In this equation, $C_{preserve}$ is the concentration of nitrogen in preservative fluid, ($C_{NH_4^+-N} + C_{NO_2^--N}$) mg·L^{-1}. $V_{preserve}$ is the volume of preservative fluid, L. M_s is the quantity of sludge stored, g. NRRs is the nitrogen removal rate of sludge, kg·kg^{-1}·d^{-1}. PT is the preservation time of sludge, d. $C_{activity}$ is the concentration of nitrogen in the activity determination solution, ($C_{NH_4^+-N} + C_{NO_2^--N}$) mg·L^{-1}. NRRv is the nitrogen removal rate of sludge, kg·m^{-3}·d^{-1}.

Table 3. The results of nitrogen conservation of ANAMMOX sludge after preservated at different time and temperature

Prese	Medium temperature			Natural environment			Refrigeration			Freezing		
rvation Ntime/d	NH_4^+-N	NO_2^--N	NO_3^--N	NH_4^+-N	NO_2^--N	NO_3^--N	NH_4^+-N	NO_2^--N	NO_3^--N	NH_4^+-N	NO_2^--N	NO_3^--N
	mg·L^{-1}											
15	95.4	113	0	58.9	84.5	0	83.8	99.5	0	105.8	111	0
30	95	120.5	1	53.1	106	7.1	85.9	109	0	91.8	119	0
45	98	122.5	1.4	78.6	104.5	0	92.7	122	3.6	106.3	98	0
150	95	120	15	84	118	0	97	121	15	107.8	105	8
365	123	129	0	120.8	125	0.5	108.5	119	0	134.8	73.75	0

RESULT AND DISCUSSIONS

Results of Activities of ANAMMOX Sludge After Different Temperatures and Time Storage

The nitrogen conservation results of preserved ANAMMOX sludge are shown in Table 3.

A little ammonia nitrogen could be converted if the sludge was stored at 30°C. With the increase of storage time, the color of the sludge turned black, accompanied with a heavy pungent odor. During preservation, no nutritional substrates supplied would force bacteria to use themselves to sustain. Then bacteria gradually disintegrated, decayed and released H_2S, resulting in the black appearance and smelly odor.

The sludge preserved in a natural environment for 150 days and those stored in refrigeration for 45 days both could convert ammonia and nitrite similarly.

The frozen sludge almost could not convert ammonia in spite of the color remaining bright red with the red effluent.

From Table 4, it was shown that the substrate conversion stoichiometric ratios of all preserved ANAMMOX sludge mixtures were not a typical ANAMMOX stoichiometry (degradation of NH_4^+-N: degradation of NO_2^--N: production of NO_3^--N= 1:1.32:0.26). Obviously, there were other reactions.

Nitrogen Transition Characteristics of Sludge after Preservation

Fig.1 was based on nitrogen balance. The ANAMMOX sludge stored at different temperatures and times all exhibited nitrification capabilities. Because some aerobic ammonium

Table 4. The Characteristics of nitrogen conservation of ANAMMOX sludge after preservated at different time and temperature

Preservation Time/d	Medium Temperature	Natural Environment	Refrigeration	Freezing
	NH_4^+-N degradation:NO_2^--N degradation:NO_3^--N production			
15	1:17: 0	1:1.11:0	1:1.88:0	-1:3.28:0
30	1:1.9:0.2	1:0.51:0.15	1:1.49:0	1:1.34:0
45	1: 3.75:0	1:1.19:0	1:1.1:0.49	-1:5.08:0
150	1:2:3	1:0.75: 0	1:3:5	-1:3.21:1.03
365	-1:0.04:0	-1:0.24: 0.02	-1:1.29:0	-1:1.62:0

oxidation bacteria often co-exist with ANAMMOX sludge[13], it declined with ANAMMOX bacteria during preservation. While aerobic ammonium oxidation bacteria could survive in the low substrate concentration, starvation or fluctuant environment, with low mortality and energy demand requirements[14,15]. So the preserved ANAMMOX sludge all had nitrification capabilities.

It was shown that nitrogen loss was completed by the ANAMMOX and endogenous denitrification processes when the sludge was preserved in refrigeration, medium temperatures or a natural environment preservation for no more than 45 days.

The ANAMMOX sludge after frozen preservation had no ANAMMOX conversation characteristics, and the nitrogen loss was caused

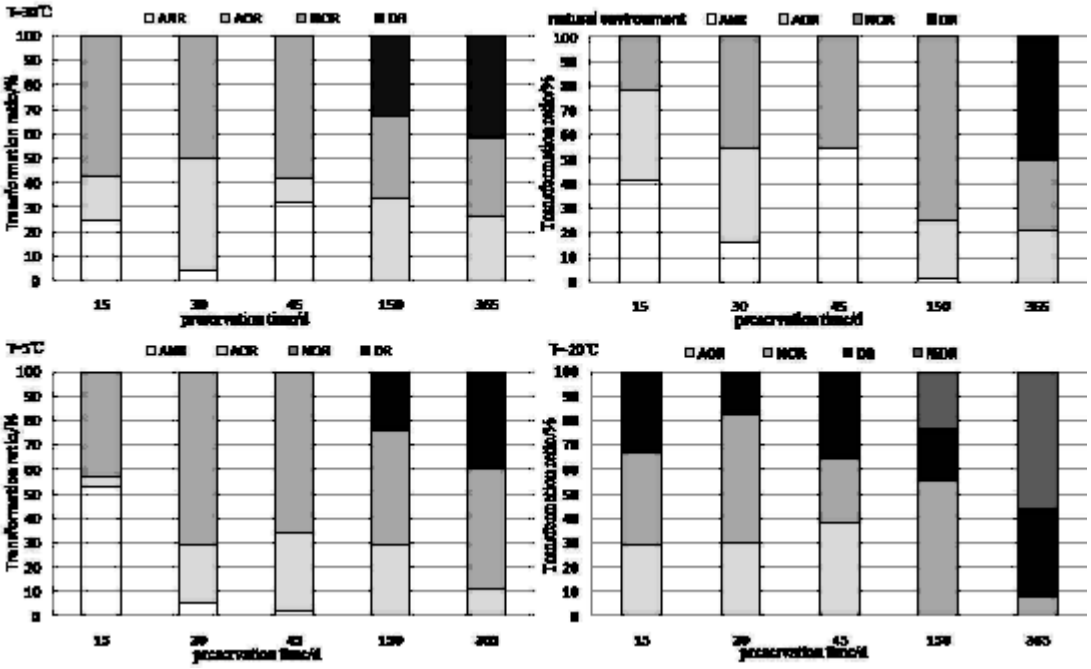

Fig. 1. Percents of different processes in ANAMMOX sludge after preservation at different conditions the proportion of the ANAMMOX process as ANR (ANAMMOX ratio); the proportion of aerobic ammonia oxidation process as AOR (ammonia oxidation ratio); the proportion of nitrite oxidation as NOR (nitrite oxidation ratio); the proportion of endogenous denitrification for nitrate as NaDR (nitrate- denitrification ratio); the proportion of endogenous denitrification for nitrite as NiDR (nitrite-denitrification ratio); the endogenous digestive process as DR (digesting ratio)

by the endogenous denitrification process. During the measurement of nitrogen removal rate, the effluent was light red, which suggested ANAMMOX bacteria had not completed endogenous digestion during storage, but the breakage of cell turns the hemachrome C inside the cell into water[13]. So it could be concluded that ANAMMOX sludge stored at freezing with hunger could not maintain ANAMMOX activity.

Nitrogen Removal Capacities of ANAMMOX Sludge After Different Preservation Strategies

From Table 5, ANAMMOX sludge could maintain 20.2% of nitrogen removal capacity after 15 days of mesophilic preservation, and 43.6% retained it after 15 days of refrigerated preservation. This indicated that sludge stored in refrigeration were better than at medium temperatures in the short term. However, both of them decline rapidly with the increase of storage time. After 150 days of storage, neither the mesophilic preserved ANAMMOX sludge nor the refrigerated preserved one could maintain any nitrogen removal capacity.

After 15d, 30d, 45d and 150d of natural environment preservation, the nitrogen removal

Table 5. The nitrogen removal capacities of ANAMMOX sludge after preservation

Preserv	Medium temperature		Natural environment		Refrigeration		Freezing	
ation time /d	nitrogen removal rate/kg·m^{-3}·d^{-1}	nitrogen removal capacity reserved /%	nitrogen removal rate /kg·m^{-3}·d^{-1}	nitrogen removal capacity reserved /%	nitrogen removal rate /kg·m^{-3}·d^{-1}	nitrogen removal capacity reserved /%	nitrogen removal rate /kg·m^{-3}·d^{-1}	nitrogen removal capacity reserved /%
15	0.0216	20.2	0.0866	80.9	0.0467	43.6	0.0132	12.3
30	0.0135	12.6	0.0638	59.6	0.0351	32.8	0.0192	17.9
45	0.0095	8.9	0.0469	43.8	0.0117	10.9	0.0257	24.0
150	0	0	0.028	26.2	-	-	0.0092	8.6
365	-	-	-	-	0.0025	2.3	0.02145	20.0

"-": value was negative.

capacity of ANAMMOX sludge remains at 80.9%, 59.6%, 43.8% and 26.2%, respectively. Dosta *et al* showed that ANAMMOX sludge still retained strong ANAMMOX activity at 15°C, because the reduction of related enzyme activities at low temperature could decline the metabolism of bacteria[17]. But 365 days of preservation in a natural environment (0~33°C) made the nitrogen removal capacity of ANAMMOX sludge reduce to zero. It could be possible that the high temperatures in summer accelerate the endogenous digestion of ANAMMOX sludge. Therefore, ANAMMOX sludge is suitable for storage in a natural environment (0~15°C) for a short time (≤45d), but not for long term preservation.

After the frozen preservation, ANAMMOX sludge still had nitrogen removal capacity, but it was caused by endogenous denitrification. Vlaeminck *et al* suggested that the preserved ANAMMOX sludge mixture could only degrade nitrate[6]. This also indicated that the preserved ANAMMOX sludge has denitrification capabilities and frozen preservation would lead to nitrogen loss via endogenous denitrification.

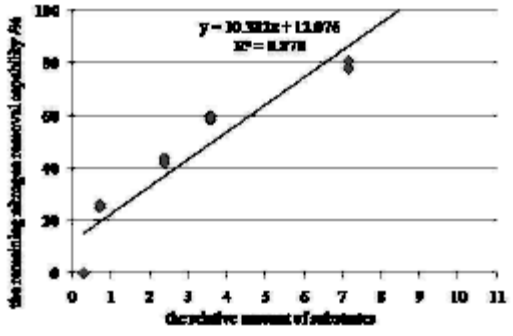

Fig. 2. Effect of relative materials quantity on ANAMMOX sludge activity after preservation

Effects of Relative Substrate on Nitrogen Removal Performance of Sludge After Natural Environment Preservation

Table 5 shows both preservation temperature and time have important impacts on nitrogen removal capabilities of ANAMMOX sludge. Preservation in natural environment (0~15°C) is a better way to maintain the activity of ANAMMOX bacteria. But the relative amount of substrate is the key for sludge preservation.

As shown in Figure 2, after natural environment preservation, there was a positive linear relationship between the remaining nitrogen removal capability of ANAMMOX sludge and the relative amount of substrates. The larger the relative amount of substrates, means the shorter the preservation time and the more preservative fluid substrates, the better the activity of sludge left after preservation. If the relative amount of substrates equals seven, the activity of sludge after preservation could remain around 80%.

An amount of 5g of wet granular ANAMMOX sludge was stored at 0~10°C and 25°Cfor 25 days respectively. Their nitrogen removal capacity was measured at around 40% retained (see table 6), close to the predicted values (maximum deviation is only 11.27%). This exhibited the nitrogen removal capacity of sludge after natural environment preservation could be predicted by the relative amount of substrates.

Table 6. Verification results for model

Batch	Temperature /°C	Nitrogen removal rate before preservation/ $kg \cdot m^{-3} \cdot d^{-1}$	relative amount of substrates	Nitrogen removal rate after preservation /$kg \cdot m^{-3} \cdot d^{-1}$	Nitrogen removal capacity reserved		Deviation / %
					actual/%	predicted/%	
I	10	0.500	3.68	0.227	45.4	51.06	5.66
II	25	0.499	3.69	0.199	39.9	51.17	11.27

CONCLUSION

The stored ANAMMOX sludge has nitrification capacities. Freezing (-20°C) not only contributes to high operating costs, but also results in microbial cell rupture, forcing sludge to lose ANAMMOX activity.

The sludge stored in a natural environment (0~15°C) could retain the highest ANAMMOX activity. Also, there is a positive linear relationship between the remaining nitrogen removal capability of ANAMMOX sludge and the relative amount of substrates. When the relative amount of substrate was above seven, approximately 80% of nitrogen removal capabilities remained. So it is a cost-effective way for ANAMMOX sludge to be preserved away from light and oxygen for a short time (≤45d) in natural environments with ambient temperatures between 0 to 15°C.

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REFERENCES

1. Tang C.J., Zheng P., Wang C H. Performance of high-loaded ANAMMOX UASB reactors containing granular sludge. *Water Res*, 2011; **45**(1):135-144.
2. Jetten M.S.M., Wagner M., Fuerst J. Microbiology and application of the anaerobic ammonium oxidation ('ANAMMOX') process. *Curr Opinion in Biotechnol*, 2001; **12**(3): 283-8.
3. Isaka K., Date Y., Sumino T. Growth characteristic of anaerobic ammonium-oxidizing bacteria in an anaerobic biological filtrated reactor. *Appl Microbiol Biotechnol*, 2006; **70**: 47-52.
4. Schmid M.C., Maas B., Dapenda A. Biomarkers for in site detection of anaerobic ammonium-oxidizing (anammox) bacteria. *Appl. Environ. Microbiol.*, 2005; **71**(4):1677–84.
5. Strous M., Heijnen J.J., Kuenen J.G. The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Appl. Microbiol. Biotechnol.*, 1998; **50**: 589-96
6. Vlaeminck S.E., Geets J., Vervaeren H. Reactivation of aerobic and anaerobic ammonium oxiders in OLAND biomass after long-term storage. *Appl. Microbiol. Biotechnol.*, 2007; **74**(1): 1376-84.
7. Rothrock Jr.M.J., Vanotti M.B., Szogi A.A. Long-term preservation of ANAMMOX bacterial. *Appl. Microbiol. Biotechnol.*, 2011; **92**(5): 147-157.
8. Van de Graff A.A., De Bruijin P, Robertron L.A. Autortophic growth of anaerobic ammonium- oxidizing micro-Organisms in a fluidized bed reactor. *Microbiol*, 1996; **142**(8): 2187-2196.
9. APHA, Standard Methods for the Examination of Water and Wastewater, 20th ed. United Book Press, 1998, USA.
10. Rittmann B.E., McCarty P.L.: Environmental Biotechnology: Principles and Applications. Beijing: Tsinghua University Press, 2002; pp: 59-135.
11. Thomsen J.K., Geest T., Raymond P.C. Mass spectrometric studies of the effect of pH on the accumulation of intermediates in denitrification by Paracoccus denitrificans. *Appl Environ Microbiol*, 1994; **60**(2): 536-541.
12. Almeida J.S., Reis M.A., Carrondo M.J. Competition between nitrate and nitrite reduction in denitrification by Pseudomonas flourescens. *Biotech & Bioengg*, 1995; **46**(5): 476-484.
13. Schmidt I., Slieker O., Schmid M. Aerobic and anaerobic ammonia oxidizing bacteria—

competitors or natural partners? *FEMS Microbiol Ecol*, 2002; **39**(3):175-181.
14. Vogelsang C., Gollembiewski K., Ostgaard K. Effect of preservation techniques on the regeneration of gel entrapped nitrifying sludge. *Water Res*, 1999; **33**(1):164-168.
15. Geets J., Boon N., and Verstraete W. Strategies of aerobic ammonia oxidizing bacteria for coping with nutrient and oxygen fluctuations. *FEMS Microbiol Ecol*, 2006; **58**(1): 1-13.
16. Van Niftrik L., Geerts W.J.C., Van Donselaar E.G. Combined structural and chemical analysis of the ANAMMOXosome: A membrane-bounded intracytoplasmic compartment in ANAMMOX bacteria. *J Struct Biol*, 2008; **161**(3): 401-410
17. Dosta J., Fernández I., Vázquez-Padín J.R. Short-and long term effects of temperature on the ANAMMOX process. *J Hazard Mats*, 2008; **154**(1-3) : 688-693.