Effect of Salt on Anammox Process

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Abstract

Effects of different growth systems and salinity on freshwater anammox bacteria were researched in this paper with SBR reactor. It was indicated that the attached growth system could retain anammox bacteria better than suspended growth system. And the nitrogen removal ratios in the attached one were obviously higher than that in the suspended one in the same degrade of sludge load. The freshwater anammox population could adapt to salinity in 7gNaCl/l. The effects of salt shock loads on freshwater anammox population were also researched. It was shown that the freshwater bacteria could afford salt shock load max to 4kgNaCl/m³ d which was function with sludge quantity. Although a substantial increase in salt load would lead to freshwater anammox bacteria unadaptation, suddenly draw-off salt load would be more serious for anammox bacteria.

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Introduction

Salinity is an important parameter for wastewater treatment because many industrial wastewaters rich in ammonium also contain high salt concentrations (total Salinity would excess 1%) , such as seafood wastewater, dairy wastewater, meat wastewater, printing and dyeing wastewater, pharmaceutical wastewater, oil wastewater, landfill leachate and leather industry, et al.. These industry wastewater were drained largely and the salinity was mainly NaCl and sulphate. Salinity could inhibit microorganism growth because cell-wall and enzymes of bacteria are destroyed. So it is difficult to be treated by microorganism and nutrient removal ratio decreases. Bacteria must adapt to high salinity so that semi-nitrification-anammox joint process could be applied to such high salinity wastewater containing ammonium.

Halophytic bacteria usually deal with high-salt wastewater more easily. Until now in natural saline ecosystems only anammox species belonging to “Scalindua” genus have been detected. These species are Candidatus Scalindua sorokinii [1], Candidatus Scalindua brodae[2], and Candidatus Scalindua wagneri
Halophytic anammox bacteria grows in the marine so that it is difficult to obtain. Thus, it would be expected that a freshwater anammox population would change dramatically during exposure to salinity. Some scholars have conducted preliminary study in this area. Dapena-Mora[3] studied the freshwater anammox bacteria would not be inhibited in NaCl <150mM (8.77g/l). Kartal et al. [4] researched the adaptation of a freshwater anammox population to high salinity wastewater in SBR reactor with the fed water which NaCl and KCl in the preparation of a ratio was 9:1. Results showed that freshwater anammox strains could gradually adapt to high salt water, and salt concentration can reach 30g/l which meet with the results of Windey et al. [5]. Fluorescence in situ hybridization (FISH) analysis revealed that the freshwater anammox species Candidatus “Kuenenia stuttgartiensis” was the dominant in salt adapted sludge. But there was rarely research about the effect of salt shock loading on freshwater anammox bacteria. This paper focused on the salinity shock impact resistance of freshwater anammox bacteria.

1. Materials and methods

1.1 Equipment

An anaerobic sequencing batch reactor with an effective capacity of 3200 ml was used (Fig. 1), which was sealed by water to avoid contacting with atmosphere and was stirred to mix wastewater and anammox sludge. SBR was operated at 30°C by immersed in a water bath and was covered with window blind to avoid restraining anammox bacteria. Influent, effluent and churn-dasher were controlled by time director per cycle. There were influent with stir/stir (time were adjusted as needed), sedimentation and drainage per cycle. The fill ratio was always 1/3 during experiment. The high activated fresh-anammox consortium was originated from a laboratory-scale anammox ASBR with non-woven fabric carriers. The reactor was filled with polyester nonwoven fabric carriers (3cm×3cm square and 4cm thick) after 45 days.

1.2 Synthetic medium

A synthetic medium was used. NH₄Cl, NaNO₂ and NaCl were added as needed. The medium also contained (per liter) NaHCO₃, 1000 mg; KHCO₃ 1000 mg; KH₂PO₄, 27 mg; MgSO₄·7H₂O, 200 mg; CaCl₂·2H₂O, 136 mg, and trace element solution I (including EDTA and FeSO₄), 1ml and trace element solution II[6], 1ml. There were no measures to reduce dissolved oxygen (DO) concentration in the feeding.

1.3 Analytical methods

Ammonium was measured by Nessler's reagent spectro-photometer[7]. The nitrite was analyzed by N-(1-naphthyl)-1, 2-diaminoethane dihydrochloride spectrophotometer [7]. Nitrate nitrogen (NO₃⁻-N) was measured by ultraviolet spectrophotometric screening method[7] using spectrophotometer (Hach DR5000, USA); pH and ORP were online measured by Mettler-Toledo 770MAX multi-parameters conveyer instrument.

Fig.1. Anammox
1.4 Sensitivity ratio

Stability is regarded as the resistance of the performance parameters (e.g. substrate conversion percentage, effluent substrate concentration) change to operational parameters (e.g. inflow rate, influent substrate concentration) variation. If a slight disturbance in inflow rate or influent substrate concentration results in alteration of the effluent substrate concentration enormously then the system is judged as sensitive. The sensitivity can be described as Eq. (1)[8]:

\[
O_i - O_b \quad I_i - I_b
\]

where, \(O_i\) and \(I_i\) are the effluent substrate concentration and influent salt load at the moment of \(i\), respectively; \(Ob\) and \(Ib\) are the effluent substrate concentration and influent salt load during the steady operation period, respectively.

2. The result of experiment

2.1 suspended growth system

During the first 20 days, 130mgNH4+-N/l and 180mgNO2--N/l were fed. One cycle of SBR was 8 hours with operating mode containing a 6 hours of reaction in which 30min agitation with filling /30min agitation without filling, 115 minutes of sedimentation and 5 minutes of drainage. Due to highly nitrogen concentrations in effluent, the reaction time was extended to 7h.

During 20d to 45d, there were 140mgNH4+-N/l and 213mgNO2--N/l in influent. One cycle of SBR was still 8 hours, but the operating mode was adjusted. After 6 hours of reaction in which 15min agitation with filling /45min agitation without filling, 45 minutes of sedimentation and 15 minutes of drainage were set. Nitrogen load was 0.353kgN/m3 d, pH was 7.71 and ORP was +114mV. The highest nitrogen concentration in effluent was 44.8mgNH4+-N/l and 80mgNO2--N/l respectively with the sludge adhering to the top of the wall of the reactor. The sludge in reactor reduced over 90% (the initial sludge volume was about 5/9 of the effective volume of reactor, but now only 1/18) at 45d because sludge was serious washout. Nitrogen removal efficiency was 85%. The color of sludge became a light red from bright red. And the block sludge gradually disintegrated to the loose floc with sludge concentration reduction to 4352mg/l.

Table 1 the experiment result of suspended growth system

| Time (d) | NH4+-N concentration (mgN/l) | NO2--N concentration (mgN/l) | Nitrogen removal (%) | Nitrogen load (kgN/m3 d) |
|----------|-----------------------------|-----------------------------|----------------------|-------------------------|
|          | influent        | effluent        | influent   | effluent    | NH4+-N  | NO2--N  |                        |                         |
| 4        | 129            | 3.45            | 180       | 1.4         | 97.3    | 99.2    | 0.309                 |                         |
| 7        | 129            | 2.585           | 180       | 1.175       | 98.0    | 99.3    | 0.309                 |                         |
| 9        | 129            | 1.05            | 180       | 2.975       | 99.2    | 98.3    | 0.309                 |                         |
| 20       | 140.6          | 44.8            | 213       | 80          | 68.1    | 62.4    | 0.353                 |                         |
| 39       | 140.6          | 26.9            | 213       | 46.25       | 80.8    | 78.3    | 0.353                 |                         |
| 45       | 140.9          | 14.85           | 212.6     | 28.9        | 89.5    | 86.4    | 0.353                 |                         |

2.2 attached growth system

In 45d, half of sludge was taken away from reactor and sludge concentration remained in the reactor was 2716mg/l. 85 pieces of nonwoven fabric (3cm × 3 cm square and 4mm thick) were placed into reactor to
clarify water. The 45d of suspended growth system was used as the first day for the implementation of Biofilm reactor. After adding non-woven filter fabric, free sludge in the water was adsorbed to the filter so that muddy water was clear and the ORP in the reactor dropped to -300mv.

In the first ten days one cycle of SBR was 12 hours. After 10 hours of reaction in which 15min agitation with filling /45min agitation without filling, 105 minutes of sedimentation and 15 minutes of drainage were set. The reactor was fed with 140mgNH$_4^+$-N/l and 213mgNO$_2^-$-N/l and nitrogen load was 0.235kgN/m$^3$.d. At the same time 1gNaCl/l was added to domestication a freshwater anammox population. The anammox reaction was not affected by salinity with NH$_4^+$-N and NO$_2^-$-N removal rate above 95%.

During 11d–26d, salinity was 3gNaCl/l. Anammox reaction was stable when fed with 140mgNH$_4^+$-N/l and 213 mgNO$_2^-$-N/l. At 13d the NO$_2^-$-N concentration was reduced to 170mg NO$_2^-$-N/l in order to cut down NO$_2^-$-N concentration in effluent. And at the same time the operation cycle was shortened to 8h with run mode containing a 7.5h reaction with 15min agitation with filling /45min agitation without filling, 15min of precipitation and 15min of drainage. The operation mode was not changed during the rest experiment. At the first five days of the time when the operation cycle was shorten from 12h to 8h,

![Fig.2](image)

**Fig.2** the result of salt adaption experiment in attached growth system

NH$_4^+$-N and NO$_2^-$-N removal rate decreased to 84% and 78%, respectively, but both increased to 90% subsequently.

The salinity was 7gNaCl/l and the fed contained 220mgNH$_4^+$-N/l and 220mg mgNO$_2^-$-N/l during 27d–52d. Removal rates of NH$_4^+$-N and NO$_2^-$-N decreased to 81.8% and 82.5%, respectively, and then resumed to more than 95%.

In the above performance period, the fillers packed offspring gas were floating and some sludge particles scattered in below solution. Sludge and water were mixed by mechanical stirring. In the sedimentation phase, the scattered sludge particles could settle to the bottom in tens of seconds.

During 53d ~ 56d, salinity was increased to 12gNaCl/l in fed water with 568mgNH$_4^+$-N/l and 354mgNO$_2^-$-N/l. The influent increased by 50% in two days so that the reactor was shocked seriously by hydraulic load, nitrogen load and salt load. Suspended granular sludge (losing from biofilm) was manifold in the reactor at the same time. From 57d, the nitrogen concentration in influent was reduced to 90mgNH$_4^+$-N/l and 186mgNO$_2^-$-N/l step by step, respectively, yet there were no signs of recovery.
The salinity in fed water was reduced to 0gNaCl/l from 63d. During 63d~80d, the nitrogen removal ratios were still very low with influent containing 171±20mgNH₄⁺-N/l and 190±10 mgNO₂⁻-N/l. During 81d ~ 85d, raw nitrogen concentration was reduced to 75mgNH₄⁺-N/l and 85 mgNO₂⁻-N/l, respectively, and then dropped to 45±5mgNH₄⁺-N/l and 52±1mgNO₂⁻-N/l after 86d. But in the above meantime the effluent NO₃⁻-N concentration was gradually increased to 90mgN/l or more, and the sludge further disintegrated from biofilm to liquid. At ultimate, very little sludge was retained in reactor with light brown color indicated the reaction complete failure.

3. Analysis and Discussion

3.1 Effect of carrier on anammox process

In the same influent nitrogen concentration, sludge nitrogen load of the suspended growth system was below 0.081kgN/kgMLSS·d, and the sludge nitrogen load of the attached growth system was about 0.0865kgN/kgMLSS·d. Anammox reaction was clearly not stable in Suspended growth system in which ORP was positive indicated that the system can not eliminate DO effectively resulting in continued loss of sludge and increased sludge load continuously to 0.081kgN/kgMLSS·d with reactor instability. While anammox reaction was stable in the attached growth system showing a higher removal efficiency than that in the suspended system. The main reason was that the attached growth system can well retain anammox sludge, and sludge at the outer biofilm could consume DO coming from raw water (ORP of system was -300mv) so that could avoid inhibiting anammox process.

Table 2 nitrogen removal of different growth system in the same influent nitrogen concentration

| growth system       | Raw water concentration mgN/l | salt gNaCl/l | Nitrogen removal (%) | Sludge nitrogen load kgN/kgMLSS·d | Volume nitrogen load kgN/m³·d |
|---------------------|-------------------------------|--------------|----------------------|----------------------------------|-------------------------------|
| suspended growth system | 140 NH₄⁺-N 213 NO₂⁻-N    | 0            | 79.46±7.58          | 75.7±8.87                        | 0.081                          | 0.353                          |
| attached growth system | 1 99.2               | 94.8         | 0.0865                | 0.235                           |

3.2 Salt loads impact on anammox process

Sensitivity ratios of biofilm reactor at different salt shock loads were listed in Table 3. When the salt concentration was increased from the 1gNaCl/l to 3gNaCl/l and from 3gNaCl/l to 7gNaCl/l respectively, the maximum sensitivity was 0.076 and 0.128 respectively shown that freshwater anammox sludge could withstand the salt concentration of 7gNaCl/l. And if ignored the growth of microorganisms because of sludge washout, the salt of sludge loading was 2.58kgNaCl/kgMLSS·d.

Then the salt concentration increased to 12gNaCl/l and the amount of influent were increased by 50% resulting salt load over 6.63 kgNaCl/kgMLSS·d which was higher than 4kgNaCl/kgMLSS·d (salt concentration was 30gNaCl/l) in research of Kartal. The reactor was instable and the maximum sensitivity ratio was 2.037 with nitrogen removal rate dropping significantly. In addition, the low capacity utilization rate was one of the reasons for reactor instable because fillers suspended in the top of reactor which led to sludge and sewage uncompleted well. It was shown in Table 3 that the max salt shock load which freshwater anammox bacteria could afford was 4 kgNaCl/m³·d in this research. When a sudden withdrawal of the salt, the sensitivity ratios further increased up to 3.44. It was shown that although a
A substantial increase in salt load would lead to freshwater anammox bacteria unadaptation, suddenly draw-off salt load would be more serious for anammox bacteria.

**Table 3** sensitivity ratios of biofilm reactor at different salt shock loads

| Influent salt Concentration (gNaCl/l) | 1→3  | 3→7  | 7→12 | 12→0 |
|---------------------------------------|------|------|------|------|
| Salt shock load (kg NaCl/m³·d)        | 1.33 | 4    | 7.5  | 12   |
| S(max)                                | 0.076| 0.128| 2.037| 3.44 |

**Conclusion**

Attached growth system equipped with carriers was prior to implement anammox process and retain sludge than the suspended growth system. Fresh-anammox sludge could also adapt to salt water in which the highest salinity was 7gNaCl/l and salt load was 2.58kgNaCl/kgMLSS·d in this experiment. The freshwater anammox bacteria could strongly compress salt shock load under 7gNaCl/l and the max was 4kgNaCl/m³·d. A substantial increase in salt load would lead to freshwater anammox bacteria unadaptation, but suddenly draw-off salt load would be more serious for anammox bacteria. The salt tolerance of anammox sludge was function with sludge concentration.

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