# Salt Acclimation Behavior of the Nitrifier Consortium for

## the Nitrification of Saline Wastewater

### SEO, JAE-KOAN and SUNG-KOO KIM

Department of Biotechnology and Bioengineering,
Pukyong National University, 599-1 Daeyeon-3-Dong, Nam-Gu, Pusan 608-737
Tel: 051-620-6188, Fax: 051-620-6180

#### Abstract

The effect of salinity on the nitrification efficiency of the nitrifier consortium was evaluated for the nitrification of saline wastewater. The nitrifier consortium, which was the activated sludge acclimated with ammonium as the only energy source, was used as the nitrifier for the salt acclimation. Airlift reactors for the nitrification of ammonia with increasing concentration in saline synthetic wastewater (35 g/l NaCl), and synthetic wastewater without salt as a control, were continuously operated with the nitrifier consortium for 43 days. The ammonia removal rate was about 23g ammonia-N/m³/day in both the absence and presence of the salt. An accumulation of nitrite was observed in the saline nitrification reactor at an early period. However, the nitrite decreased to less than 1 mg/l after 39 days of operation. The salinity increased the acclimation time of the nitrifier consortium to obtain a stable marine nitrification system. However, the salt acclimated system showed the efficient removal of ammonia which was same as that without salt.

#### Introduction

Effluents containing ammonia can cause serious environmental problems, such as an increase in biological oxygen demand and eutrophication of coastal sea, as well as rivers or lakes. Such effluents are also toxic to aquatic life and demand a greater amount of chlorine during water treatment for public supply<sup>5)</sup>.

Biological nitrification is the most usual process for the treatment of such wastewater. It consists of the biochemical oxidation of ammonia and is promoted by two bacterial strains *Nitrosomonas* sp. and *Nitrobacter* sp.; the first strain oxidizes ammonia to nitrite, which is subsequently converted to nitrate by the other strain. Both microorganisms are autotrophic, deriving their energy from ammonia and nitrite, respectively<sup>5)</sup>. These bacteria have a low growth rate and are very sensitive to environmental conditions, such as temperature, pH, dissolved oxygen level and salinity<sup>5)</sup>. Salinity is known to affect bacterial metabolic activity, reducing microbial growth and ammonia oxidation rate. Although the nitrification in freshwater has been extensively

studied for domestic and industrial wastewater treatment, there are few studies on the nitrification of brackish and seawater<sup>4</sup>. However, nitrification of saline wastewater is of great significance to many industrial fields, such as aquaculture, concentrated wastewater treatment and the petroleum industry<sup>5</sup>.

The present study evaluated the ammonia oxidation characteristics of the nitrifier in saline wastewater (35 g/l NaCl) with that in fresh wastewater as a control.

## Material and Method

The airlift bioreactor consisted of a nitrification tank and sedimentation tank and the working volumes of each tank were 35 l and 10 l, respectively. Air was supplied at a flow rate of 0.1 vvm (vessel volume per minute) at the bottom of the reactor, and the influent ammonia concentration was increased with operating times. The nitrifier consortium was obtained by the acclimation of activated sludge from Soo Young Wastewater Treatment Facility in Busan, Korea.

The composition of the feeding solution with a hydraulic retention time (HRT) of 24 hrs is shown in Kim et al., 2000, and Seo et al., 2001. All experiments were conducted at  $27\pm1$  °C. Dissolved oxygen (DO), pH, ammonium nitrogen (NH<sub>3</sub>-N), nitrate nitrogen (NO<sub>3</sub>-N), nitrite nitrogen (NO<sub>2</sub>-N), and mixed liquor suspended solid (MLSS) were determined according to the methods described in the standard methods<sup>1)</sup>. These experiments were replicated and the results are the average of at least three determination.

## Results and discussion

Through microscopic observation of the strains isolated in Petri dishes, it could be determined that the colonies had characteristics of nitrifiers. Generally, the organism formed round, convex colonies, 0.5-3.0 mm in diameter. Colonies of nitrite oxidizers grew after 36-48 h of incubation time and colonies of ammonia oxidizers grew after 48-72 h. Some characteristics of the nitrifying bacteria were observed. Ammonia oxidizers and nitrite oxidizers were a cell size (µm) of 11.5 and 0.51 and color of colonies was transparent or white and white or reddish brown, respectively. Both microorganisms were gram negative.

The continuous operation of an airlift bioreactor with nitrifiers at increasing ammonia loading is shown in Fig. 1. An airlift bioreactor was operated for 43days. Influent ammonia loading was increased stepwise from 5 to 25 g/m³/day with a 5 g/m³/day interval when stable ammonia removal efficiency was observed. The initial ammonia oxidation rate decreased for a short time when ammonia loading was increased. However, influent ammonia was almost completely removed during the 43 days of the operating periods except the initial period of the ammonia loading increase. The presence

of NaCl negatively affects bilfilms development in aerated biological filters. High salt concentration caused physiological changes in the cells and that affected the microbial metabolism, thus reducing the ammonia removal efficiency. Therefore, the decrease in efficiency could be observed by salt shock. However, these salt shocks could be recovered in 3-5days by the acclimation of cells. This indicates that the nitrifiers became more sensitive to environmental changes when salt was added.

The ammonia conversions to nitrite and nitrate were determined as shown Fig. 1. Nitrate concentration increased with the ammonia addition in the control, the fresh wastewater system. But the nitrate concentration in the salt-added reactor decreased from 12 days of operation due to the nitrite formation and that increased from 18 days of operation.

The saline reactor shows that nitrite built up in the reactor, although nitrite oxidation is reported to occur more rapidly than ammonia oxidation. That is due to a greater susceptibility of the nitrite oxidizers to environmental conditions such as reduced temperatures, salt concentration, limited supply of oxygen, high pH, and presence of inhibitors. However, nitrite concentration increased up to 8 mg/l during the first 18 days of operation and decreased to less than 1 mg/l after 39 days of operation. The reactor with high salt concentration took a long period for the stable system without the formation of nitrite. Nitrification occurs in two steps<sup>3,4)</sup>. Nitrosomonas sp. converts ammonia to nitrite and Nitrobacter sp. convert nitrite to nitrate. Therefore, it could be presumed that Nitrosomonas sp. was more rapidly acclimated than Nitrobacter sp. by salt according to the nitrite accumulation. On the contrary, the reactor with the fresh wastewater control completely converted the ammonia to nitrate without nitrite formation.

#### Conclusion

In conclusion, salt concentration in the wastewater seriously damaged the activity of *Nitrobacter* sp. and the initial nitrification process. The initial accumulation of nitrite was potentially due to the cell damage of *Nitrobacter* sp. rather than *Nitrosomonas* sp. by salt. However, nitrite concentration decreased from 18 days of operation and decreased to less than 1 mg/l at 39 days of operation. Even though the reactor system with high salt concentration took a longer period for the system stabilization without the formation of nitrite, the salt acclimation could be achieved with almost the same ammonia oxidizing capacity to the fresh water nitrification process.

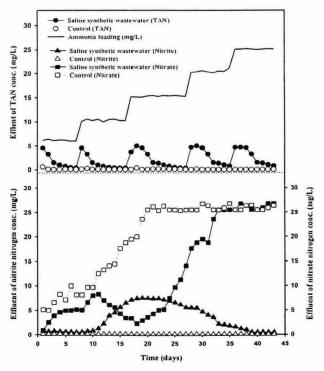


Fig. 1. Continuous operation of airlift bioreactor with nitrifier consortium (2.5 g/L dry cell weight) as increasing ammonia loading at 24hrs of HRT

# Reference

- 1. Anthonisen, A. C., C. R. Loehr, T. B. S. Prekasam, and E. G. Srinath, "Inhibition of nitrification by ammonia and nitrous acid" (1976), *J. Wat. Pollut. Cont. Feder.*, 48, 835–850.
- 2. APHA (American Public Health Administration). 1995. Standard methods for the examination of water and wastewater. 19th ed. American public health administration. Washington. DC.
- 3. Kim, S. K., I. S. Kong, B. H. Lee, L. S. Kang, M. G. Lee, and K. H. Suh, "Removal of ammonia-N from a recirculation aquacultural system using an immobilized nitrifier" (2000), Aquacul. Engin., 21, 139-150.
- 4. Seo, J. K., I. H. Jung, M. R. Kim, B. J. Kim, S. W. Nam, and S. K. Kim. "Nitrification performance of nitrifiers immobilized in PVΛ for a marine recirculating aquarium system"(2001), *Aquacul. Engin.*, 24, 181–194
- 5. Morsyleide, F. R., A. L. F. Angela, T. A. Ricardo, G. F. L. Selma, and A. M. Ricardo, "Biofilm development and ammonia removal in the nitrification of a saline wastewater" (1998), *Biore. Technol.*, 65, 135-138.