

Effect of Saline Concentrations on Biological Nitrification in Batch Reactor

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Abstract

This study was carried out on 4 batch reactors to determine the specific ammonium oxidizing rate (SAOR), specific nitrate forming rate (SNFR) and inhibitory degree of nitrifying activities with saline concentrations. Under salt free condition ammonia was consumed during the reaction period within 200 min. When the salt level increased to 10, 20 and 30 g NaCl L⁻¹ in reactor, ammonia depletion took 250, 300 and above 350 min, respectively. During concentration above 10 g NaCl L⁻¹, there was nitrite accumulation. Also, at 30 g NaCl L⁻¹ ammonia did not depleted and NO₂-N accumulated until the final reaction. Nitrate formation rates decreased with increasing salt concentration. SAOR and SNFR showed a decreasing trend as salinity concentrations were increased. The SAOR was reduced from 0.2 to 0.08 mg NH₄⁺-N g⁻¹VSS day⁻¹ as the salt concentration increased from 0 to 30 g NaCl L⁻¹. Similarly, the SNFR decreased from 0.26 kg NO₃⁻-N kg⁻¹VSS day⁻¹ at saline free to 0.1 kg NO₃⁻-N kg⁻¹VSS day⁻¹ at saline 30 g L⁻¹. A severe inhibition of nitrifiers activity was observed at increased salt concentrations. The inhibition ratio of specific ammonium oxidation rates were 17, 47 and 60% on the reactor of 10, 20 and 30 g NaCl L⁻¹ added, respectively. The inhibition ratio of specific nitrate forming rates also were inhibited 30, 53 and 62% on the reactor of 10, 20 and 30 g NaCl L⁻¹ added, respectively. As the salinity concentrations increased from 0 to 30 mg NaCl L⁻¹, the average MLSS concentration increased from 1,245 to 1,735 mg L⁻¹. The SS concentration of supernatant in reactor which settled about 30 minutes was not severely difference between concentration of salt free reactor and one of those high salt contained reactors.

Key words : Nitrification, NaCl Inhibition, Nitrifying Activity, Saline Wastewater

1. Introduction

During recent years, aquaculture industry (especially, shrimp aquaculture) has been grown rapidly and become an industry. Nowadays, there are about 5000 shrimp farms in over of Vietnam. This value is equal to value of 6 million hectares of rice. The shrimp farm produced wastewater containing some pollutants like as nitrogen, salt and organic compounds. Pollutants of shrimp farm is produced by uneaten feeds and fish waste, and it is toxic to the fish cultured in the aquac-

ulture system (Roger and Klementon, 1985). Also, wastewater from this farm has made some serious problems to water and soil, due to high saline concentration.

Nitrification is the biological oxidation of ammonia to nitrate via nitrite by two groups of chemolithotrophic bacteria, ammonia oxidizers and nitrite oxidizers; both groups have low specific growth rates (Bock et al., 1991; Prosser, 1986). Nitrifying bacteria and the process of nitrification are sensitive to environmental factors such as temperature, dissolved oxygen concentration, pH, available substrate, product inhibition and inhibitory compounds (Antoniou et al., 1990; Hellinga et al., 1998; Moussa, et al., 2006; Sharma and Ahlert, 1977).

Nowadays salt is considered as a common stress factor in WWTPs, especially in the industrial sector. Industries such as pickling, cheese manufacturing, seafood processing, tanning, the production of chemicals and pharmaceuticals, oil and gas recovery produce high inorganic salt concentrations in their wastewater. Other

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(Received : May 24, 2011, Revised : June 17, 2011,

Accepted : June 20, 2011)

sources of saline wastewater include infiltration of sub-surface water in the coastal areas, landfill leachates and contaminated ground water (Moussa, et al., 2006).

The salt-induced inhibition of nitrifying bacteria is a wellknown phenomenon (Ludzack and Noran, 1965). But these results have only recently been refined by multiple studies. But, studies on the effect of salt on nitrification are difficult to compare and show contradictory results (Campos et al., 2002; Catalan-Sakairi et al., 1996; Dahl et al., 1997; Dincer and Kargi, 1999; Hunik et al., 1992, 1993; Intrasungkha et al., 1999; Pan-swad and Anan, 1999a, b; Vredendregt et al., 1997; Yu et al., 2002). Reasons for these contradictions might be: (1) the system configuration and instability in the experimental conditions with respect to temperature, pH, presence of inhibitory compounds or factors; (2) the way of salt introduction to the system as a pulse or by gradual increase; (3) the species involved, use of pure or mixed cultures and of adapted or non-adapted bacteria.

Therefore, this study aim is to investigate specific ammonium oxidizing rate and nitrate forming rate and inhibitory degree of nitrifying activities with saline concentrations.

2. Methods and materials

2.1. Batch reactor and operational conditions

Batch tests were performed to evaluate the variations of nitrification activities of activated sludge on each saline concentration in the bioreactor. Five 8 L batch reactors with a working volume 6 L were operated at room temperature (see Fig. 1 and 2). And pH was initially adjusted to 7.6 with NaOH 0.1 N. During the experiment, the pH and dissolved oxygen (DO) level were monitored every 1.5hr. Dissolved oxygen (DO) level was monitored around 7-8 mgL⁻¹ every day. The experiment was carried out without controlling the DO because nitrification from high salinity wastewater favors high DO levels (Glass and Silverstein, 1999; Hwang et al., 2006). The reactor was inoculated with of activated sludge from the aeration tank, which is the basin mainly responsible for nutrient removal in the Truc Bach domestic wastewater treatment plant (Hanoi, Vietnam). The Truc Bach domestic wastewater treatment plant receives municipal wastewater in the amount of 23,000 m³day⁻¹, which is treated mechanically and

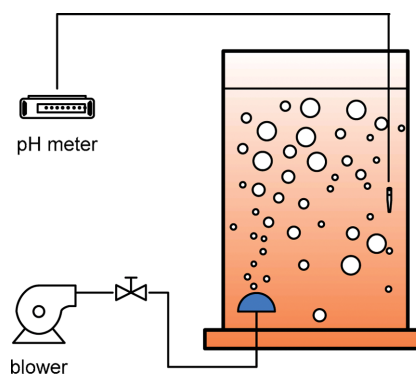


Fig. 1. Schematic configuration of batch reactor.



Fig. 2. Photograph of batch reactors.

biologically. The biological processes are conducted in three zones reactors working in the A2O mode (see Fig. 3). Sludge was cultivated in laboratory for 1 month (4weeks). The experiment was carried out on 4 batch reactors with 1,300-1,400 mgL⁻¹ of MLSS. The reactor operation is briefly described as follows : Reactor A was operated with no salt addition as blank reactor during the experiment. At the first step, reactor B, C and D were operated with 10 g NaClL⁻¹ during the period (5-9 weeks). On the second step, salts of reactor B, C and D were increased from 10 to 15, 20 and 30 g NaClL⁻¹ for each reactor, respectively, during the period (10-12 weeks). At the beginning of the test, a predetermined amount of stock NH₄Cl solution was added to give an initial NH₄⁺-N concentrations of 10 mgL⁻¹ and NaHCO₃ of 0.15 gL⁻¹ at each reactor.

2.2. Determination of nitrifiers activity

Specific ammonium oxidizing rate (SAOR) and specific nitrate forming rate (SNFR) were determined the

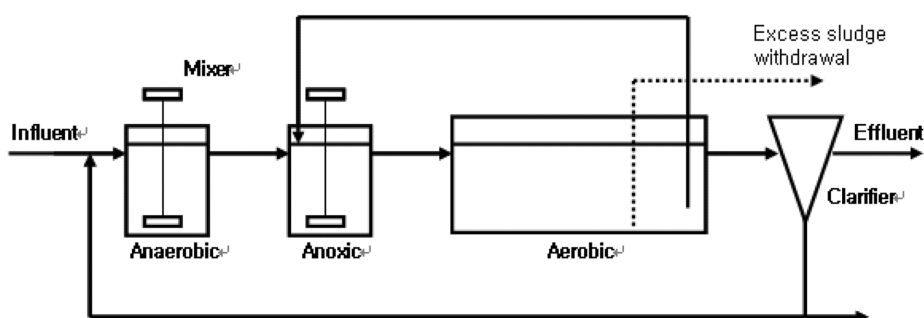


Fig. 3. Schematic diagram of Truc Bach wastewater treatment plant (Hanoi, Vietnam)

concentration slope of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$, the reaction time, and the average MLVSS concentration, respectively (Halling-Sorensen and Jorgensen, 1993). Nitrifying activity was measured without the presence of salts (maximum activity), then successive amounts of the salt tested were added in order to calculate the nitrifying activity for every salt concentration. Inhibitory percentages were calculated as the ratio between the nitrifying activity for every salt concentration and the maximum activity ($A=A_{\text{max}}$) (Campos et al., 2002).

2.3. Analytical methods

Samples were taken three times per week and ammonium, nitrite, nitrate concentrations were analyzed spectrophotometrically following filtration according to the Standard Methods (APHA, 1998). Mixed liquor volatile suspended solids (MLVSS) were determined by filtering the sample through a glass fibre filter. The filter dried 2 hours at 103°C and weighed (MLSS). The filter was further heated at 550°C for 15 min and weighed again. The difference in these weights represented the MLVSS (Greenberg et al., 1992).

3. Result and Discussion

3.1. Performance nitrification

Four reactors containing salt free, 10, 20 and 30 g L^{-1} on each reactor, respectively, were operated during the experimental period. Variation of ammonia, nitrite and nitrate average concentrations is shown in Fig. 4 for each reactor performance under different saline concentrations. As shown in Fig. 4(a) under salt free condition ammonia was consumed during the reaction period (5 h) within 200 min. When the salt level increased to 10, 20 and 30 g NaCl L^{-1} in reactor, ammonia depletion

took 250, 300 and above 350 min, respectively; this depletion rate did not change during the next 4 weeks (see Fig. 4; b, c and d). During concentration above 10 g NaCl L^{-1} , there was nitrite accumulation. Also, at 30 g NaCl L^{-1} ammonia did not depleted and $\text{NO}_2^-\text{-N}$ accumulated until the final reaction. It means that high salinity might affect the metabolic behavior of ammonia oxidizers. Chen et al. (2003) also found that that when the chloride concentration exceeded $10,000\text{ mg Cl L}^{-1}$ both the ammonia- and nitrite-oxidizers might be inhibited initially; however, after a 4-week period with the higher chloride concentrations, saline-resistant nitrifiers, particularly ammonia oxidizers, may be built up (Vredendregt et al., 1997).

Nitrate formation rates decreased with increasing salt concentration. Nitrite produced up to $2\text{ mg NO}_2^-\text{-NL}^{-1}$ under salt free and 10 g NaCl L^{-1} conditions at 80 min during the reaction period (5 h). Increasing the salt level in reactor up to 20 and 30 g NaCl L^{-1} nitrite produced $3.4\text{ mg NO}_2^-\text{-NL}^{-1}$ (150 min) and $5.3\text{ mg NO}_2^-\text{-NL}^{-1}$ (250 min), respectively. Kim et al. (2003) observed that nitrite accumulations caused by overloads could be explained because nitrite oxidizers are stronger inhibited by free ammonia than ammonia oxidizers. Nitrite accumulations could not be merely attributed to an inhibition by free ammonia but perhaps to an increase of salt concentrations during concentration overloads (Campos et al. 2007). Also, some authors found that nitrite oxidizers are more affected by high salt concentrations than ammonia oxidizers (Pen et al., 2005; Vredendregt et al., 1997; Catalan et al., 1997). On the contrary, Moussa et al. (2006) observed that ammonia oxidizers were more sensitive to short and long-term salt stress compared to nitrite oxidizers. This agrees with results of Campos et al. (2002) who found no nitrite accumulations working

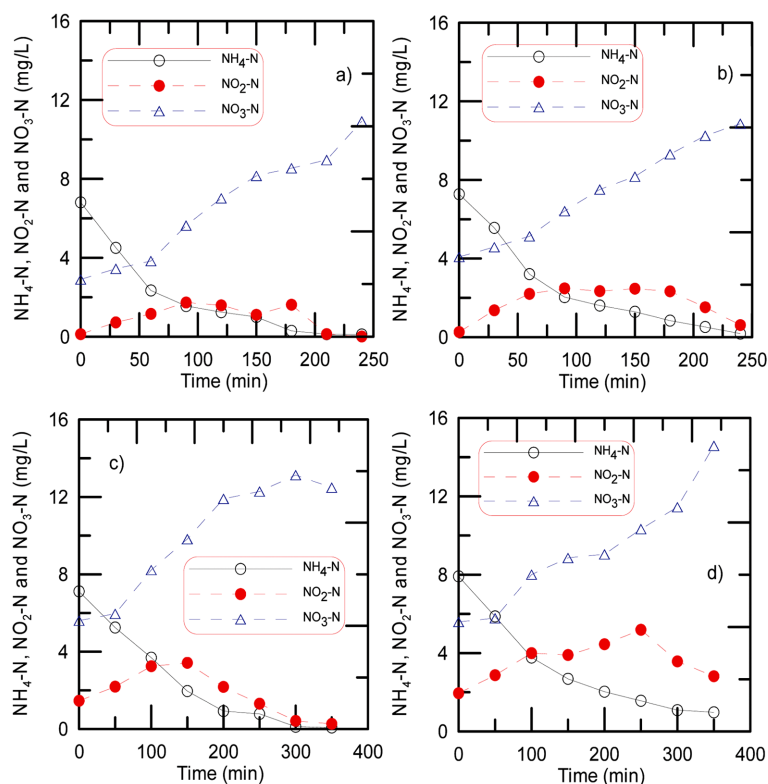


Fig. 4. Variation of ammonia, nitrite and nitrate average concentrations at each reactor. (a)= saline free, (b)=NaCl 10 gL⁻¹, (c)= NaCl 20 gL⁻¹, (d)= NaCl 30 gL⁻¹

at high salts concentrations (525 mM) during long time periods.

3.2. Variations of specific ammonium oxidizing rate and specific nitrate forming rate

The specific ammonium oxidizing rate (SAOR) and specific nitrate forming rate (SNFR) with salt concentration were measured to monitor the nitrifier activities, as shown in Fig. 5 and 6. The SAORs showed a decreasing trend as the experimental salinity concentrations were increased. The SAOR was reduced from 0.2 to 0.08 mg NH₄⁺-N g⁻¹VSS day⁻¹ as the salt concentration increased from 0 to 30 g NaCl L⁻¹. Similarly, the SNFR decreased from 0.26 kg NO₃⁻-N kg⁻¹VSS day⁻¹ at saline free to 0.1 kg NO₃⁻-N kg⁻¹VSS day⁻¹ at saline 30 g L⁻¹. Despite the decreasing trend of SAOR and SNFR above 20 g NaCl L⁻¹, complete nitrification was achieved due to the relatively low initial NH₄⁺-N loads in Fig. 5 and 6. This agrees with results of Dincer and Kargi (2001) who found the nitrification dropped with increas-

ing salt content. In this study nevertheless salt-acclimated nitrifiers (during four weeks at 10 g NaCl L⁻¹) were used to monitor the activity, nitrifiers activity above 20 g NaCl L⁻¹ was lower than other reported results. Panwad and Anan (1999) observed only a moderate reduction in the nitrifying activity from 4 to 3 mg N-NH₃ oxidised g⁻¹ of SS h⁻¹ after NaCl concentration increased from 5 to 30 gL⁻¹, provided that the sludge was acclimated to high NaCl concentrations. In addition, Dahl et al. (1997) showed that a rapid increase in the chloride concentration had an inhibiting action on the nitrifying bacteria. Thereafter, Campos et al. (2002) showed a combined salt- and ammonia-induced inhibition of nitrification: the accumulation of ammonia started at an ammonia loading rate of 3 g N-NH₃ L⁻¹ d⁻¹ and a salt concentration of 525 mM (13.7 g NaCl L⁻¹, 19.9 g NaNO₃ L⁻¹ and 8.3 g Na₂SO₄ L⁻¹). On the other hand, SAOR and SNFR at 15 g NaCl L⁻¹ result were higher than activities of 0 and 10 g NaCl L⁻¹ (data not shown). This partially implied that slow increase in the chlorine con-

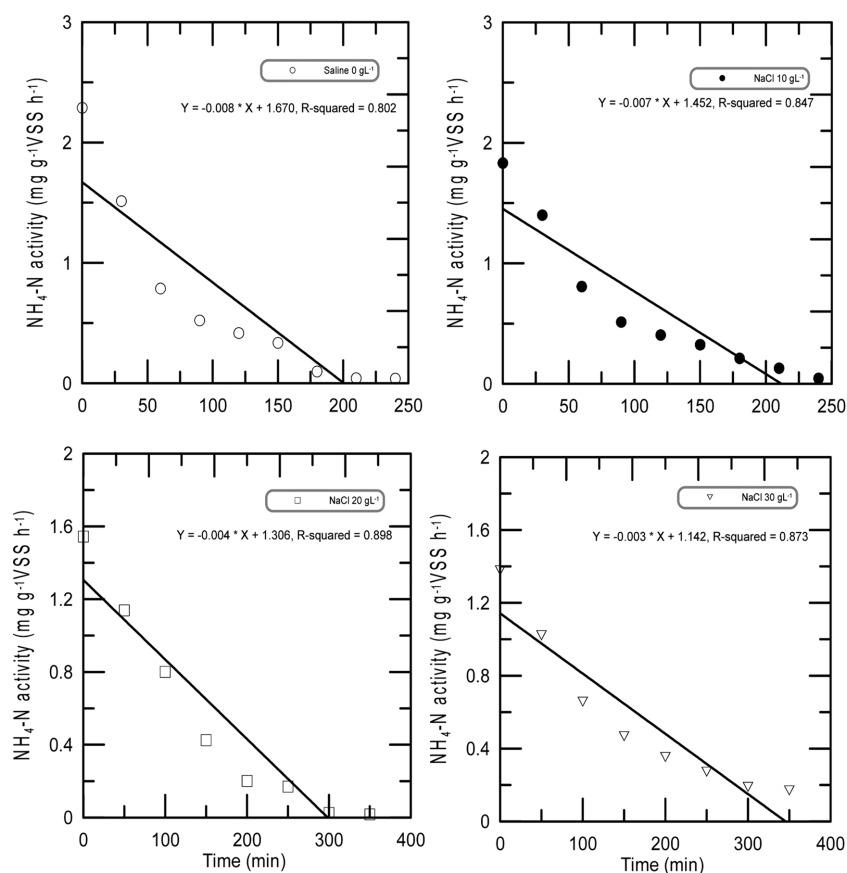


Fig. 5. Variations of ammonium oxidizing rate in reactor.

centration needed to reduce inhibition of nitrification by salt. From the comparison of the specific nitrifying activities in the reactor and in batch tests, an acclimation of the sludge to high saline concentrations in the case of continuous operation was observed. Panswad and Anan (1999) also found that salinity produces a greater inhibition on non-adapted nitrifying organisms. These authors observed that the stepwise increase from 0 to 18 g Cl L⁻¹ caused nitrifying activity losses of 55% and 30% for non-adapted and adapted organisms, respectively.

3.3. Inhibition of nitrifying activity

The inhibitory effects of salts on nitrifiers activity were studied as shown in Fig. 5 and 6. The inhibition ratio on ammonium oxidation rate was correlated with a linear equation in the following form ($r^2 = 0.98$):

$$A/A_{\max} = 1.005 - 0.021 \cdot (S_c) \quad (1)$$

where A/A_{\max} is the ratio of specific ammonium oxidation ($\text{mg NH}_4^+-\text{N g}^{-1}\text{MLVSS h}^{-1}$) and (S_c) is salt concentration (g NaCl L^{-1}).

The inhibition ratio on nitrate forming rate was correlated with a linear equation in the following form ($r^2 = 0.95$):

$$A/A_{\max} = 0.951 - 0.0209 \cdot (S_c) \quad (2)$$

where A/A_{\max} is the ratio of specific nitrate forming ($\text{mg NO}_3^--\text{N g}^{-1}\text{MLVSS h}^{-1}$) and (S_c) is salt concentration (g NaCl L^{-1}).

A severe inhibition of nitrifiers activity was observed at increased salt concentrations. The inhibition ratio of specific ammonium oxidation rates were 17, 47 and 60% on the reactor of 10, 20 and 30 g NaCl L⁻¹ added, respectively. The inhibition ratio of specific nitrate forming rates also were inhibited 30, 53 and 62% on the reactor of 10, 20 and 30 g NaCl L⁻¹ added, respectively.

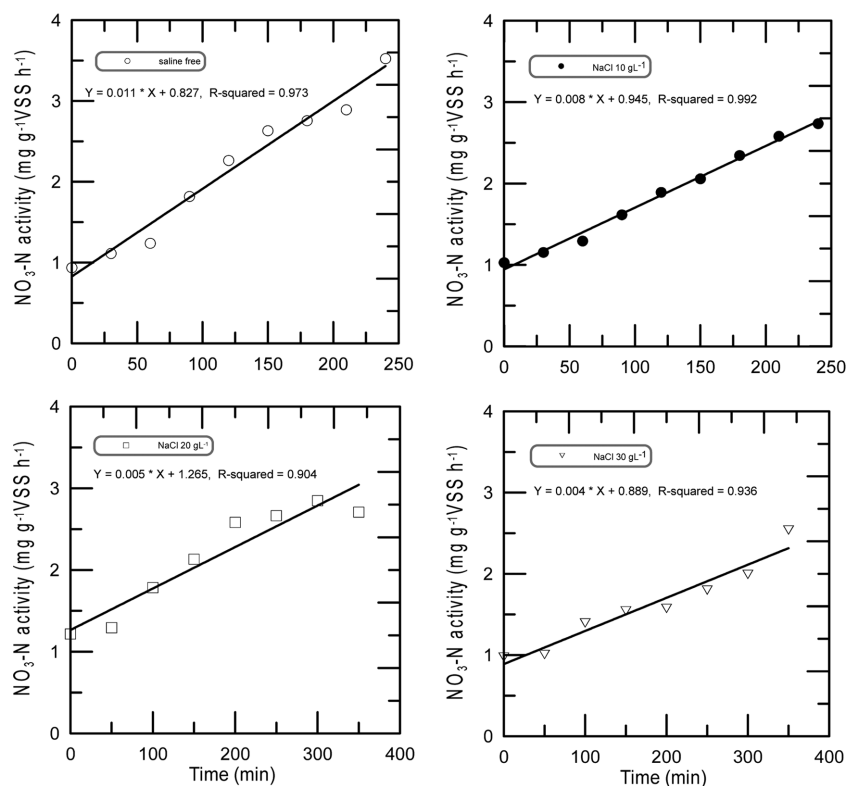


Fig. 6. Variations of nitrate forming rate in reactor.

Table 1. Reported results on the impact of salt on the nitrification activity in various systems and under different environmental conditions

Impact of salt on activity of nitrifiers inhibition (%) ^a	Salt		Environmental conditions			Nitrifiers Seed ^e	System used ^d	Reported observation	
	Type	g Cl L ⁻¹	pH	Temp	Medium ^b				Refs ^e
5-60	NaCl	6, 18, 30, 36	8	25	SW	EN (No adapted)	LA	>18gCl/L SRTmin is 25 days, at 0 g Cl/L	5
10-20	NaCl	18, 30	8	25	SW	EN (No adapted)	LA	NO ₂ accumulation above 12 g Cl/L	6
31-55	NaCl	3, 6, 12, 18	nm ^f	27-33	SW	DA (No adapted)	LA	MLSS decreased with increased NaCl	8,9
<5	NaCl	3	nm	20-22	SW	DA (No adapted)	LS		7
17-60 (AOR) 35-62 (NFR)	NaCl	3, 6, 12	8	30	SW	DA (No adapted)	LBA	MLSS increased with increased NaCl. On the other hand, MLVSS slightly decreased with increased NaCl	This study

^aAOR=Ammonium oxidation rate, NFR=Nitrate forming rate.

^bSW=Synthetic wastewater.

^cEN=*Nitrosomonas* and *Nitrobacter* in mixed culture, DA=Domestic activating sludge performing nitrification.

^dLA=Lab-scale activated sludge unit, LS= Lab-scale sequencing batch reactor, LBA=Lab-scale batch reactor

^e5=Dincer and Kargi,1999; 6=Dincer and Kargi, 2001; 7=Intrasungkha et al., 1999; 8,9=Panswad and Ana,1999a,b.

^fnm=Not measured.

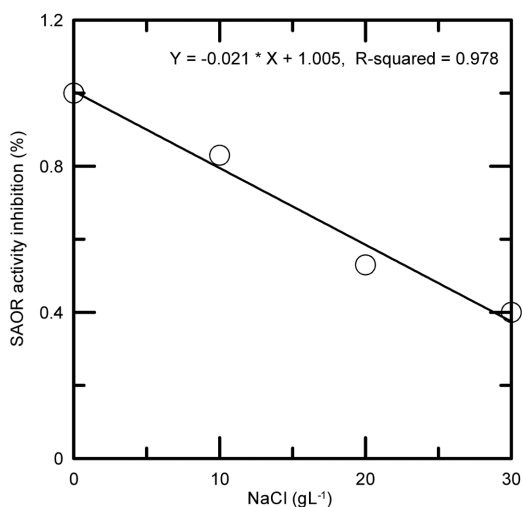


Fig. 7. Inhibitory effect on salt of ammonium oxidizing rate.

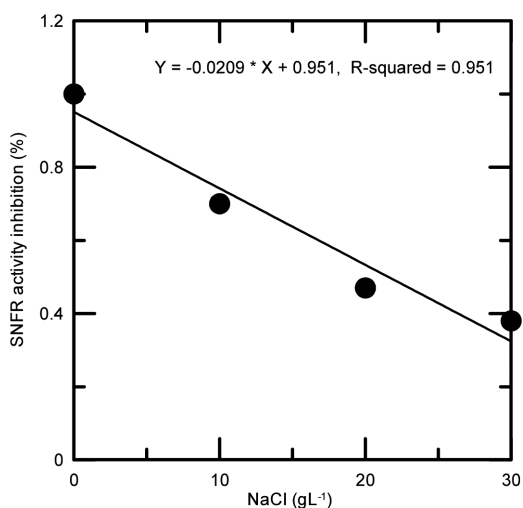


Fig. 8. Inhibitory effect on salt of nitrate forming rate.

These results agree with those of Hunik et al. (1992), who while studying the activity of *Nitrosomonas europaea* found the same effect for NaCl. Table 1 summarizes the reported results on the impact of salt on the nitrification activity in various systems and under different environmental conditions and obtained from this study.

3.4. Characteristics of MLSS and sludge settling with saline concentration

As the salinity concentrations increased from 0 to 30 mgL⁻¹ of NaCl in this experiment, the average MLSS

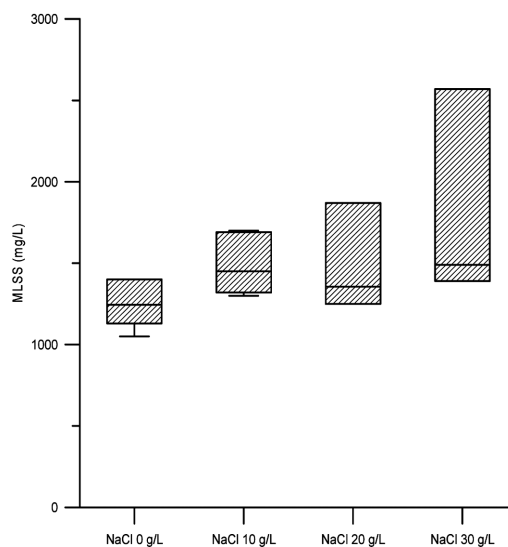


Fig. 9. Variations of MLSS with different salt concentrations.

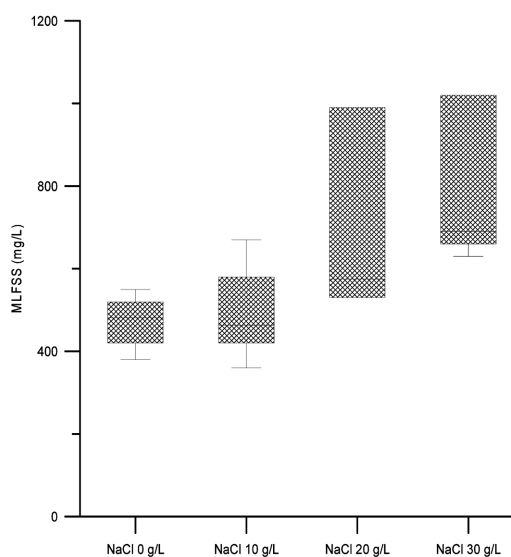


Fig. 10. Variations of MLFSS with different salt concentrations.

concentration increased from 1,245 to 1,735 mgL⁻¹ (see Fig. 9). However, MLVSS was not greatly decreased with increasing salt concentration in contrast to the phenomena of a drastic increment of MLSS concentration (see Fig. 10). This implied that the reason of increasing MLSS with salt concentration was caused by salt concentration and MLFSS (Generally, MLSS = MLVSS + MLFSS), and the salt used which was sun dried salt contained many kinds of mineral more than chemical

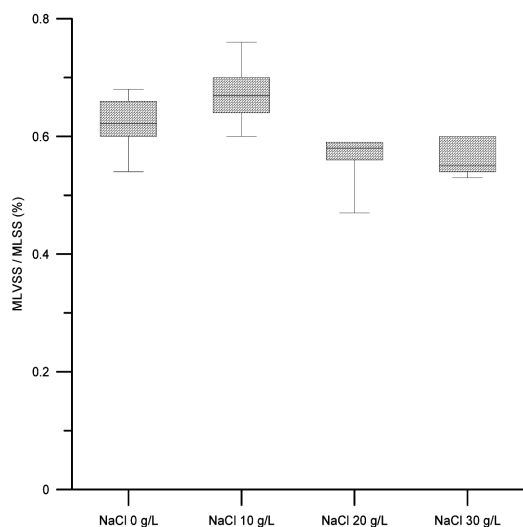


Fig. 11. Variations of MLVSS/MLSS ratio with different salt concentrations.

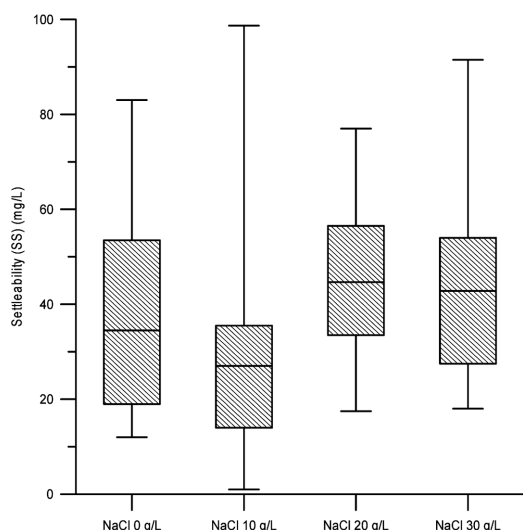


Fig. 12. Variations of SS of each reactor with different salt concentrations.

salt (see Fig. 11). This observation was similar to the result from the study of Hamoda and Al-Attar (1995) which found higher MLSS at higher salinity for activated sludge process. The SS concentration of supernatant in reactor which settled about 30 minutes was not severely difference between concentration of salt free reactor and one of those high salt contained reactors (see Fig. 12). The experimental results can be explained by the following two mechanisms. (1) Due to increased salt levels and consequently an increased water density,

the dense flocs which leads to more bigger flocs in the reactor. (2) The increase of flock size is caused by a combination of electrostatic and hydrophobic interactions with the floc. Christopher et al. (1998) observed the same change in floc characteristics due to increased wastewater salinity. In their study the floc achieved new characteristics in about 15 min after increasing salinity. The increase in salt concentration reduced the electric double layers, thereby reducing the overall repulsive force between particles. The microbial aggregates then approached close enough so that increased hydrophobic interactions resulted in increased aggregation and the formation of larger flocs. These two mechanisms can be utilized to achieve good floc characteristics. Gradual increase in salinity will stimulate the selection of dense flocs with minimum washout. On the other hand, sudden increases in salinity increases water density, causing excessive wash out of biomass. Also, high salt concentrations cause cell plasmolysis and death of microorganisms usually present in sewage due to the increase of osmotic pressure, which results in a reduction in particle size and density (Kargi and Dincer, 1997). Nonetheless, our experiment by stepwise increase salinity from 10 to 20 and 10 to 30 g NaCl L⁻¹ resulted in a slightly reduction of biomass with increasing salt content.

4. Conclusion

The experiment was carried out on 4 batch reactors to determine the specific ammonium oxidizing rate (SAOR), specific nitrate forming rate (SNFR) and inhibitory degree of nitrifying activities with saline concentrations. The results are summarized as follows :

1. Under salt free condition ammonia was consumed during the reaction period within 200 min. When the salt level increased to 10, 20 and 30 g NaCl L⁻¹ in reactor, ammonia depletion took 250, 300 and above 350 min, respectively; this depletion rate did not change during the next 4 weeks. During concentration above 10 g NaCl L⁻¹, there was nitrite accumulation. Also, at 30 g NaCl L⁻¹ ammonia did not depleted and NO₂⁻-N accumulated until the final reaction. Nitrate formation rates decreased with increasing salt concentration.

2. SAOR and SNFR showed a decreasing trend as salinity concentrations were increased. The SAOR was reduced from 0.2 to 0.08 mg NH₄⁺-N g⁻¹VSS day⁻¹ as the

salt concentration increased from 0 to 30 g NaCl L⁻¹. Similarly, the SNFR decreased from 0.26 kg NO₃-N kg⁻¹ VSS day⁻¹ at saline free to 0.1 kg NO₃-N kg⁻¹ VSS day⁻¹ at saline 30 g L⁻¹.

3. A severe inhibition of nitrifiers activity was observed at increased salt concentrations. The inhibition ratio of specific ammonium oxidation rates were 17, 47 and 60% on the reactor of 10, 20 and 30 g NaCl L⁻¹ added, respectively. The inhibition ratio of specific nitrate forming rates also were inhibited 30, 53 and 62% on the reactor of 10, 20 and 30 g NaCl L⁻¹ added, respectively.

4. As the salinity concentrations increased from 0 to 30 mg L⁻¹ of NaCl, the average MLSS concentration increased from 1,245 to 1,735 mg L⁻¹. However, MLVSS was not greatly decreased with increasing salt concentration in contrast to the phenomena of a drastic increment of MLSS concentration. The SS concentration of supernatant in reactor which settled about 30 minutes was not severely difference between concentration of salt free reactor and one of those high salt contained reactors.

Acknowledgements

This work was sponsored by KOICA project and fund of selective study of Institute of Environmental Technology, Vietnam.

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