The stability of nitrite nitrification with strong nitrogenous wastewater: effects of organic concentration and microbial diversity

Z. Yun*, Y.-H. Jung, B.-R. Lim**, E. Choi*** and K.S. Min******

* Department of Environmental Engineering, Korea University, 5-1 Anam-dong, Life Science Bldg. #243, 5-1 Anam-dong, Seoul 136-701, Korea (E-mail: *zyun@korea.ac.kr*)

**Korea Institute of Science and Technology, Hawoelgok-dong, Sungbuk-ku, Seoul, Korea 136-791 Korea

***Department of Civil and Environmental Engineering, Korea University, 5-1 Anam-dong, Seoul 136-701, Korea

**** Department of Environmental Engineering, Kyungpook National University, Taegu, Korea

Abstract A stable achievement of nitritation with strong nitrogenous wastewaters is considered as a difficult task in practice, probably due to the fate of interaction between dominating heterotrophs and nitrifier species. An experimental study was carried out to examine the organic effects in lab-scale biofilm nitritation reactors. The control unit without organic addition showed a stable nitritation performance for more than 220 days of operating period. The nitritation activity gradually failed at the reactors with an organic addition, but the nitritation activity eventually recovered with a prolonged aeration. It was not possible to explain the nitritation recovery with neither free ammonia inhibition concept nor DO competition hypothesis in these cases. The results suggest that the nitritation with organic requires a long start-up period for acclimation. In addition, the results of quinone profile analysis were in agreement with nitritation activity in reactors. The diversity of microbial community in the nitritation reactors could be described by the quinone profiles.

Keywords Biofilm; DO competition hypothesis; microbial community; nitritation; organic effects; quinone profile

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Introduction

The scientific understanding of the nitrite nitrification (or nitritation) process is increasingly important in the areas of biological nutrient removal (BNR) systems for sewage treatment as well as various strong nitrogenous waste treatments including leachate and animal wastes. However, a stable achievement of nitritation is considered as a difficult task in reality, probably due to the fate of interaction between heterotrophs and two categories of nitrifier species (ammonium oxidizer and nitrite oxidizer). In general, heterotrophs are considered as a more dominant organism than nitrifiers at various environmental conditions including temperature and toxic substances. In between two categories of nitrifiers, the nitrite oxidizers are also considered as more powerful and dominant species than ammonium oxidizer, so that produced nitrite is readily converted to nitrate. In other words, it is difficult to accumulate nitrite in ordinary conditions.

However, recent efforts to save both aeration energy and precious carbon source for nutrient removal demonstrated that nitrite accumulation in biological reactor is possible in many ways. Up to now, the achievement of a nitritation with strong nitrogenous wastewater could be explained with two different concepts. First concept is the free ammonia (FA) inhibition mediated accumulation of ammonium oxidizer as suggested by Anthonisen *et al.*'s early work (1976). With the strong ammonium concentration condition and alkaline pH condition, the liberated FA in bulk liquid acts as an inhibitor for the growth of nitrite oxidizers, resulting in an accumulation and domination of the ammonium oxidizer in the

reactor. As a result, high nitrite concentration in reactor effluent could be achieved. The second concept is the temperature-induced selection of ammonium oxidizer over nitrite oxidizer on the basis of different growth rates of two species at higher temperature (usually more than 35 ^{o}C), as claimed by Hellinga *et al.* (1998). This concept has been further developed as the combined Sharon and Anammox process for the removal of nitrogen in the sludge recycle water.

However, both concepts could not be fully applied to explain the failure of a sensitive nitritation system. The sensitivity of the nitritation system is considered as a fate of the nitrifier, since the nitrifier responded sharply to the environmental parameters including DO, HRT, temperature, alkalinity, toxic substances, and so on. In real conditions, strong nitrogenous waste contains both ammonium and high strength organics, such as are seen in sludge recycle water, leachate, and animal waste. The nitritation often fails when organic shock load is introduced to the system, as is common in cases of high nitrogenous wastes. The reason for the failed nitritation operation in this case was mainly speculated to be due to the DO competition between dominant heterotrophic consortia and oxygen-sensitive autotrophic nitrifier. For instance, Sternstorm and Song (1991) who worked with activated sludge for sewage treatment applied the DO competition concept to explain the nitrification potential. This concept is based on two assumptions: first, the nitrifier is not strong enough to compete with heterotrophs; second, the number of nitrifiers is always smaller than heterotrophs as shown in ordinary activated sludge. The DO competition concept seems reasonable to explain the failure of nitritation at the organic shock loading condition that is often observed in real conditions when wastewater organic and nitrogen concentration vary widely, but it has not been examined in detail with strong nitrogenous wastes.

The objectives of this research were therefore focused on the effects of organic concentration in nitritation. In order to achieve the objective, a series of laboratory experiments was devised with the biofilm nitritation system. A biofilm carrier with hydrophilic surface characteristics has been used in this experiment, since this type of carrier surface could more easily attract autotrophic nitrifier than heterotrophs resulting in an accumulation of nitrifier biomass. With an accumulation of nitrifier in the biofilm, organic effects on nitritation would be easier to observe. The feed wastewater was synthetically made up of NH_4^+-N

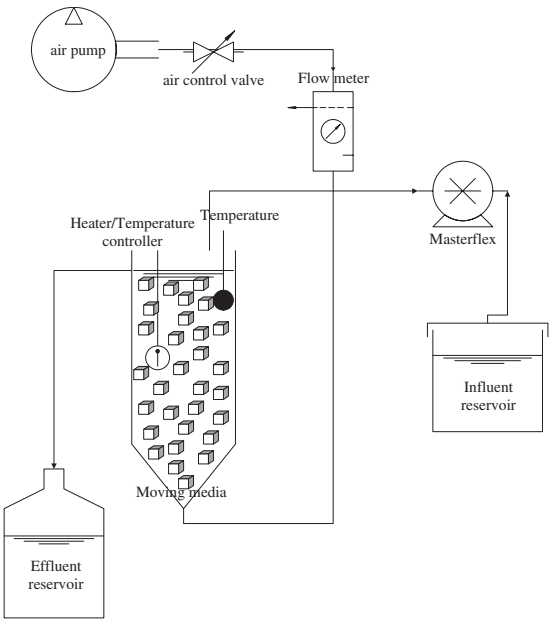

Figure 1 Schematic diagram of laboratory reactor system

concentration of 300 mg/L that represented the average nitrogen concentration of recycle water in wastewater treatment plants in Korea. The organic concentration varied from 0 to 300 mg COD/L with an addition of glucose. Throughout the experimental periods, sufficient DO level (4–6 mg/L) and temperature (35 ^{o}C) were maintained with an average HRT of 0.5 days. In order to determine the changes of microbial community, the quinone profile in biofilm has been monitored. The microbial countermeasure was performed with quinone profiles of the nitritation systems.

Material and methods

Laboratory reactor

Figure 1 shows the schematic diagram of a lab-scale nitritation reactor. Three identical reactors were operated during the experimental periods. The total reactor volume was 4.5 L including 4 L of reaction volume and 0.5 L of overhead space. The aeration and mixing achieved by an air compressor (Medo vacuum pump, NITTO KOHKI, 5 L Cat. #106502) with up to 3 L of air/L of reactor/min. The aquatic heater and controller maintain the reactor temperature of 35 °C. A cube type media made of polyvinyl alcohol (PVA) was placed in the reactor with 10% of apparent reactor volume. And the feed substrate was pumped by a Masterflex pump (Cole-Parmer 7521-50).

Operating conditions

Three identical lab reactors were continuously operated for 9 months. Reactor 1 fed without organic addition was used as the control unit. The glucose was added to the feed for Reactors 2 and 3 with final COD concentration of 156 mg/L and 299 mg/L. According to previous study (Yun and Jung 2003), the minimum HRT to obtain sufficient nitritation in the biofilm reactor was found to be 0.5 days. Sufficient mixing and aerobic condition were maintained in the lab reactor by the aeration. DO level was maintained in the range 3.5–4.0 mg/L.

Wastewater

A synthetic wastewater was used in this research in order to minimize the adverse effects when real recycle water is used. The composition of the feed substrate used for the lab-experiment is shown in Table 1. NH_4^+-N was supplied in forms of NH_4HCO_3 with a concentration of 300 mg NH_4^+-N/L. The alkalinity was supplied by both NH_4HCO_3 and $NaHCO_3$.

Sampling and analysis.

The samples were analyzed on a weekly basis during the operating period. All water quality parameters were measured in accordance with *Standard Methods* (1998). An ion

Table 1 Composition of the feed substrate used for experiments

		Chemicals	Concentration
Nitrogen source		NH_4HCO_3	33.857 gr/20 L (300 mg NH_4-N/L)
Alkalinity		$NaHCO_3$	37.968 gr/20 L (2,200 mg Alk/ mg NH_4-N)
Organic source	Glucose	Reactor 1	as COD 0 mg/L
		Reactor 2	as COD 156 mg/L
		Reactor 3	as COD 299 mg/L
Inorganic salts		$MgSO_4 \cdot 7H_2O$	0.01 gr/L
		$CaCl_2 \cdot 2H_2O$	0.002 gr/L
Trace metals		*	1 mL/L

* Trace metals: $FeCl_3 \cdot 6H_2O$ 1.5 g/L, $MnSO_4 \cdot 5H_2O$ 1.5 g/L, $ZnSO_4 \cdot 5H_2O$ 0.1 g/L, $CoCl_2 \cdot 2H_2O$ 0.1 g/L, $(NH_4)_6Mo_7O_{24} \cdot 5H_2O$ 0.1 g/L

chromatography (DIONEX DX-500) was used to analyze nitrite and nitrate. SEM (field-emission scanning electron microscope, S-4700, Hitachi) was used for media surface. Quinone analysis was performed in accordance to the modified methods developed by Hu *et al.*(1999).

Results and discussion

Effects of organic addition on nitritation

The variation of NH_4-N, NO_2-N, and NO_3-N concentration in influent and effluent of biofilm reactors are shown in Figures 2, 3 and 4. Nitrogen profiles in Figure 2 show the performance of the control (Reactor 1) without glucose addition. Figure 3 and Figure 4 represent the nitrogen conversion performance in Reactor 2 (COD = 156 mg/L) and Reactor 3 (COD = 299 mg/L), respectively.

Without an addition of COD, the control unit showed a stable nitritation performance for more than 220 days of operating period (Figure 2). In this case, most of effluent nitrogen is nitrite (NO_2-N). Reactor 2 and Reactor 3 were initially operated without glucose addition as Reactor 1. Thus, NO_2 accumulation behaviour in Reactors 2 and 3 is almost the same as in Reactor 1, up to 80 days of operating period. When organic is introduced in Reactors 2

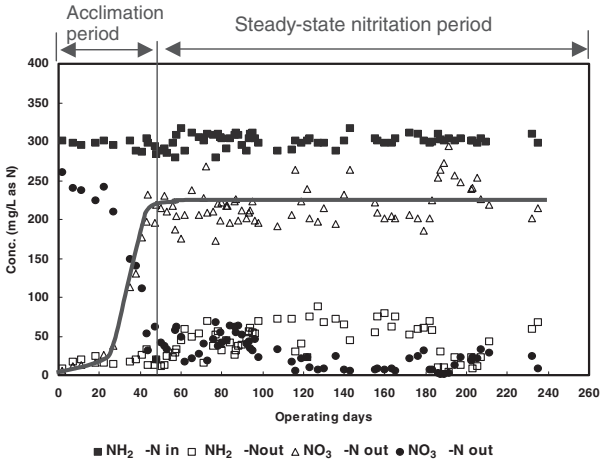

Figure 2 Nitrogen profiles in nitritation reactor 1 with COD 0 mg/L and NH_4^+-N 301 mg/L

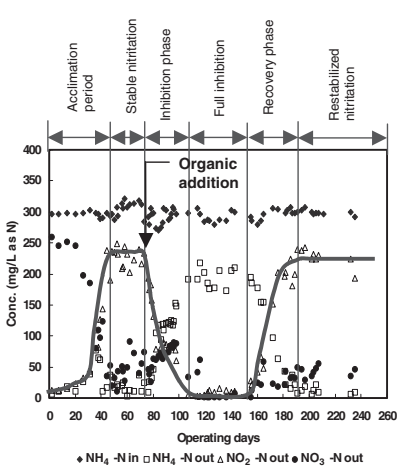

Figure 3 Nitrogen profiles in nitritation reactor 2 with COD 156 mg/L and NH_4^+-N 297 mg/L

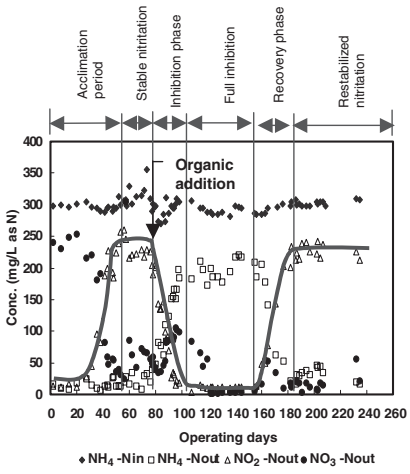

Figure 4 Nitrogen profiles in nitritation reactor 3 with COD 299 mg/L and NH_4^+-N 298 mg/L

and 3, the nitrite accumulation performance gradually failed. It took about 20 days to reach a complete loss of nitrification activity. Up to this period, it can be concluded that organic definitely inhibited the nitrification. The failure of nitritation could not be explained by either the FA inhibition concept or the temperature selection concept, since both nitrifiers are completely inhibited by the addition of organic.

Although the nitritation gradually failed with an organic addition as shown in Figures 3 and 4, the nitrite accumulation activities eventually recovered with a prolonged aeration. Both Reactors 2 and 3 took about 80 days to recover the full nitritation activities. The initial inhibition of nitritation definitely seemed due to the addition of organic, thus, it could be only postulated that the powerful mutational (or adaptation) ability of the nitrifier leading to the recovery of nitritation activity even at adverse environmental conditions co-existed with heterotrophs. In addition, the result also suggests that the hypothesis of DO competition between aerobic heterotrophs and autotrophic nitrifier could not accurately describe the retardation and recovery of nitritation in this experiment with high DO condition. During the nitrification inhibition periods, the reactor DO gradually increased up to 6 mg/L because of the loss of nitrification. The result suggests that a high DO condition is a prerequisite to achieve the nitritation with the presence of organic. Thus, it would be therefore reasonable to suggest that the maintenance of low DO condition (less than 2 mg/L) would not be a good strategy to achieve a stable nitritation. Based on the repetition of the experiment with the parallel running reactors (results not shown in this paper), the recovery pattern is almost the same, as long as sufficient DO is maintained in the reactor.

Operating results in average concentration of nitrogenous compounds from nitritation reactors are shown in Table 2. The organic inhibition to nitritation can be categorized into: (1) initial steady state condition; (2) inhibition phase; (3) full inhibition phase; (4) recovery phase; and (5) re-stabilization of nitritation phase. With an organic addition, recovery of nitritation activity took more than 100 days. According to the result, organic concentration of either 156 mgCOD/L or 298 mgCOD/L did not show much difference in terms of recovery. In practice, however, the nitritation reactor with up to 300 mgCOD/L would require more than 80 days to stabilize the nitritation potential. It suggest that the nitritation reactors with organic requires a long start-up period. Also, in order to reduce the adverse effects of organic shock load to the nitritation reactor, an installation of an equalization tank would be a simple approach to achieve the stability of the nitritation.

Table 2 Operating results of biofilm nitritation reactors with and without organic addition

Period	Parameters	Reactor 1 COD = 0mg/L NH_4^+-N = 301mg/L	Reactor 2 COD = 156mg/L NH_4^+-N = 297mg/L	Reactor 3 COD = 299mg/L NH_4^+-N = 298mg/L
	Operating days	191 days	26 days	27 days
Initial steady-state	Eff. NO_2-N	218.6 mg/L	221.1 mg/L	223.9 mg/L
effluent concentration	Eff. NO_3-N	29.0 mg/L	49.1 mg/L	47.1 mg/L
	Eff. NO_4-N	42.9 mg/L	17.0 mg/L	21.6 mg/L
Inhibition phase		↓	30 days	19 days
	Operating days		48 days	62 days
Full inhibition period	Eff. NO_2-N	↓	11.6 mg/L	11.3 mg/L
	Eff. NO_3-N		11.8 mg/L	25.9 mg/L
	Eff. NH_4-N		193.8 mg/L	195.1 mg/L
Recovery phase		↓	37 days	22 days
	Operating days		16 days	25 days
Re-stabilized nitritation	Eff. NO_2-N	↓	230.4 mg/L	227.8 mg/L
effluent conc.	Eff. NO_3-N		42.2 mg/L	13.8 mg/L
	Eff. NH_4-N		13.6 mg/L	32.9 mg/L

Microbiological community analysis

Photographs of scanning electron microscopy of the media surface at the steady state condition of the restabilization period are shown in Figure 5. The nitritation reactor without COD addition showed some empty surface with colonization of nitrifier (Figure 5 (a1)). Magnified surface showed a similar pattern (Figure 5 (a2)). Large cluster of colonies can be seen on the media surface with an organic addition (Figure 5 (b) and Figure 5(c)). These clusters are probably inhabited by a large population of heterotrophs with nitrifiers, since up to 90% of COD removal was achieved in Reactors 2 and 3.

The quinone profile in biofilm seems a useful tool for microbial community analysis. Quinones are aromatic dioxo compounds, which are typically colored and are constituents of many natural pigments. Microorganisms contain a range of quinones, the nature of which can be useful taxonomically. Quinones function in aerobic and anaerobic electron transport chains and photosynthesis so that their profile becomes a useful tool to analyze the nature of a microbial community without cumbersome incubation and identification procedures (Collins and Jones, 1981; Hedric and White, 1986). The quinone profile can further be used to identify the dominance and diversity of microbial species in the microbial community with nutrient removal systems with aerobic and anoxic conditions (Lim *et al.*, 2001). The quinone profile of biofilm together with nitrogen effluent quality could be used to identify the diversity of the microbial species in the reactor.

In order to verify the microbial community changes with an organic addition, the quinone profile of the biofilm was measured as shown in Fig. 6. The ubiqinone-8 (UQ-8) can be found in both the heterotrophs and the ammonium oxidizer, but not in most of the nitrite oxidizer. Since Reactor 1 did not have any organic addition, measured UQ-8 is mostly originated from the ammonium oxidizer since there is no organic removal activity. Ubiquinone-10 (UQ-10) is, however, found in most of the nitrite oxidizer and heterotrophs, but not in the ammonium oxidizer. The UQ-8 content in the ammonium oxidizer decreased from 151.4 nmol/L at Reactor 1 to 121.4 nmol/L at Reactor 3. Conversely, heterotrophic UQ-8 content increased to 61.6 nmol/L at the reactor with 299 mgCOD/L. Another heterotrophic UQ-10 also increased to 33.0 nmol/L at Reactor 3. However, content of UQ-10 in the nitrite oxidizer decreased to 2.4 nmol/L with an increase of organic addition.

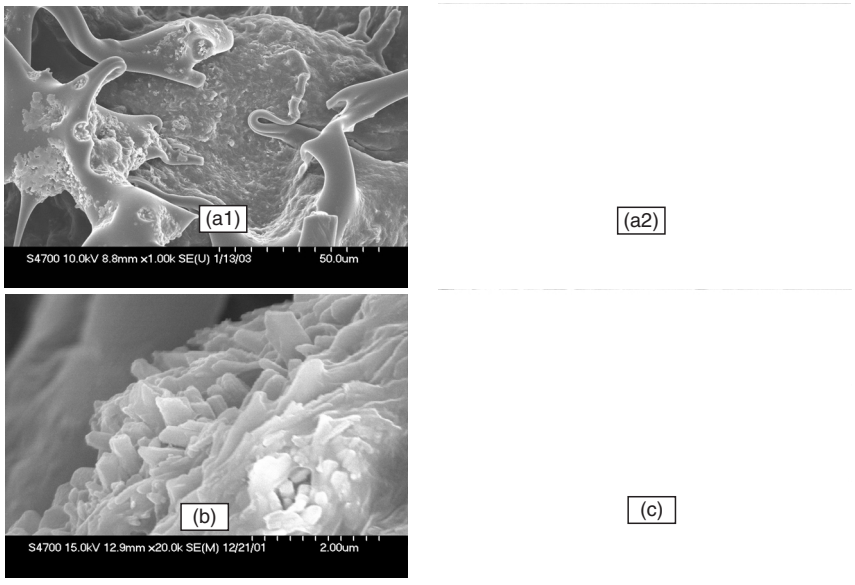

Figure 5 SEM photos of biofilm carrier surface with various organic concentrations. (a1 and a2) = Reactor 1 (COD = 0 mg/L). (b) Reactor 2 (COD = 156 mg/L). (c) Reactor 1 (COD = 299 mg/L)

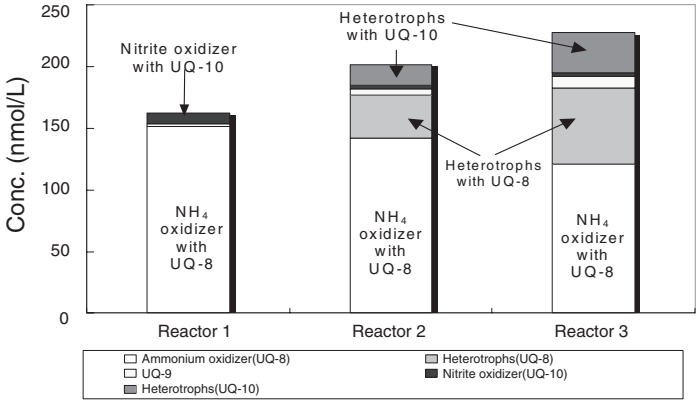

Figure 6 Quinone profile of microbial community in biofilm with a recovered nitritation activity (after 220 days of operation)

In general, the quinone profiles in reactor biofilm showed that the activity of NH_4 oxidizer decreased with an increase of organic concentration along with an increase of heterotrophic activity. The profile in Reactors 2 and 3 also indicates that the activity of NO_2 oxidizer reduced with a decrease of the NH_4 oxidizer's activity. According to the effluent nitrogen profile in Reactor 1 without organic addition, the ammonium oxidation was the dominating activity. The operating result is in agreement with the quinone profile analysis. It seems that the massive presence of ammonium oxidizer is obvious in Reactors 1–3 with a stable nitrite accumulation activity according to the quinone profile analysis, but the nitrite oxidizer UQ-10 content seemed not to be correlated with the nitrite oxidation. It should be noted that the quinone content does not accurately respond to the actual microbial activity or amount of biomass. However, it is interesting to observe that the overall nitrite accumulation activity in the reactors with organic addition was not reduced with the co-existence of heterotrophs and autotrophs on the biofilm. The findings suggest that the stability and performance of the nitritation system critically depended on the activity and presence of an ammonium oxidizer rather than the presence of heterotrophs. The result means that the stability of the nitritation process is directly related to the activity of the ammonium oxidizer itself.

Conclusions

During the course of the experiment, it has been found that the control unit without organic addition showed a stable nitritation performance for more than 220 days of operation. The nitritation activity gradually failed at the reactors with an organic addition. But the nitritation activity eventually recovered with a prolonged aeration. The organic inhibition on nitritation can be clearly categorized into: (1) initial steady state nitritation condition; (2) inhibition phase; (3) full inhibition phase; (4) recovery phase; and (5) re-stabilization of nitritation phase. It can be postulated that the recovery of nitritation activity might be due to the adaptation ability of the nitrifier under the adverse environmental conditions co-existing with heterotrophs. It was not possible to explain the nitritation recovery with either free ammonia inhibition concept or DO competition hypothesis in these cases. In addition, the variation and diversity of the microbial community in the nitritation reactors could be described with the quinone profile analysis.

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