

[S6-5]

Molecular Analysis of Bacterial Community in Nitrifying Biofilm Reactors under Different Hydraulic Retention Times

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In order to treat nitrogen compounds from the wastewater, biological nitrogen removal processes have been widely used to over the past decades. Biological nitrogen treatment processes are often broken down because nitrifying bacteria are extremely slow-growing microorganisms that are sensitive to disturbances in process operating conditions. To overcome this problem biofilm reactors are often used as an effective process for removal of nitrogen compounds from wastewater, because the biofilm process can maintain the slow-growing nitrifying bacteria in biofilms and flexibly respond to variations in process conditions. The nitrogen removal efficiency of biofilm process is significantly affected by the community structures and activities of nitrifying and heterotrophic microorganisms. The competition between nitrifying and heterotrophic bacteria results in a stratified biofilm structure, the fast growing heterotrophic bacteria can form a layer above the slow-growing nitrifying bacteria in the biofilm. The oxygen consumption and resistance to mass transfer in the heterotrophic layer creates disadvantage for the nitrifying bacteria and affects the nitrification performance negatively. The structure of the heterotrophic layer would be affected by hydraulic retention time (HRT) and the types of organic compounds in influent wastewater. A better understanding of the microbial community structure of nitrifying and heterotrophic bacteria in biofilm is essential for improving process performance and control.

In this research, One set of three biofilm reactors (A1, A2 and A3) were supplied with artificial wastewater containing glucose as carbon source and NH₄Cl as nitrogen source, while other set of three reactors (D1, D2 and D3) were supplied with raw domestic wastewater. Each set of reactors was operated at three different HRTs; 8 h (for A1 and D1), 4 h (for A2 and D2), and 2 h (for A3 and D3), respectively, at 25°C for about 4 months. PCR-DGGE was applied to investigate variation of heterotrophic and nitrifying bacterial community in biofilms depending on treating wastewater types and HRTs. The community variation was compared with statistical similarity coefficients and analyzed with traditional diversity statistics to evaluate richness, evenness and diversity indices. Another molecular method of FISH

was used to investigate variation of some representative groups of nitrifying bacterial population such as *Nitrosomonas* spp., *Nitrobacter* spp. and *Nitrospira* spp. in biofilms under different HRTs.

Biofilm reactor performance

The averaged removal efficiency of $\text{NH}_4^+\text{-N}$ decreased as HRT decreased in both sets of biofilm reactors. In the artificial wastewater, A1 and A2 reactors showed stable ammonia removal efficiency as high as 94%, but A3 reactor showed unstable ammonia removal efficiency as low as 50%. The domestic wastewater reactors showed similar results except that the ammonia removal efficiencies of reactors with HRT 8 h and 4 h (D1 and D2, respectively) were relatively lower than those of artificial reactors. These results suggest that the decrease of HRT leads to an excess growth of biomass, especially fast growing heterotrophs, in the outer layer of biofilm. This may result in the depletion of oxygen and resistance to mass transfer, and eventually, a decrease in ammonia removal efficiency in the biofilm. Most of the removed $\text{NH}_4^+\text{-N}$ was transferred to $\text{NO}_3^-\text{-N}$ in all biofilm reactors in both types of wastewater, suggesting that the activity or population of nitrite oxidizing bacteria (NOB) is high enough to transfer NO_2^- to NO_3^- in the biofilms.

Diversity in heterotrophs and nitrifying bacterial community

The Eubacterial DGGE profile suggested that dominant bacterial populations varied according to an increase of HRT in both wastewaters and that the bacterial diversity in biofilms grown on domestic wastewater was greater than that on artificial wastewater. The high bacterial diversity in domestic wastewater might result from the fact that various species of bacteria can grow on various compositions of compounds in domestic wastewater. The reason of the relatively low diversity in artificial wastewater seemed to be that the sole carbon source, glucose, allowed the growth of limited kinds of bacteria.

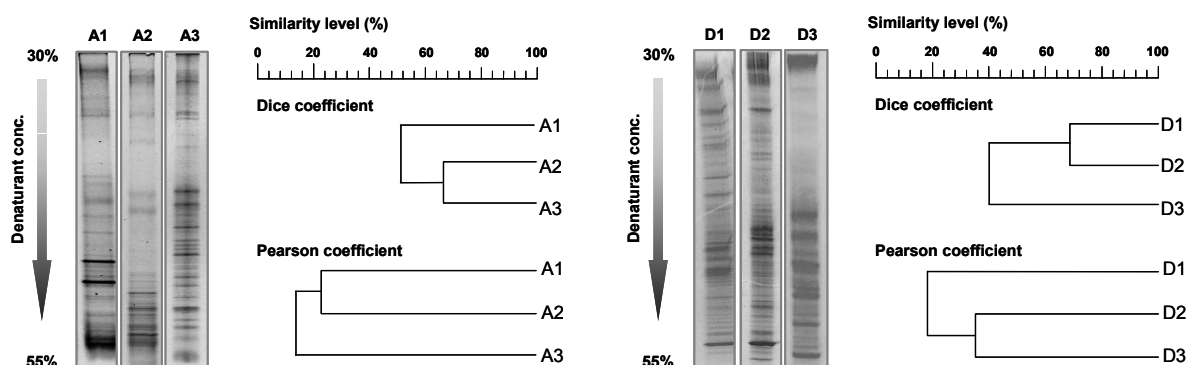


Figure 1. DGGE profiles of eubacterial 16S rDNA fragments in biofilms from each artificial wastewater reactor with different HRT (A1, 8 h; A2, 4 h; A3, 2 h) and domestic wastewater reactor with different HRT (D1, 8 h; D2, 4 h; D3, 2 h) and dendrograms based on similarity coefficients; Dice coefficients and Pearson correlation coefficients

However, the growth of biofilms, according to an increase of HRT, resulted in the formation of a concentration gradient of compounds such as organics and oxygen, this might allow the growth of more various kinds of bacteria.

Nitrifying bacterial population dynamics

The relative abundance of nitrifying bacteria to Eubacteria in biofilms grown on both types of wastewaters was quite higher than that of the activated sludge used as an inoculum. In the artificial wastewater, 70% of ammonia oxidizing β -Proteobacteria was *Nitrosomonas* spp. (detected with Nsm156), while the portion was as high as about 90% in domestic wastewater. This result insists that the remaining portion of ammonia oxidizing β -Proteobacteria should be occupied by the presence of the other AOB belonging to nitrosospiras (including the genera *Nitrosospira*, *Nitrosolobus* and *Nitrososvibrio*) but any sequence affiliated with nitrosospiras was not detected from sequence analysis of DGGE bands. Among NOB, the population of *Nitrospira* spp. (detected with Ntspa662 as about 12.5% of Eubacteria) was higher than that of *Nitrobacter* spp. (detected with Nit3 as about 8.6% of Eubacteria) in both types of wastewater. The high numbers of *Nitrospira* spp. have been reported in other biofilm systems (Schramm *et al.*, 2000). The strategy of *Nitrospira* strains to present in biofilm was thought that they have high affinity for oxygen and can maintain their ribosome content for a long period of inactivity. These characteristics allow them to survive at the lower layer in the biofilm and act as seed population that immediately recovers its activity in other places when detached (Gieseke *et al.*, 2003). Therefore, both NOB groups may separate their niches in space due to the gradient of nitrite concentrations in biofilm. The hypothesis supported the result that *Nitrobacter* strains were present in high numbers; even though they were lower than those of *Nitrosospira*.

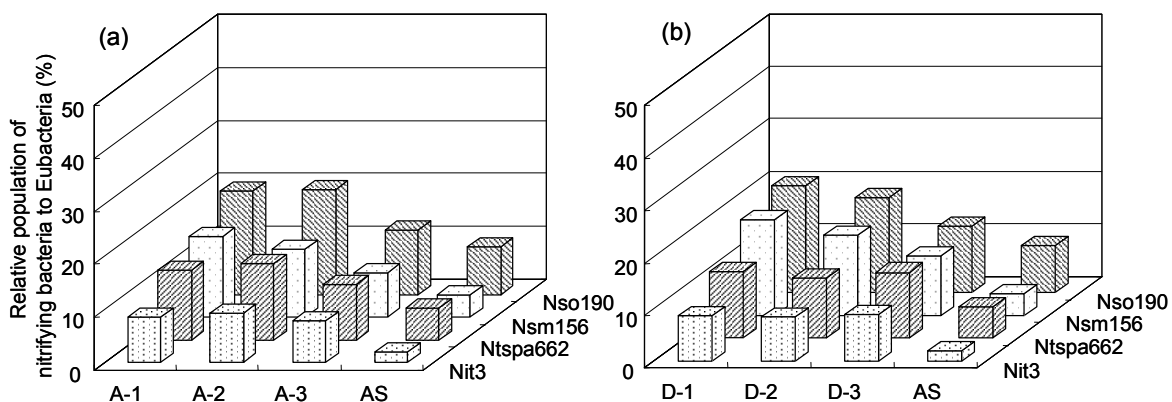


Figure 2. Relative populations of AOB and NOB in the activated sludge used as an inoculum and biofilms under different HRTs. (a) the reactors to treat artificial wastewater (b) the reactors to treat domestic wastewater.

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