Development of nitrogen removal system for freshwater breeding in aquarium

Nagaoka University of Technology（学） ○Nur Adlin, Watanabe Takako, Hatamoto Masashi, Yamaguchi Takashi

1. Introduction

The application of recirculation aquaculture system (RAS) is becoming a trend. Not only for fish breeding industry, this recirculating aquaculture system is also applicable to zoos and aquariums worldwide. Generally, in order to maintain the water quality in an aquarium, it is common to perform 10~20% water exchange of tank capacity to reduce the toxicity of NH_4^+-N. Although treating and reusing the water by nitrification can reduce the toxicity of NH_4^+-N, it is still problematic to reduce the accumulation of nitrate nitrogen (NO_3^--N) in the water. Since freshwater animal are reported to be more sensitive than seawater animals, the accumulation of NO_3^--N conc. more than 30 mg-N/L can bring an adverse effect to them. In this study, we had developed a biological nitrogen removal system for freshwater aquarium that consists of Down-flow Hanging Sponge (DHS) and Up-flow Sludge Blanket (USB) reactor that carry both nitrification and denitrification process. The objective of this study is to verify the performance of DHS-USB system in removing nitrogen components in freshwater breeding water. In addition, 16S rRNA gene sequencing of microorganisms in the retained sludge in the reactors was undertaken to identify the microbial communities present.

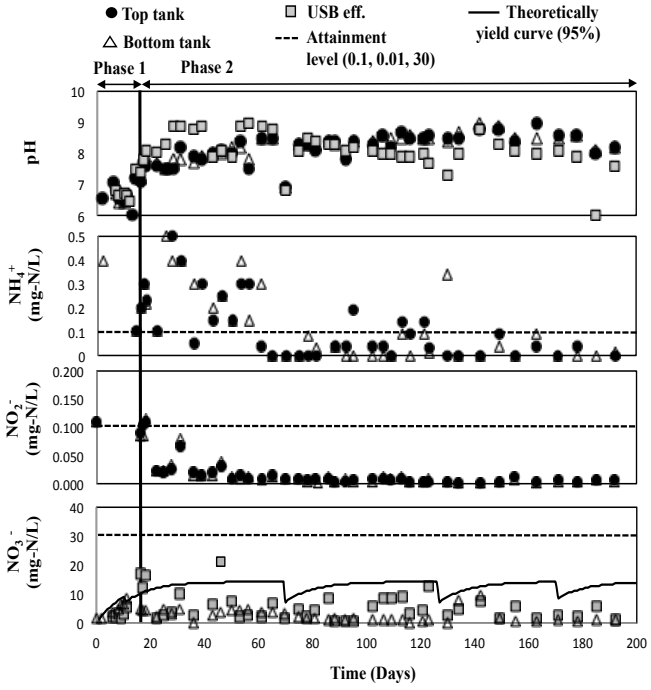

Fig. 1 The change in NH_4^+-N, NO_2^--N and NO_3^--N concentration against day

2. Method & Materials

The system consists of 600 L tank with DHS (volume: 80 L, sponge volume: 12 L, HRT: 0.01 h) and USB (volume: 8 L, HRT: 2.7 h) reactor. Water temperature is maintained between 27℃~30℃, and pH is maintained at 7.0～8.0 The breeding carp (*Cyprinus Carpio*) with density 3.0 kg/m^3 was fed with 0.014 kg/m^3/day commercial solid food. DHS-USB tank undergo two phases throughout the experiments. In phase 1, organic matter was not added but in phase 2, organic matter was added to USB reactor with ratio 7: 3 of sodium acetate and acetic acid. The standard level for this study is (NH_4^+-N: 0.1, NO_2^--N: 0.1, NO_3^--N: 30 [mg/L]) while the addition organic matter quantity is calculated as equation 1.

Addition organic matter quantity (mg-C/L) = gC/gN ratio X inflow NO_3^--N conc. + 0.375 (gC/gO) X inflow DO conc. ...eq. (1)

DNA was extracted using a Fast DNE SPIN Kit for Soil (MP Biomedicals) following the manufacturer's instructions. PCR amplification was performed using universal primers for whole bacteria and archaea for 515F and 806R. Massive parallel 16S rRNA gene sequencing was carried out using Miseq reagent kit v.2 with the Miseq system (Illumina). Sequence data analysis was conducted using QIIME software package v.1.7.0.

3. Results and Discussion

3.1 Water quality

Fig. 1 shows the change in NH_4^+-N, NO_2^--N and NO_3^--N conc. against day. The optimum pH for *Nitrosomonas* that is the ammonia oxidation bacteria is between pH 8.0~9.0, while *Nitrobacter,* nitrite-oxidizing bacteria is around pH 7.5[2)]. Therefore, pH was maintained at 7.9 ± 0.4 throughout this experiment. Besides, in comparison of Phase 1 and Phase 2, a decrease in NO_2^--N conc. in Phase 2 after the addition of organic matter can be seen. Thus, other than the ability to control the pH level, the influence of organic matter in ammonia oxidation and nitrite oxidation process can also be confirmed. Water temperature is maintained at 28.2 ± 1.7℃ and DO was maintained at 5.0 mg-O_2/L and above. In comparison of Phase 1 and Phase 2, a decrease in NO_2^--N conc. in phase 2 after the addition of organic matter can be seen. Thus, other than the ability to control the pH level, the influence of organic matter in denitrification processes can also be confirmed. In addition, water was changed 4 times throughout the experiment. At day 11, 20% of the tank water capacity was changed and for the rest 3 times 50% of the tank water capacity was changed. At day 11, 20% of tank water capacity was exchange because of high ammonia conc. in the tank. This is due to low presence of microorganisms in DHS reactor that causes ammonia conc. at day 11 quite high. DHS reactor became stable after day 60. Then, for the rest 3 times, 50% water exchange was done. This is for diluting the color of the water in the tank. Color of water in the tank changes over time due to excess feeding material provided to the fishes. The average NH_4^+-N and NO_2^--N concentration were maintained under standard level (NH_4^+-N: 0.1, NO_2^--N: 0.1 [mg/L]) by (NH_4^+-N: 0.10 ± 0.09 and NO_2^--N: 0.01 ± 0.01 [mg/L]) while NO_3-N was also kept below 30 mg/L. Based on Fig.1, NO_3^--N

Keywords: nitrogen removal, water quality, aquarium
Contact details: Nagaoka University of Technology, 〒940-2188 新潟県長岡市上富岡町 1603-1 TEL：0258-46-6000

conc. is seen much lower than theoretical yield. This might has been due to increase in the conc. of the organic resulted from excess feed material that has decomposed during the experiment.

3.2 Microbial community analysis

Fig. 2 and 3 shows the abundance of microbial community in both USB and DHS reactors. In both USB and DHS reactors, *Proteobacteria* is the most dominant community. The bacterial genera responsible for the oxidation ammonia and nitrite such as *Nitrosomonas* and *Nitrospira* that is also a chemolithoautotrophic member of the phylum *Proteobacteria* was detected in DHS reactor. The outstanding result with 97% ammonia removal in DHS reactor shows that nitrite-oxidizing bacteria such as *Nitrospira* and ammonia oxidizing archaeans such as *Nitrososphaera* in DHS sponge has effectively converts ammonia to nitrites and nitrates over the total operational period. Their abundancy of *Proteobacteria* in this study increases from 31.0% on day 64 to 36.3% on day 102. However, it reduces to 13.4% on day 163, suggesting that it has been outcomes by *Cyanobacteria*. They are mostly originated from the algae that flow from top tank straight to DHS reactor. *Nitrospira* has been reported to grow best at 28~30℃[3]. The presence of *Nitrospira* can be observed in this study because average temperature in this study was maintained at favourable condition for *Nitrospira* growth that was 28.2 ± 1.7 ℃. However *Nitrobacter,* another important nitrite oxidizing bacteria was not detected anywhere in DHS reactor. Besides, prefers higher salinity condition, *Nitrobacter* also prefers pH around 7.5 while the average pH level in this study is 7.9 ± 0.4 that is slightly higher for their optimum growth[4]. In USB sample, *Cyanobacteria* did not outcome *Proteobacteria.* This is likely due to the algae in top tank did not flow directly into the reactor. It is also interesting to observe sulfur-oxidizing bacteria, *Desulforhabdus amnigenus* belonging to *Proteobacteria* was detected in USB reactor. These bacteria grow optimally with the presence of acetate. The used of sodium acetate and acetic acid as external carbon source for USB reactor may have promote the growth of this bacteria. The presence of nitrifier and denitrifier has enhanced the performance of the reactor.

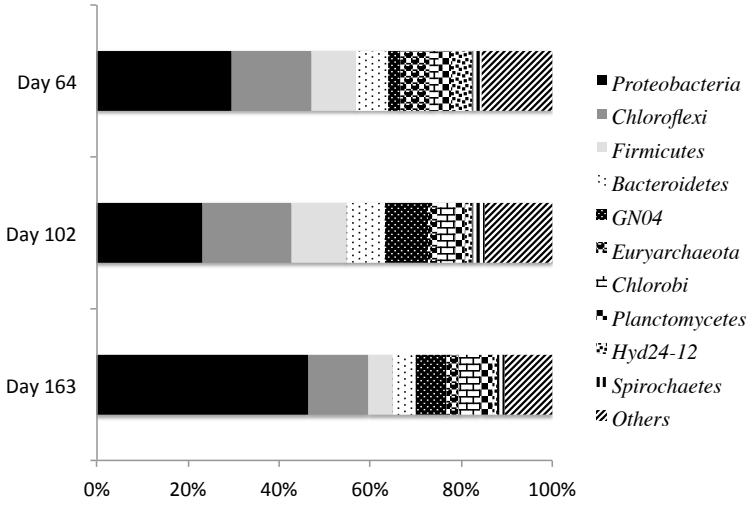

Fig. 2 Microbial community composition in USB (phylum)

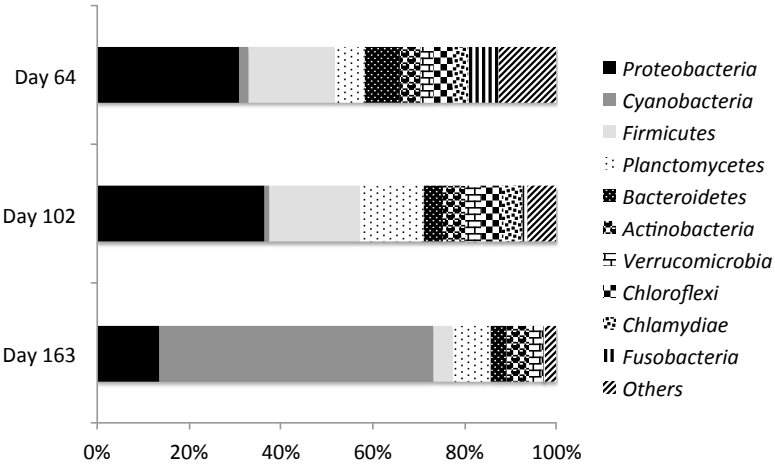

Fig. 3 Microbial community composition in DHS (phylum)

4. Conclusion

Based on the result from Fig. 1, we can conclude that nitrogen removal performance under conditions of 2% DHS and 1% USB tank capacity is proven suitable in a fresh water breeding. We had maintained an average of NH_4^+N, NO_2^--N and NO_3^--N concentrations at 0.10 ± 0.09 mg/L, 0.01 ± 0.01 mg/L and 1.93 ± 2.17 mg/L that are below standard level for freshwater breeding. Besides, we can also say that the presence of nitrifying and denitrifying bacteria in the reactors has influenced the performance of reactors over time. DHS-USB system is suggested to be an effective and efficient system for freshwater breeding.

References:

1. 濵口威真ら:海洋生物飼育を対象とした循環型窒素除去システムの開発,環境工学研究論文集,Vol. 47, pp. 297-302, 2013

2.Guisasola, A. et al: Inorganic carbon limitations on nitrification: Experimental assessment and modeling, Water Research Vol. 41, pp. 277-286, 2007

3. Off. S et al: Enrichment and Physiological Characterization of a Novel *Nitrospira*-Like Bacterium Obtained from a Marine Sponge, Applied Environment Microbiology, Vol.76, No. 14, pp. 4640-4646, 2010

4.Moussa M.S, et al: Long term effects of salt on activity, population structure and floc characteristics in enriched bacterial cultures of nitrifiers, Water Research, Vol. 40, No. 7, Pages 1377–1388, 2006