

Effects Of Feeding Cycles On The Microbial Community In The Biofilter Of A Recirculating Aquaculture System

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Extended Abstract

Water purification filters in recirculating aquaculture systems provide a microbiologically rich environment. They are colonized by groups similar to those in wastewater treatment plants. They contain numerous nitrifying bacteria: ammonia oxidizing bacteria and archaea (AOB and AOA) and nitrite oxidizing bacteria (NOB), which oxidize toxic ammonia to harmless nitrates [1]. In the next phase, denitrifying bacteria convert nitrates into dinitrogen gas. As the well-being of the fish bred in the RAS system must be guaranteed, it is equally important to maintain the right bacterial community in the filter. The bacterial community is influenced by many factors for example dissolved oxygen concentration, temperature or the concentration of nitrogen compounds in influent water [2]. In fish farming, the concentrations of these compounds rise and fall cyclically, which is due to the cyclical feeding of the fish. In the period after feeding, the concentration of organic compounds and fish metabolites in the incoming water increases significantly. Shortly before feeding, however, the concentration of these compounds can be much lower. Such fluctuations can have a significant influence on the bacterial community of the biofilter. The aim of this study was therefore to identify the main bacterial groups responsible for the transformation of nitrogen compounds in the biofilter of the RAS system and to determine the extent to which fluctuations in the composition of the influent affect their activity and abundance.

All samples were taken from an external EHEIM filter that filtered the water from an indoor rearing tank. The typical water temperature in the tested system was (mean \pm standard deviation) $18.7\text{ }^{\circ}\text{C} \pm 0.2$ and the dissolved oxygen concentration was $8.7\text{ mg/l} \pm 0.3$. The fish in the aquarium of the tested RAS system were fed every third day with a dose of about 2% of their weight. Biomass samples from the filter were taken the day before and the day after feeding. Samples of the water flowing into and out of the filter were taken at the same time as the biomass samples to determine the concentration of the individual nitrogen compounds. The bacterial community was analyzed by shotgun metagenomics and metatranscriptomics.

178 bacterial and archaeal phyla and 3741 genera were identified in the analyzed metagenomes. In all samples, the most abundant phyla were *Proteobacteria* (55-70 %) and *Bacteroidetes* (8-14 %). Bacteria of the genera *Nitrospirae*, *Verrucomicrobia*, *Planctomycetes*, *Actinobacteria* and *Acidobacteria* were also quite common (2 - 5 %), although with different frequencies in individual samples. Statistically significant differences were observed for the bacterial community at the genus level. The pre-feeding samples contained significantly more *Aeromonas* and *Mycobacterium* bacteria, while the post-feeding samples contained more *Pseudomonas* bacteria (White's non-parametric t-test). In addition, all samples contained numerous bacteria of the genus *Nitrospira* (3.3% – 5.2%), which are the only representatives of nitrifying bacteria. There were also numerous representatives of denitrifiers, such as *Flavobacterium* and *Acidovorax*. Alpha diversity was analyzed using the Chao1 and Shannon_H biodiversity indices. The decrease in the values of these coefficients in the "After feeding" group indicates that in the post-feeding periods some bacterial groups gained predominance and their numbers in the population increased at the expense of a decrease in the numbers of other groups. However, Permutation multivariate analysis (PERMANOVA) based on the Bray–Curtis dissimilarity matrix showed no statistically significant differences between the population before and after feeding (Bonferroni corrected p-values: 0.33). Genes and transcripts related to nitrification, denitrification and dissimilatory nitrate reduction were abundant in all samples. Comammox *Nitrospira* was the only microorganism responsible for nitrification. Its activity was highest before feeding, when the ammonia concentration

was lower. In the samples after feeding, the abundance of genes and transcripts related to denitrification and dissimilatory nitrate reduction increased.

The microbial population in the biofilter shows a relatively stable composition despite cyclic changes in nitrogen compound concentrations. However, the activity of these microorganisms fluctuates with the feeding cycles, highlighting the dynamic nature of microbial processes in response to environmental changes. This changes underscore the functional adaptability of the microbial community in maintaining water quality in RAS.

References

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