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Nitrifying biofilms critical for water quality in intensive shrimp RAS

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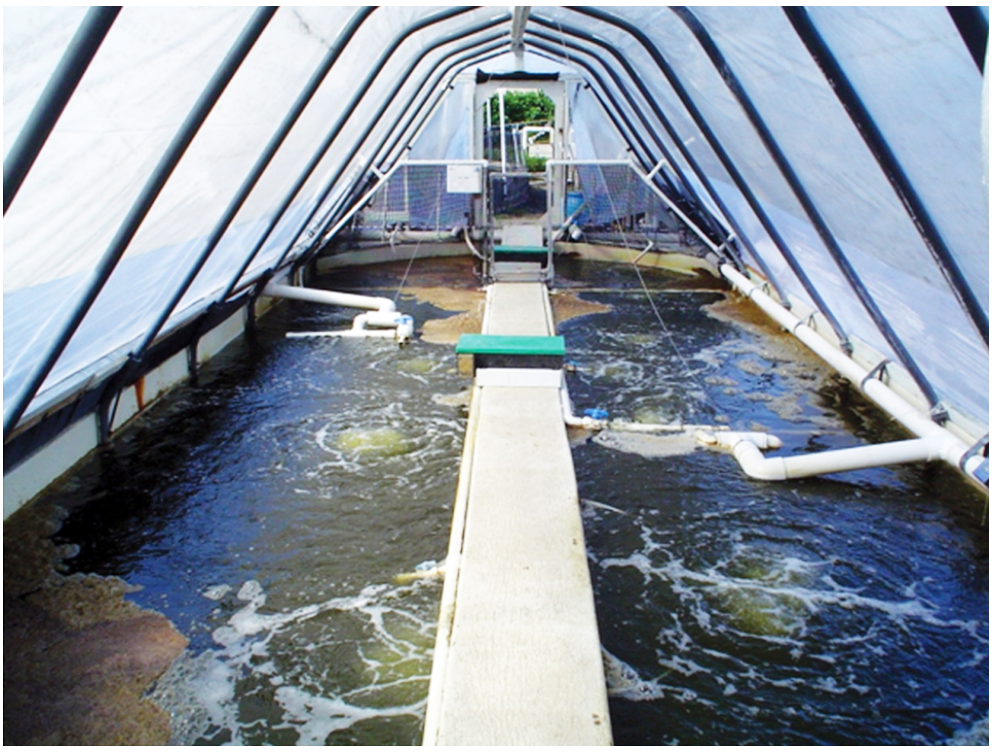
Genetic diversity and elevated rates of nitrification illustrate the critical importance of biofilms



The Oceanic Institute in Hawaii, USA, uses RAS systems for the high-intensity production of *Litopenaeus vannamei.*

The use of recirculating aquaculture systems (RAS) for shrimp represents a major paradigm shift from current methods that rely on open, coastal ponds and flow-through water exchange to maintain water quality. RAS rely on the biogeochemical capabilities of the in situ microbial community to provide acceptable water quality for the target species.

In super-intensive RAS where little to no water is exchanged, nitrification and uptake of dissolved inorganic nitrogen (DIN) by phytoplankton are critical for the detoxification and removal of ammonia and nitrite. However, interrelationships among various functional groups within the microbial community are complex and poorly understood.

Because of this complexity, optimal microbial community nitrogen cycle functions have not been well characterized for RAS. Critical control variables that promote high rates of ammonium oxidation, nitrite oxidation and phytoplankton DIN have yet to be elucidated for super-intensive aquaculture. A clear understanding of these control variables and their implementation in RAS management strategies will be highly effective in promoting shrimp growth and survival under environmentally sound conditions.

Oceanic Institute study

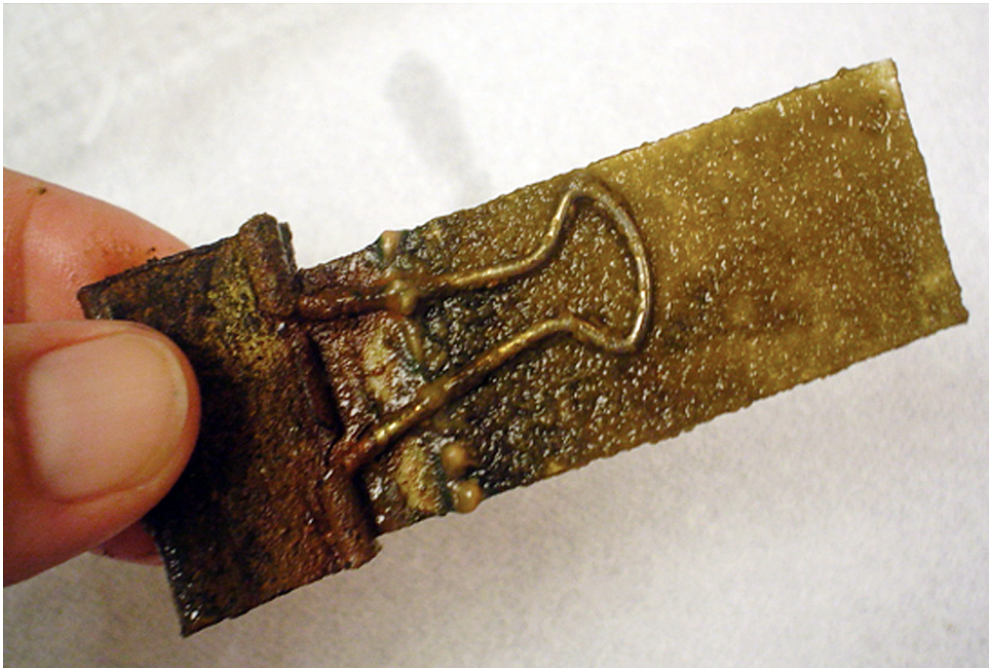
With the support of the U.S. Marine Shrimp Farming Program and the Hawaii Pacific University Trustees Scholarly Endeavors Program, scientists at the Oceanic Institute have measured ammonia removal and oxidation rates in water column samples over the course of several shrimp production trials in super-intensive RAS.

Results indicated that the ammonia oxidation in water column samples ranged from 0.92 to 2.60 µmole/L/hour for a mean of 1.7 ± 3.4 µmole/L/hour. This rate exhibited little variation after the initial acclimation period.

However, nitrogen budget analysis based on the production and accumulation of nitrate, the end product of nitrification, suggested that ammonia oxidation in the water column at these rates cannot account for the accumulation of nitrate over time. Therefore, nitrification must be occurring in locations other than on water column particles. Nitrifying bacteria readily form biofilms on surfaces, and colonization by these important bacteria on the interior walls of the RAS production unit likely provided an additional source of nitrification.

To investigate this, the authors submerged microscope slides in the RAS water column to allow the formation of biofilms. The time points encompassed the initial acclimation period as well as after ammonia and nitrite concentrations became stable. Because full nitrification of ammonia to nitrate is critical to RAS water quality, these time points allowed researchers to ascertain the arrival and proximity of ammonia and nitrite oxidizers to each other in space and time.

These filmed slides were subsequently exposed to ^{15}N-ammonia or ^{15}N-nitrite during three-hour incubations. Changes in the concentration of ammonia, nitrite and nitrate were measured, and uptake of the ^{15}N label into the biofilm itself was measured to quantify phytoplankton and heterotrophic uptake. The authors also conducted fluorescent in situ hybridization (FISH) assays and extracted DNA from the biofilm samples to determine key groups of nitrifying organisms and relate them to the measured nitrification rates.



Submerged microscope slides were colonized by the RAS microbial community.

Results

Interestingly, the first incubation on day 5 indicated not oxidation, but production of both ammonia and nitrite over the course of the three-hour incubation. However, volumetric rates of ammonium oxidation by biofilms in all subsequent incubations ranged from 0.18 to 1.7 µmole/L/hour with a mean of 0.88 ± 0.69 µmole/L/hour over the entire course of the shrimp grow-out.

The mean volumetric rate of biofilm nitrite oxidation ranged from 0.16 to 1.8 µmole/L/hour with a mean of 0.71 ± 0.77 over the course of the grow-out. Therefore, mean volumetric rates of ammonia oxidation by biofilms were not significantly different from rates measured in water column particles. Similar volumetric rates may reflect the fact that both the $^{15}NH3$ amendment at 10 µmole/L and the incubation conditions were held constant for both water column and biofilm incubations, and may well reflect potential, rather than actual rates of oxidation on any given day.

Despite the similarity in volumetric rate measurements, when the rate of ammonia oxidation by a biofilm colonizing 34-square-centimeter slide was scaled up to the surface area of the interior RAS walls, 24 square meters, ammonia oxidation would be nearly 60 times the rate of the water column particles.

This is likely an overestimation, as this calculation assumes that the entire surface area of the RAS is uniformly covered by the biofilm and that the entire surface area will oxidize ammonia at the same rate. However, potential ammonia oxidation by biofilms appears to be more than enough to account for nitrate accumulation over time and allows us to close the nitrogen budget for RAS.

FISH analyses on disaggregated biofilm samples indicated the quantifiable presence of ammonium-oxidizing □-proteobacteria, nitrite-oxidizing bacteria from the genus Nitrospira and bacteria of the order Planctomycetales. The latter contains the anaerobic ammonium-oxidizing bacteria. These groups were present throughout the grow-out trial after the first incubation on day 5.

Ongoing DNA extractions as informed by the FISH analyses indicate the presence of a highly diverse suite of ammonia-oxidizing bacteria and Archaea. Taxon-specific primers, which indicate the presence of potential ammonia- or nitrite-oxidizing organisms, were positive for ß-proteobacteria *Nitrosomonas* or *Nitrosospina, Nitrobacter, Nitrospina*, bacteria of

the order Planctomycetales and of the phylum Nitrospira, and Group 1 Archaea. All of these bacteria and Archaea were present in biofilm samples as early as four weeks into the shrimp grow-out.

The authors have also used enzyme-specific primers that explicitly indicate the presence of the gene that codes for the enzyme ammonia mono-oxygenase, which catalyzes the oxidation of ammonia to nitrite, the first step in nitrification. Polymerase chain reaction test results indicated that this gene was present three weeks into the grow-out for ß-proteobacters and Group 1 Archaea, and four weeks into the grow-out for these two groups as well as the ß-proteobacter *Nitrosococcus*.

Perspectives

Clearly, there is great diversity in the ammonia oxidation pathway in naturally occurring biofilms in recirculating aquaculture systems, which denotes functional redundancy of nitrification and ammonia oxidation, in particular, and demonstrates the potential resiliency of the microbial nitrifying pathway. Genetic diversity combined with elevated rates of nitrification illustrate the critical importance of biofilms for water quality remediation in super-intensive shrimp RAS.

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