# COMPARISON, IDENTIFICATION, AND ROLE OF MICROBIAL COMMUNITIES IN RECIRCULATING SYSTEMS IN THE NORTH CENTRAL REGION[10]

Project ***Termination Report*** for the Period September 1, 2009 to August 31, 2011

**NCRAC FUNDING:** $65,000 (September 1, 2009 to August 31, 2011)


**PARTICIPANTS:**

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**REASON FOR TERMINATION**

Project completed.

**PROJECT OBJECTIVES**

(1) Characterize the microbial communities in established production scale marine and freshwater recirculating aquaculture systems (RAS). These systems have been operational and producing aquatic organisms for more than one year.

(2) Once these microbial communities have been identified, the role(s) of these microbial communities within the nitrogen cycle will be quantified with the goal of increasing the efficiency of the RAS (increased survival, growth, and density, etc. of aquatic organisms).

(3) Coordinate the results of this project with the Technical Committee Extension Subcommittee of NCRAC.

[10]This 1-year funded project was chaired by Lutgarde M. Raskin and began September 1, 2009. In other areas of this report, this project is referred to as RAS Microbial Communities.

**PRINCIPAL ACCOMPLISHMENTS**

*OBJECTIVE 1*

Freshwater Recirculating Aquaculture Systems (RAS)

One of the recirculating systems is located at the Great Lakes Water Institute at the University of Wisconsin-Milwaukee (UW- Milwaukee). This indoor, freshwater RAS, housing approximately 10,000 yellow perch, has been in operation for approximately 10 years. The system is comprised of a production tank housing the yellow perch, followed by an ozonation step, pH correction using sodium bicarbonate, a floating plastic bead filter designed for solids removal, a fluidized bed sand filter, and a whiffle ball aerator. The plastic bead filter and the sand filter were analyzed for biological activity.

DNA was extracted from biomass samples obtained from both the plastic bead filter and the sand filter of the freshwater RAS. Clone libraries were generated targeting the 16S rRNA gene of *Bacteria*, *Archaea*, and *Planctomycetes*.

The bacterial clone libraries for the plastic bead filter and the sand filter resulted in 231 and 239 sequences, respectively. The plastic bead filter contained a diverse bacterial community. It was primarily comprised of *Alphaproteobacteria*, *Acidobacteria*, and *Planctomycetes*. However, it contained members of the *Actinobacteria, Verrucomicrobia, Bacteroidetes, Oputitae, Nitrospira, Deinnococcus, Fusobacteria, Flavobacteria, Betaproteobacteria, Gammaproteobacteria,* and *Deltaproteobacteria*. The bacterial community in the sand filter was less diverse. The *Alphaproteobacteria* made up over 50% of the sequences in this library. This community also contained members of the *Acidobacteria, Planctomycetes, Sphingobacteria, Actinobacteria, Verrucomicrobia, Bacteroidetes, Nitrospira, Deinnococcus, Flavobacteria, Betaproteobacteria, Gammaproteobacteria,* and *Deltaproteobacteria*. Surprisingly, no ammonia oxidizing bacteria (AOB) were detected in either of the clone libraries, whereas nitrite oxidizing bacteria (NOB) were contained with the phylum *Nitrospira*.

The archaeal clone libraries for the plastic bead and sand filters contained 47 and 60 sequences, respectively. The archaeal diversity in both of the filters was low. The detected *Archaea* belonged almost exclusively to the *Thermoprotei*. No ammonia oxidizing archaea (AOA) were detected in either filter.

*Planctomycetes* clone libraries were constructed specifically to detect the presence of anaerobic ammonia oxidizing (anammox) bacteria. The clone libraries for the plastic bead and sand filters contained 78 and 37 sequences, respectively. The plastic beads contained members of the genera *Planctomyces, Zavarzinella, Pirellula, Rhodopirellula,* and *Blastopirellula*. The sand filter community contained members of the *Planctomyces* and *Blastopirellula.* None of these genera contain any known species that perform the anammox metabolism.

In order to supplement the clone library data, 454-pyrosequencing was performed, targeting the bacterial and archaeal 16S rRNA genePyrosequencing resulted in 460 and 248 bacterial sequences for the plastic bead and sand filter samples, respectively. The plastic bead filter was comprised primarily of *Alphaproteobacteria*, which made up more than 50% of the sequences. Other groups that were present were *Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Actinobacteria, Acidobacteria, Planctomycetes, Nitrospira,*

*Fusobacteria, Bacteroidetes, Chloroflexi, Firmicutes*, and *Deinnococcus-Thermus*. The sand filter contained *Alphaproteobacteria, Bacteroidetes, Acidobacteria,* and *Nitrospira* at high abundances. The remainder of the community was comprised of *Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria, Verrucomicrobia, Gammatimonadetes, Planctomycetes, Deinnococcus-Thermus, Actinobacteria,* and *OP10*. No AOB were detected in either sample. In both samples, *Thermoprotei* was the only archaeal group that was detected and classified.

Marine RAS

The marine RAS located at Seafood Systems, Inc. in Okemos, Michigan consists of nine culture tanks in series followed by a trickling filter. Because the facility is located away from the coast, artificial seawater was prepared from a commercial salt solution. The marine RAS relies on biofiltration in a multi-stage, nitrifying trickling filter that contains two types of biofilm attachment media: plastic bioballs and crushed oyster shells. Microbial biomass from five biofilters and water samples from the effluent of the culture tanks and biofilter compartments were collected on August 10, 2010. The first five compartments contained bioballs, while the last compartment contained oyster shells.

At the time of sampling, the pH in the system ranged from 8.52–8.67. The alkalinity in the system ranged from 308–470 mg/L as $CaCO_3$, and the total chemical oxygen demand (COD) ranged from 44–63 mg/L. The ammonia concentrations were very low (in the µg-N/L range) and showed a generally decreasing trend across the tanks and the biofilter compartments. Both the ammonia and nitrite concentrations were below the toxicity level.

The composition of the microbial community in the biofilter compartments was determined by analysis of the bacterial 16S rRNA gene sequences obtained from 454-pyrosequencing. Analysis of the sequences revealed that the most abundant groups at the phylum level were the *Bacteroidetes*, *Nitrospira*, *Planctomycetes*, and *Proteobacteria*. Populations of interest within the microbial community included AOB, anaerobic ammonia oxidizing (anammox) bacteria, NOB, and *Vibrio* species. AOB of the genera *Nitrosococcus* and *Nitrosospira* were present. The abundance of AOB in all compartments was less than 1% of sequences analyzed. NOB from the phyla *Proteobacteria* and *Nitrospira*, genera *Nitrobacter,* and *Nitrospira*, respectively, were detected. The most abundant NOB in all compartments were the nitrite-oxidizing *Nitrospira*. All sequences in the *Nitrospira* phylum were nitrite-oxidizing NOB. *Nitrospira* were the dominant NOB at the time of sampling, which was likely due to the low nitrite concentrations in the biofilter. Also, *Vibrio* species were not detected in any of the samples. The genus *Vibrio* is of importance because it contains shrimp pathogens, specifically *Vibrio harveyi*.

*OBJECTIVE 2*

The freshwater RAS was monitored for various water quality parameters, including ammonia, nitrite, nitrate, COD, alkalinity, total suspended solids (TSS), and volatile suspended solids (VSS). Samples were taken at five points throughout the system: the production tank, the pH adjustment tank, the plastic bead filter effluent, the sand filter effluent, and the whiffle ball aerator effluent. The total ammonia nitrogen (TAN) and nitrite concentrations were consistently removed to levels of 0.05 mg $NH_4^+$-N/L and 0.2 mg $NO_2^-$-N/L, respectively.

To determine the nitrification capacity and the ability of the system to respond to a high ammonia concentration, the system was spiked with ammonia and the concentrations of ammonia and nitrite were monitored over the course of several hours. The ammonia concentration decreased from 6.3 mg/L $NH_4^+$-N (450 μM) to less than 0.35 mg/L $NH_4^+$-N (25 μM) in just under two hours and a significant reduction in ammonia concentration was observed within the first half hour. The nitrite concentrations were initially 0.003 mg/L $NO_2^-$-N (0.2 μM) but increased to just over 0.014 mg/L $NO_2^-$-N (1 μM) within the first hour. The concentration stabilized at this level and decreased to pre-spike levels within 2.5 h. These data indicate that the nitrification capacity of this system is sufficient to allow the system to respond to high ammonia concentrations. This suggests that the system would be able to respond to an ammonia shock or could accommodate denser fish stocking or a higher feed rate in the production tank.

Batch experiments were also conducted on the media from both the plastic bead filter and the sand filter to determine the ammonia and nitrite oxidizing activity of the biologically active media. In these experiments, media (either plastic beads or sand) was sampled from the biological filters, placed in bottles with water sampled from the system and spiked with either ammonia or nitrite. For the bottles spiked with ammonia, ammonia and nitrite concentrations were measured to determine how quickly the ammonia was removed and whether there was nitrite buildup in the water. In the assay with biofilter sand, the ammonia level dropped from an initial concentration of 13.3 mg/L $NH_4^+$-N (950 μM) to below 2.8 mg/L $NH_4^+$-N (200μM) in 40 h and began to level off. After 130 h, the ammonia level was down to 1.8 mg/L $NH_4^+$- N (130 μM). The nitrite concentration in this assay remained less than 0.02 mg N/L for the duration of the experiment. Ammonia and nitrite oxidation rates of the biofilter sand were proportional to the amount of sand added to the batch experiment, with the rates increasing linearly with increased sand concentration.

Similar experiments were performed using plastic beads from the floating bead filter. For the beads, the ammonia was removed almost as quickly as in the assay with the sand. However, the nitrite was not removed quickly enough to counteract the nitrite production from ammonia oxidation, resulting in a buildup of nitrite in the system. Based on these experiments, one can conclude that both the sand and the plastic beads have some ammonia-oxidizing capacity. However, the sand has significantly greater capacity for ammonia oxidation than the beads. This is consistent with the results from the monitoring of the RAS. Also, given that there was no nitrite buildup in the sand experiments and that the plastic beads had little nitrite oxidation capacity, one can conclude that the sand filter is the primary location of nitrite oxidation. The clone library analysis of the two filters showed similar abundances of *Nitrospira* in both the plastic bead and sand filters (3.1 and 3.5%, respectively). However, pyrosequencing revealed a much greater abundance of *Nitrospira* in the sand filter (12.9%) versus the plastic bead filter (3.5%). This supports the finding in the batch experiment that the sand is primarily responsible for nitrite oxidation.

Unfortunately, the marine RAS system was not available during the time period for which experiments were planned for nitrification capacity experiments.

*OBJECTIVE 3*
Information garnered from this project will be reviewed by members of the Extension Workgroup in developing possible new materials for the industry.

**IMPACTS**

- Improve knowledge of nitrogen cycle in RAS and generate means for stable management of toxic nitrogen compounds.
- Understand how operational changes (e.g., backwashing, change in ammonia load) affect biofilter operation.
- Characterize the microbial communities in the two biological filters.
- Improve economic viability of RAS by finding ways to improve process efficiency (i.e., underutilized nitrification potential suggests that a greater stocking density or a larger production tank is possible for the current water treatment system).
- In collaboration with a Mathematics- Biology Initiative at UW-Milwaukee, an undergraduate student has made progress on a model using physical characteristics of the RAS, flow rates, bench-derived process rates, and time series chemical analysis.

**RECOMMENDED FOLLOW-UP ACTIVITIES**
To better understand the microbial community dynamics in a RAS, it would be necessary to perform a detailed assessment of the abundance and activity of ammonia and nitrite oxidizers in biofilters over an extended time period. To optimize the efficiency of this type of RAS, it would be important to understand the stability of the system under higher ammonia loadings. This could provide information to investigate the possibility of increased stocking densities and thus greater economic efficiency. It would also be useful to understand the system's response to spikes in ammonia or nitrite loading to evaluate its ability to respond to sudden changes in water quality and protect the fish from acute toxicity.

**SUPPORT**
NCRAC has provided $65,000 which is the total amount allocated for this project.

**PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED**
See the Appendix for a cumulative output for all NCRAC-funded RAS Microbial Communities activities.