COMPARISON, IDENTIFICATION, AND ROLE OF MICROBIAL COMMUNITIES IN RECIRCULATING SYSTEMS IN THE NORTH CENTRAL REGION

Chairperson: Lutgarde M. Raskin, University of Michigan

Industry Advisory Council Liaison: Russ Allen, Okemos, Michigan

Extension Liaison: Mark Clark, North Dakota State University

Funding Request: $65,000

Duration: 1 Year (September 1, 2009 - August 31, 2010)

Objectives:

1. Characterize the microbial communities in established production scale marine and freshwater recirculating aquaculture system (RAS) units. 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.

Proposed Budgets:

Institution	Principal Investigator(s)	Objec-tive(s)	Year 1	Total
University of Michigan	Lutgarde M. Raskin & James S. Diana	1, 3	$32,517	$32,517
University of Wisconsin-Milwaukee	Russell L. Cuhel & Carmen Aguilar	2	$32,483	$32,483
Totals			**$65,000**	**$65,000**

Non-funded Collaborators:

Facility	Collaborator
Seafood Systems Inc.	Russ Allen
Great Lakes WATER Institute	Mark Clark

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JUSTIFICATION

Aquaculture of edible fish and shellfish can become a tremendous asset to the economy of the North Central U.S. and fill a need no longer supported by the natural environment. Small business in this field can be profitable, but factors contributing to stability and resilience need to be studied for the industry to be sustainable, i.e., profitable in the long term. The first step in this direction is to establish a thorough understanding of how closed-system aquaculture and each of its components operates. Biofiltration systems for removing toxic ammonia and nitrite comprise the core of recirculating aquaculture systems (RAS).

Indoor shrimp aquaculture is an infant industry in the United States. The bulk of shrimp product imports into the U.S., valued at $3.8 billion in 2003 (Harvey 2006), originate from countries with warm climates and extensive coastlines. The industry has gone through dramatic boom and bust cycles, which is a pattern not indicative of sustainable growth (Arquitt et al. 2005). There is potential for the industry to expand in the U.S., but limitations imposed by climate and land cost dictate that indoor operations away from the coasts be considered. This will require (i) a greenhouse or indoor system with heating and temperature control, (ii) the availability of local brackish water sources or the artificial preparation of salt water, and (iii) water treatment to allow recycling of the water. Using currently available technologies, the energy and material costs of such a system exceed those of outdoor production facilities in warmer areas with ready access to salt water. An economic feasibility study (Van Wyk 1999) concluded that shrimp from recirculating aquaculture cannot directly compete with imported shrimp in the wholesale frozen market. It is clear that developing a viable indoor shrimp aquaculture industry in the U.S. will require operations to be highly efficient, predictable, and stable in order to compete with inexpensive imports. One way to achieve this goal will be through microbial resource management (Verstraete et al. 2007).

The water in RAS is treated in biofilters, which are expensive compared to other technologies (Gutierrez-Wing and Malone 2006). Therefore, operating RAS may become profitable only when biosecurity is a prime concern and the final product is of high value (such as is the case for shrimp and yellow perch). The heart of an RAS facility lies in the biofilters, which convert ammonia excreted by fish and shellfish to nitrate through nitrification. The process is carried out by chemoautotrophic nitrifiers, i.e., microbes that fix their own C while obtaining energy by oxidizing ammonia (ammonia oxidizing bacteria [AOB] or ammonia oxidizing archaea [AOA]) or nitrite (nitrite oxidizing bacteria [NOB]). The reliance of RAS on chemoautotrophs is also its weakness, because the microbes that accomplish this task encompass a narrow phylogenetically range, are slow growing, possess limited metabolic versatility, and are sensitive to a variety of environmental factors (Prosser 1989).

There also remain incompletely answered questions regarding nitrogen toxicity to fish and shellfish, as either ammonia or nitrite, and the mechanisms leading to their excessive appearance. This research will directly and systematically address crucial aspects of biofiltration. The use of a combination of molecular methods to determine microbial community composition and experimental methods to evaluate nitrogen cycling and budgets will provide an opportunity to identify system components favoring specific aspects of microbial nitrogen detoxification. The work will be made accessible to local, regional, and national consumers through the North Central Regional Aquaculture Center (NCRAC) Work Group. Manuscripts will be submitted to aquaculture and environmental microbiology journals. It is the intention of work group participants to stress practical outreach to a developing regional industry.

The proposed research will develop greater practical understanding of the fate of ammonia-nitrogen in RAS with biofilters. Specific goals include (i) determining the loading capacity and resilience of ammonia-oxidizing bacterial biofilters; (ii) determining the lag in response time of nitrite-oxidizing bacteria during high input circumstances; and (iii) assessing relationships among bacterial components and biochemical processes for RAS.

RELATED CURRENT AND PREVIOUS WORK

Chemoautotrophic ammonia removal in RAS makes use of nitrification, which is a two step process in which ammonia is first oxidized to nitrite and then to nitrate by two different microbial guilds: AOB or AOA and NOB. During water treatment, these microbes are allowed to grow on filter media (biofilm attachment media) with high surface areas in so called biological filters or biofilters. After passage through the

biofilters, water is returned to the production tanks, making it a RAS. The sensitivity of AOB and NOB to a wide variety of environmental factors is well known; so much so that nitrification has been regarded as the "Achilles heel" of wastewater treatment (Daims et al. 2006). The same sensitivity has been observed in aquaculture settings, where problems with high concentrations of ammonia and nitrite, low dissolved oxygen levels, pH outside the optimal range for nitrifying microbes (7.5–8.6), and trace amounts of toxic sulfides have been reported (Joye and Hollibaugh 1995; Masser et al. 1999; Ling and Chen 2005). Although some heterotrophic breakdown of organic matter is expected in RAS, heterotrophic bacteria are not actively selected for, because they can out-compete nitrifiers for oxygen and space on biofilters (Michaud et al. 2006). Since their discovery around 100 years ago, the nitrifiers were believed to belong exclusively to the domain *Bacteria*. The AOB encompass a very narrow phylogenetic range and both AOB and NOB display a very limited metabolic diversity. The only known AOB belong to the genera *Nitrosomonas*, *Nitrosospira*, and *Nitrosococcus* (Purkhold et al. 2000). This limited diversity makes this group of bacteria an ideal target for microbial community studies because low community diversity reduces the microbial matrix complexity without ignoring genuine *in situ* community level interactions.

The recent discovery of the existence of AOA has changed the century old dogma that nitrification can be accomplished only by microbes belonging to the domain *Bacteria*. Methanogens and representatives isolated from extreme environments (the so-called extremophiles) are the best recognized microbes belonging to the domain *Archaea*. However, mesophilic *Archaea* of the class *Crenarchaeota* are now recognized as ubiquitous and abundant in a variety of environments (Chaban et al. 2006). Their possible role in ammonia oxidation was first suggested by the detection of the ammonia monooxygenase gene (*amoA*) associated with crenarchaeal DNA in two different metagenomic studies in seawater (Venter et al. 2004) and soil (Treusch et al. 2005). The capability of an archaeon to oxidize ammonia was definitively established with the cultivation of the first AOA, designated *Nitrosopumilus maritimus*, which was isolated from a saltwater aquarium (Konneke et al. 2005). Since then, AOA have been detected (usually by detecting *amoA*) in marine waters and sediments (Francis et al. 2005), soils (Leininger et al. 2006), corals (Beman et al. 2007), and wastewater treatment plants (Park et al. 2006). Recent findings on sponge-associated AOA suggest that they are vertically transmitted, therefore forming a stable association with their host (Steger et al. 2008), despite undergoing prolonged periods of anoxia (Hoffmann et al. 2008).

There is currently no experimental test or procedure to differentiate between ammonia oxidation due to bacterial or archaeal sources. Recent findings show that a traditionally used bacterial nitrification inhibitor nitrapyrin, is also effective against archaeal nitrification (Bayer et al. 2008). However, it is widely recognized that *Archaea* and *Bacteria* differ considerably in antibiotic sensitivities (Mankin and Garrett 1991; Nieto et al. 1993; Yonath 2005). Therefore, the differential inhibition of the two ammonia oxidizing groups can form the basis of a specific test. Because a high degree of variation in antibiotic sensitivities of various members of the *Archaea* can be expected, the genome sequence data of *Nitrosopumilus maritimus*, which was recently completed (http://www.jgi.doe.gov/genome-projects/pages/projects.jsf) will be evaluated although this achievement has not yet been published. Because the sequence is publicly available, this can be searched for genes or domains involved in antibiotic resistance or sensitivity (see below).

The marine RAS (MRAS) in Okemos, Michigan

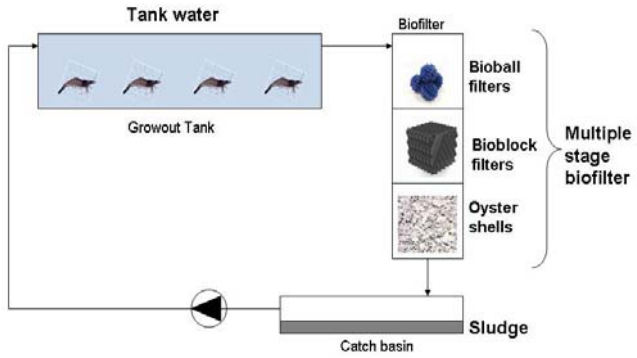

Figure 1. Diagram representing maturation system of Seafood Systems, Inc. in Okemos, Michigan.

The presence of AOA was recently detected in the maturation system of a pilot scale shrimp farm managed by Russ Allen in Okemos, Michigan. The marine RAS (MRAS) relies on biofiltration using a sequence of biofilters containing two different types of biofilm attachment media: plastic bioballs/bioblocks and crushed oyster shells. In this system, water from the production tank is pumped and filtered by gravity first through the bioballs/bioblocks followed by the oyster shells.

Biomass sludge is allowed to settle in a catch basin, from which the water is pumped back to the production tank (Figure 1). Previously described PCR primers (Francis et al. 2005) were used to detect AOA-specific *amoA* in biomass samples collected from each of these attachment media as well as from the water column and sludge collected from a basin underneath the biofilters. The initial PCR result indicated the presence of AOA-*amoA*, and revealed the highest detection signals in the samples obtained from crushed oyster shells (data not shown). These results were confirmed quantitatively in this system by designing quantitative real time PCR primers for the presumptive AOA present in this system (Figure 2). The abundance of archaeal *amoA* in the biomass attached to oyster shells is around 10-fold higher than in the biomass associated with bioballs or in the sludge. No archaeal *amoA* was detected in the water column. Sequence analysis of the archaeal *amoA* PCR products showed that most of the sequences amplified are related to the previously described *Nitrosopumilus* species (Konneke et al. 2005), while other sequence types could not be reliably classified. These results were confirmed by analysis of 16S rRNA genes PCR amplified from the same farm samples (data not shown). 16S rRNA gene clone libraries of *Archaea*, *Bacteria,* and *Planctomycetes* were also constructed. Analysis of the bacterial clone libraries (Table 1) failed to detect any representatives of the known AOB or anaerobic ammonium oxidizing bacteria (anammox bacteria) (Mulder et al. 1995). However, when a PCR experiment targeting Betaproteobacterial *amoA* (Rotthauwe et al. 1997) was conducted, the AOB were faintly detected, but only in bioball samples (data not shown). NOB-like sequences belonging to the *Nitrospirae* were detected in both bioballs and oyster shells (Table 1).

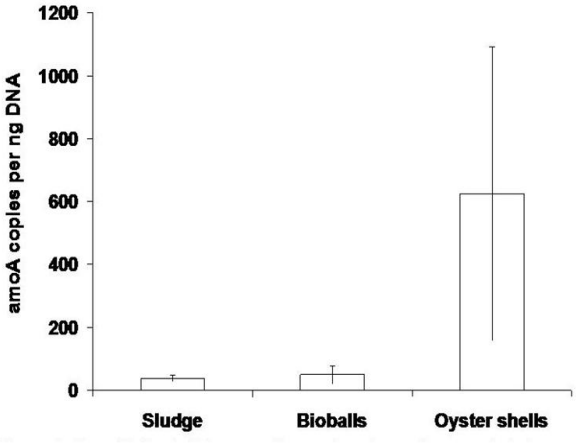

Figure 2. Quantitative PCR measuring archaeal *amoA* gene of sludge and biofilter media in a RAS shrimp farm. No archaeal *amoA* was detected in the water column.

Table 1. Phylum level affiliations of bacteria PCR amplified from different RAS compartments

	RAS compartments			
Phylum level classifications	Water column	Bioballs	Oyster shells	Biofilter catch basin sludge
Bacteroidetes	45.3%	2.9%	6.8%	18.6%
Alphaproteobacteria	29.7%	28.6%	22.7%	19.6%
Betaproteobacteria	N.D.	N.D.	N.D.	1.0%
Gammaproteobacteria	9.4%	17.1%	9.1%	26.5%
Deltaproteobacteria	N.D.	4.3%	5.7%	7.8%
Planctomycetes	1.6%	4.3%	25.0%	2.0%
Firmicutes	N.D.	1.4%	2.3%	8.8%
Actinobacteria	3.1%	4.3%	4.5%	2.0%
Nitrospirae	N.D.	14.3%	3.4%	N.D.
Chloroflexi	N.D.	2.9%	2.3%	2.9%
Chlorbi	N.D.	N.D.	N.D.	1.0%
Verrucomicrobia	N.D.	N.D.	2.3%	1.0%
Gemmatimonadetes	N.D.	N.D.	2.3%	N.D.
unclassified	10.9%	20.0%	13.6%	8.8%
Number of phyla identified	6	10	12	12

No. of clones compared were 64-102
N.D. = not detected

Finding the presence of AOA in a marine RAS demonstrates another level of diversity of microbes involved in nitrification in a RAS using multi-stage biofiltration. Based on this result and the apparent ubiquity of crenarchaeal *amoA* in environments surveyed so far (Francis et al. 2005; Leininger et al. 2006; Park et al. 2006; Beman et al. 2007), this additional diversity is likely important in the N cycle in this engineered system. It is interesting to note that the two types of ammonia oxidizing microbes appear to be spatially separated: AOA were much more abundant in the biomass associated with oyster shells, while AOB were only detected in bioball biofilms. If confirmed, this spatial separation should make future studies on their ecophysiology easier. The sequence data collected will now serve as a useful library to develop a higher throughput method of microbial community profiling such as terminal restriction fragment length polymorphism (T-RFLP) (Marsh 1999; Briones et al. 2007b).

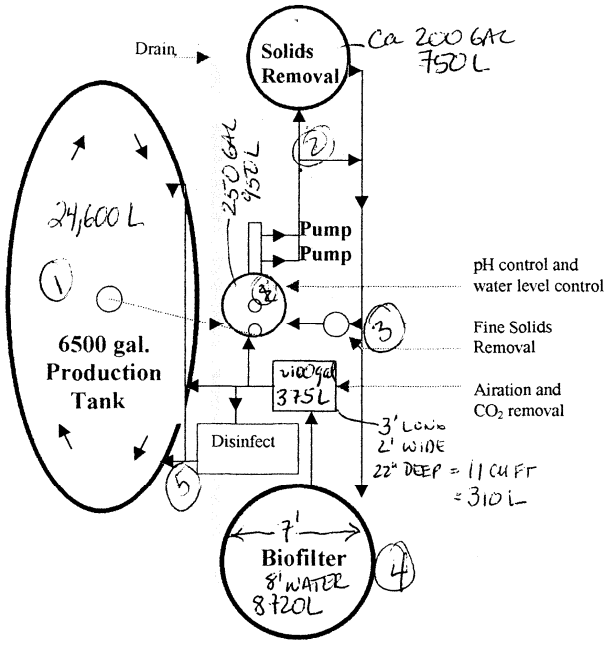

Figure 3. Diagram representing freshwater RAS at UW-Milwaukee Aquaculture Facility

The freshwater RAS (FWRAS) at the University of Wisconsin-Milwaukee (UW-Milwaukee)
In late February 1999 a 35,000-L (9,250 gallons) FWRAS was finished at the UW-Milwaukee Aquaculture Facility and filled with dechlorinated Milwaukee city water (Lake Michigan source). The freshwater system, managed by Fred Binkowski, uses a fluidized sand bed biofilter with a separate plastic bead solids removal component (Figure 3). The solids removal unit is the only zone capable of producing anaerobic conditions suitable for the anaerobic oxidation of ammonia by anammox bacteria; (Mulder et al. 1995). Permission was granted to conduct experiments prior to fish (yellow perch) addition.

To prime nitrifier populations, a 10-L mixture of ammonium chloride and sodium nitrite was added initially as a pulse to assess additions to the RAS prior to fish addition. To prime nitrifier populations, a 10-L mixture of ammonium chloride and sodium nitrite was added initially as a pulse to assess mixing rates and early transformation. After initial oscillation, a uniform concentration of ammonium (ca. 1200 μM or 16.8 ppm N) and nitrite (100 μM; 1.4 ppm N) was attained in 30 min based on 5 sampling sites (see Figure 3). During the first 100 h, at pH 9-9.2, ammonium decreased without any change in either product nitrate or nitrite. Because chloride remained at the original enrichment, this suggests evasion (volatilization) as gas from the aeration system at high pH (see Figure 4). After 100 h, the pH had decreased to less than 8.5, and a steeper rate of ammonium decrease was accompanied by an increase in nitrite. Between 7 and 13 days, ammonium was eliminated completely, with a removal rate of 6 μmol/L/h (2 ppm/day). Nitrite increased to 400 μM (5.6 ppm) near day 10 and then dropped to zero by day 15 at 3.3 μM/h (1.1 ppm/day), significantly more slowly than ammonium loss. Because of the pH changes, it was difficult to balance a nitrogen budget during this first spike, though it was apparent that nitrite oxidation to nitrate was substantially slower in the first two weeks of activity. Nitrate increase accounted for the added nitrite plus 700 μM (9.8 ppm) of the ammonium, indicating that as much as 500 μM (7 ppm) of ammonia evaded into the atmosphere. During subsequent spikes, pH remained at or below 8.2 and evasion as gas was minimal. For the second experiment, nitrite alone (550 μM; 7.7 ppm) was added. After a short lag, nitrite was removed continuously at about 6.9 μM/h (2.32 ppm/day) with a mirror-image increase in its oxidation product nitrate. This was more than twice the rate attained in the first experiment, and because nitrite does not vaporize at higher pH, it indicated that the nitrite-oxidizing bacteria were growing in to the system. During the consumption of 550 μM (7.7 ppm) nitrite, no detectable decrease in the bacterial carbon source CO_2 occurred.

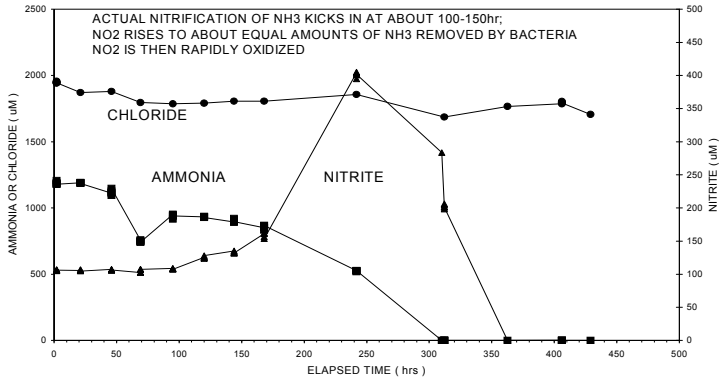

Figure 4. N transformations in FWRAS after pulse additions of ammonium chloride and sodium nitrite

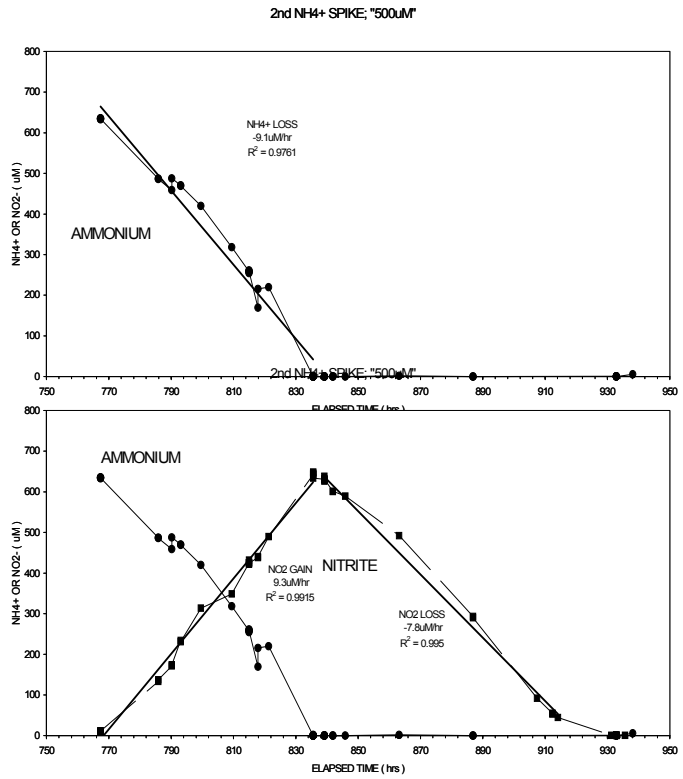

Figure 5. N transformations in FWRAS after spiking with ammonium chloride

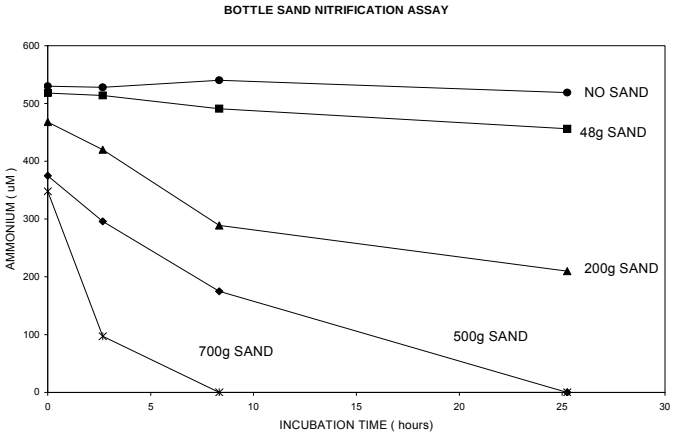

Figure 6. Changes in ammonium concentrations in FWRAS water supplemented with different levels of biofilter sand

The third trial (see Figure 5) consisted of an ammonium chloride spike of 650 μM (9.1 ppm) at pH 8.0-8.2. Ammonium oxidation started immediately (Figure 5, top panel) at about 8.2 μM/h (2.76 ppm/day) and proceeded to completion with concomitant increase in nitrite to 645 μM (9.0 ppm) after 3 days. Nitrite removal began as soon as, *but not before,* ammonia was completely removed. Like ammonia, nitrite was eliminated at a constant rate (7.9 μM/h; 2.65 ppm/day) to undetectable levels. During nitrite oxidation the product nitrate increased at 7.3 μM/h (2.45 ppm/day) with essentially complete recovery. Total CO_2, the carbon source for nitrifying bacteria, decreased consistently during ammonia removal and then remained constant.

To test the importance of the sand associated biofilms in the biofilter, incubation experiments with different amounts of sand addition were performed. The rates of ammonium removal were proportional to the added sand, reaching as high as 94 μM/h (1.3 ppm/h) with 700 g wet weight sand in 460 mL total volume (roughly 85 vol% sand, similar to the bottom of the biofilter tank) (Figure 6).

Rates of dark carbon dioxide fixation, using $^{14}CO_2$ additions, also demonstrated both the role of sand-associated biofilm bacteria and their response to added nitrogen. Incubations with biofilter inflow water with or without 1 g wet weight of biofilter sand were initiated just prior to and during the last ammonium spike experiment. Prior to addition, when NH_4^+ and NO_2^- were absent, dark carbon fixation was uniformly low throughout the system (Figure 7, top panel): water-only incubations showed no short-term response while *sand-amended samples increased by more than 5-fold during the first day of high ammonium levels*. That the sand-associated bacteria were only inactive and not dormant is shown in the center panel, where ammonium or nitrite caused significant stimulation in sand but not water prior to system enrichment. A growth inhibitor of bacteria reduced control activity ("CAP") and prevented stimulation by added nitrogen ("+NCAP"). Nitrite stimulated much less, consistent with the absence of total CO_2 decrease during nitrite consumption (second experiment, above). After enrichment (bottom panel), when ambient ammonium was high, further added nitrogen was not stimulatory, and nitrite even inhibited fixation slightly in the sand. Water-only samples still showed no response, and were far lower in activity than sand-amended samples. These experiments, along with cultivation and microscopic analysis (data not shown), unequivocally demonstrate that sand-associated bacteria dominate nitrogen transformations in this FWRAS.

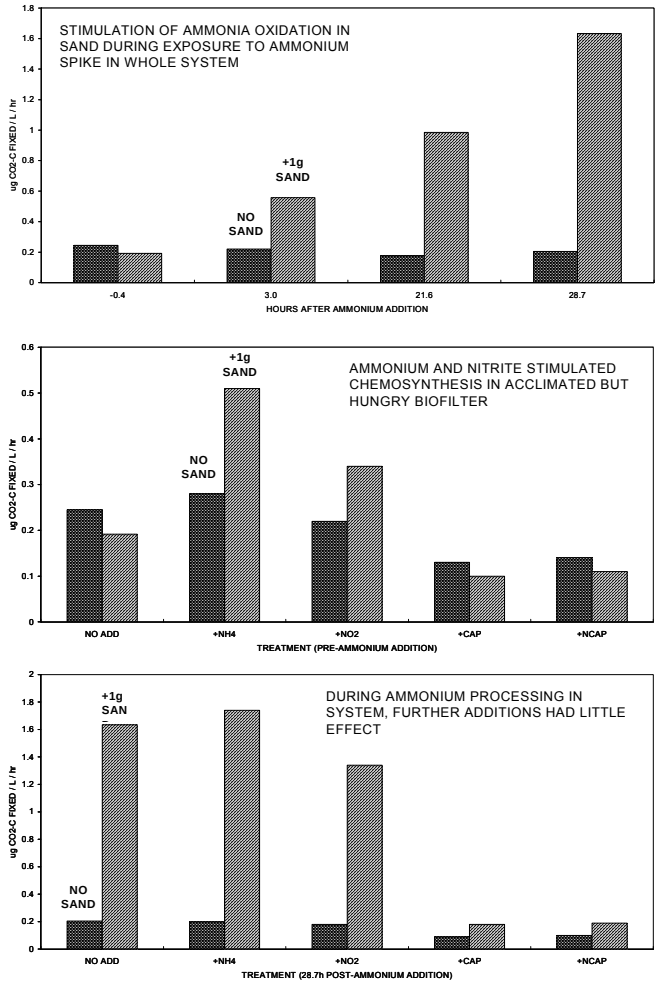

Figure 7. Rates of carbon dioxide fixation in FWRAS water alone or supplemented with biofilter sand

Several important observations were made during experiments with the FWRAS. First*, nitrite consumption began only after ammonia was totally exhausted*. In natural, as well as in optimized engineered systems, these two processes operate concurrently with little accumulation of nitrite. Second, although an equal amount of ammonia and nitrite was oxidized*, total CO_2 depletion was found only during the ammonia oxidation phase*, demonstrating a much less efficient growth of nitrite oxidizers consistent with bioenergetics considerations. Also, nitrite produced much lower levels of stimulation in dark CO_2 fixation experiments. Third, *sand-associated biofilm bacteria dominate the nitrogen conversion activity* with nearly undetectable activity in the water itself. Fourth, attainment of a quantitative mass balance for $NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$ reinforces the conclusion that evasion rather than biological removal was important in the first experiment at higher pH.

Following the addition of 10,000 yellow perch, the degree of variability in system characteristics (feeding, backwashing, refilling, occasional flow-through mode, etc.) increased to the extent that further unfunded preliminary work was not practical. Nonetheless, a lower level of effort was continued which proved valuable because the exact problems described in the first few paragraphs developed in this system. That is, irregular spikes of alarming levels of nitrite occurred, causing conversion to flow-through mode at one point. A moderate spike of nitrite (to 15 μM; 0.21 ppm) occurred early in the fish-loaded system. A much larger increase (to 35 μM; 0.49 ppm) occurred again at about 700 h, though total ammonia remained consistently low throughout. Samples were often taken as biofilter inlet/outlet pairs. In all such cases, when ammonia was present, nitrite was higher after passage through the filter, while it was significantly less when ammonia levels were very low (<4 μM; 0.06 ppm).

Of particular interest was the time course of the ultimate end product of nitrification, nitrate, during fish growth. During emergency flow-through mode, nitrate remained low, then climbed consistently upon return to recirculating mode. In aerobic systems such as this, nitrate is stable and its build-up is a measure of integrated system nitrification. In this example, the increase in nitrate (11.1 μM/h; 3.73 ppm/day) accounts for about 130 g nitrogen/day for the whole system. Given the feeding rate, food composition, and an estimate of uneaten food from the bottom, food conversion efficiency may be calculated.

ANTICIPATED BENEFITS

To our knowledge, the level of diverse expertise we have assembled has never been applied to perform a comparative study of a marine and freshwater RAS. The goal of the proposed work is to collaboratively develop a thorough understanding of the microbiology and physiology of nitrogen removal systems, and to investigate their behavior in the presence of fish and shrimp populations for freshwater and marine applications, respectively. This collaboration makes sense: we expect our combined efforts to result in generating means for the stable management of potentially toxic nitrogen compounds such as ammonia and nitrite, which is second only to oxygenation in necessity for economical viability of any RAS-based food production system. We intend to recommend changes in management or system design by

communicating directly with NCRAC extension personnel (thus addressing Objective 3). Recently described microbial groups that have not yet been studied in RAS, such as AOA and anammox bacteria, may be active or might be encouraged through system modification if results so warrant. The basic knowledge we gain from the ecophysiology of these systems will be useful in engineering resilient bioprocessing to reduce the frequency of "surprises". Localization of key nitrogen detoxifying activities within the functional components of the differing systems (e.g., solids removal of sludge catch basin, biofilter compartments with different biofilm support media, grow-out tanks, etc.) will provide greater ability to maintain optimal conditions for nitrogen removal and identify any unexpected zones of (in)activity. We expect to generate much useful data related to N cycling during this one-year project. In the future, we hope to investigate other aspects of RAS ecophysiology; for example, certain microbes may have more direct interactions with the aquatic animals themselves, either as food or for disease suppression. This study will provide a jumping point for us investigate these issues through longer-term grants.

OBJECTIVES

Two functioning RAS systems representing freshwater fish culture and marine shrimp culture will be studied for nitrogen cycling processes. The oyster shell component in the MRAS has a large, porous, irregular surface area capable of supporting anaerobic microzones. An important difference between the two (FWRAS vs. MRAS) is that fluidization and backwashing of the biofilter and solids removal components, respectively of FWRAS interrupt the stability of anaerobic conditions, whereas the structure of the oyster shell component and differences in backwashing strategies may maintain microzones of anaerobiosis in the MRAS. In this study, we intend to:

1. Characterize the microbial communities in established production scale marine and freshwater recirculating aquaculture system (RAS) units. 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.

PROCEDURES

Characterize Microbial Communities (Objective 1)

Task 1.1

Construction of clone libraries of bacterial and archaeal nitrifiers in biofilters of marine and freshwater RAS supporting shrimp and yellow perch, respectively and monitoring the diversities and dynamics through time.

Although nitrifiers play a central role in the performance of RAS, very little is known about the diversity of nitrifiers in aquaculture systems. Moreover, almost nothing is known about the ecological interactions (i.e., competition, functional complementation) between AOB and AOA in any system (natural or engineered). The microbial community composition will be determined in environments through the analysis of 16S rRNA gene clone libraries. Application of this method to characterize the microbial communities in the MRAS shrimp facility revealed that AOA were the predominant ammonia oxidizers in the system. More targeted PCR-based tools have been developed (e.g., primers for denaturing gradient gel electrophoresis (DGGE) and quantitative real-time PCR) to study the diversity and dynamics of AOA *in situ*, as well as in batch experiments intended at investigating their activities under different environmental conditions. This approach will also be used to similarly characterize the various compartments of the FWRAS in Milwaukee, Wisconsin. Although only recently discovered, AOA are now proving to be ubiquitous, and, therefore, it is entirely possible that freshwater-adapted populations exist in yellow perch RAS. To better understand N cycling in RAS, this approach will also be used to characterize the traditional ammonia- and nitrite-oxidizing bacteria (AOB and NOB), anammox bacteria, and the major

denitrifying bacteria in both systems. Each major system component (solids removal of sludge catch basin, biofilter compartments with different biofilm support media, grow out tanks, etc.) will be assessed for specific microbial communities and transformation capacities. These studies will provide useful information for development and maintenance of consistent nitrogen detoxification processes in RAS.

Microbial diversity will be measured in each of the RAS. PCR primers targeting bacterial and archaeal 16S rRNA genes (Gurtler and Stanisich 1996; Grosskopf et al. 1998) and the ammonia monooxygenase (*amoA*) gene will be used to construct clone libraries using samples taken from the attached biofilm from bioballs and oyster shells of MRAS and sand and plastic bead biofilters from the FWRAS. The levels of diversity and similarities among the libraries will be analyzed statistically using DOTUR (Schloss and Handelsman 2005) and ∫-LIBSHUFF (Schloss et al. 2004) analysis software. These clone libraries will provide an initial estimate of archaeal and bacterial community diversity, identify the most predominant populations, and allow the conduct of *in silico* PCR for developing qPCR and DGGE primers. Another option for a faster community profiling protocol is terminal restriction fragment length polymorphism (T-RFLP) analysis (Marsh 1999). This method is well-established in the Raskin laboratory (Briones et al. 2007b; Padmasiri et al. 2007). The relative ease of generating a microbial community profile using this method will make the monitoring of microbial community changes through time more tractable. Multivariate statistical methods are also available to allow analysis of T-RFLP data (Thies 2007). The Raskin laboratory has applied correspondence analysis to monitor the microbial community changes in multi- and single-compartment anaerobic bioreactors (Briones et al. 2007b).

Task 1.2.

Visualization of the spatial relationships between nitrifying populations from biofilter material through fluorescence in situ hybridization (FISH)
The use of fluorescently labeled oligonucleotide probes targeting cellular 16S rRNA molecules allows the highly specific staining of populations of microorganisms (Amann et al. 1990). During processing of microbial biomass samples for DNA extraction (Task 1.1), a subset of sample material will be set aside for fixation in 4% paraformaldehyde. Thereafter, established FISH protocols will be followed (Amann and Ludwig 2000) to microscopically visualize the different bacterial and archaeal nitrifying populations using fluorescently labeled probes targeting Betaproteobacterial AOB (Mobarry et al. 1996), AOA (Konneke et al. 2005; Steger et al. 2008) and NOB (Daims et al. 2000). FISH experiments will be critical to understanding how important spatial relationships are between ammonia and nitrite oxidizers, i.e., whether unpredictable spikes of nitrite can be related to disturbances in the juxtapositioning between ammonia oxidizers and nitrite oxidizers.

Quantifying the Role of Microbial Communities (Objective 2)

Both freshwater and marine RAS are currently in production phase. Therefore, system-scale perturbations (spikes of ammonia and/or nitrite as shown in background examples) are not possible, so all metabolic activities will be measured in bottle incubations.

Task 2.1

Measuring the stoichiometries of nitrogen transformations in freshwater and marine RAS

A similar approach for N cycling study will be taken for both systems. Subsamples from both complete systems will be subjected to a variety of treatments to assess capacity and resiliency following the concepts developed in the background section. Initially, pulses of ammonia, nitrite, or both will be added to delineate capability to transform the individual and mixed supplements. Water samples from each major component (growth tanks, biofilters, solids removal) will be collected into 1.0-L (0.26-gal) polycarbonate bottles. For each component containing sand, beads, bioballs, or oyster shells, replicates with and without solid phase material will be prepared. Aqueous phase samples will be filter-sterilized for nutrient analysis (ammonia, nitrite, nitrate, phosphate) by micro-scale standard methods (APHA 1996). Total carbon dioxide ($\sum CO_2$) will be assayed by the Teflon flow injection method of Hall and Aller (1992). Replicated 1.0–2.0-L (0.26–0.52-gal) roller-incubated bottles will be spiked with meaningful concentrations of ammonia (up to 1 mg/L) or nitrite (ca. 0.1 mg/L), and subsamples will be filtered and assayed in geometric time series (0, 2, 4, 8, 16, 24, 36, and 48 h) and adjusted as necessary for a total of about 10 time points. Subsample removal (25 mL) will have little impact on surface:volume ratios early in

the incubations and will be taken into account in rate calculations. This will provide evidence for the involvement and response time of each component in general or specific nitrogen cycling activities. Labile ammonia, nitrite, and $\sum CO_2$ samples will be analyzed on-site.

The basic analyses provide two necessary results for interpretation of nitrogen cycling in RAS. First, rates of oxidation can be applied to budget the systems in terms of food conversion, excretion, and sustainability. Second, the stoichiometry (ratios of reactants to products) will identify the presence or absence of novel anaerobic N metabolism in which lesser amounts of the terminal product nitrate are produced than in "traditional" nitrification processes. The stoichiometry links perfectly to molecular community structure analyses to delineate persistent microbiological N detoxification activities.

For incubations of 5 days, up to a 32-fold increase in ammonia oxidizing bacteria could occur. Therefore, the end-point of the incubation will be sampled to quantify the increase in specific populations using molecular techniques as described in Objective 1. Likewise, enrichment of newly-discovered AOA will be investigated using moderate-term (weeks) ammonia addition end-points. More targeted enrichments for this purpose are described below.

Task 2.2

Studying the activities, stress responses, and dynamics of nitrifiers from different systems and attachment media

Experiments will be conducted to address (1) anaerobic ammonia oxidation potential (anammox) and (2) the effect of oxygen limitation on AOB and AOA activities. In the case of anammox, the microbial characterizations to address Objective 1 will determine if there are significant levels of anammox bacteria present in the FWRAS. Preliminary characterizations of the MRAS have so far failed to detect anammox bacteria.

Effects of dissolved oxygen

RAS by nature must be highly aerobic systems to support the densities of oxygen-respiring fish or shellfish. However, anaerobic conditions likely exist in some components of RAS (e.g., solids removal basin) and it is possible that anaerobic ammonia oxidation contributes to N cycling in RAS. Should microbial characterization of FWRAS prove positive for anammox bacteria, then the systems' potential for anammox metabolism will be tested by incubating argon-sparged water containing solid-phase components in specially-constructed 1.5-L (0.4-gal) polycarbonate syringes. Subsamples for N stoichiometry can be removed as above without introduction of headspace, and at the end-point the solid phase can be processed for microbial community characterization. The rationale for these tests has to do in part with amelioration of long-term nitrate accumulation. One method for nitrate removal might be shunting of a portion of system water into an intentionally anaerobic incubation chamber where denitrification (anaerobic nitrate respiration) could occur, with concomitant removal of dissolved organic carbon metabolites. With anammox communities, a similar shunt could remove ammonia and nitrite without nitrate buildup. However, it would be challenging to rely completely on anammox in practical circumstances because continuous response to fluctuation N levels is necessary in aquaculture systems. The presence of anammox bacteria, however, would provide proof that some portion of the RAS system maintains anaerobic conditions, something that should be of great value to system design.

A similar approach will be used to study the N stoichiometries of different biofilter samples (bioballs and oyster shells in MRAS; sand and plastic beads in FWRAS) in response to a range of oxygen levels (e.g., 10–100% saturation). Previous authors have suggested that AOA may be more competitive under low oxygen conditions (Francis et al. 2005; Park et al. 2006; Coolen et al. 2007; Francis et al. 2007). Preliminary data suggest AOB and AOA are associated with the bioballs and oyster shells, respectively (see Background section). The hypothesis that the presence of anaerobic microsites in oyster shells may provide a competitive advantage for AOA will be examined by measuring nitrification generation rates as described above. Specific populations of AOA and AOB will be quantified by quantitative PCR (qPCR) targeting the ammonia monooxygenase gene (Lam et al. 2007; Mincer et al. 2007; Santoro et al. 2008), while NOB will be monitored by qPCR targeting the 16S rRNA gene (Kindaichi et al. 2006) or the functional nitrite oxidoreductase (*nxrA*) gene (Wertz et al. 2008). The same primer sets used for qPCR may be modified for use in denaturing gradient gel electrophoresis (DGGE) (Muyzer and Smalla 1998),

which will be employed to monitor the changes in diversity of AOB, AOA, and NOB through time (Rotthauwe et al. 1997; Coolen et al. 2007; Wertz et al. 2008). The diversities within each of these populations can also be monitored by terminal restriction fragment length polymorphism (T-RFLP) (Marsh 1999; Briones et al. 2007b). Participants of this project have extensive experience with DGGE and T-RFLP through other projects (Briones et al. 2003; 2007b; 2008b).

Coordinate Results with the Technical Committee Extension Subcommittee of NCRAC (Objective 3)

Results will be reported to and communicated with the Technical Committee Extension Subcommittee of NCRAC.

FACILITIES

Milwaukee, Wisconsin

A ten-year-old fully operational freshwater yellow perch (*Perca flavescens*) RAS with sand-based fluidized bed nitrification and polyethylene bead particle removal systems will be the object of a non-invasive study. Complete academic laboratories with facilities are in place for nutrient analysis (flow injection analysis [FIA] and spectrophotometry for ammonia; FIA for nitrate and nitrite; FIA for total CO_2; ion chromatography for chloride, and others), external sample incubation for process studies (e.g., environmental chambers), radioisotope counting equipment for nitrification growth rate measurements, anaerobic incubation hoods, and resources for other needs.

Okemos, Michigan

University of Michigan researchers have been conducting microbial community studies on a pilot scale facility raising marine shrimp (*Litopenaeus vannamei)* in Okemos, Michigan. The facility is located about an hour away from Ann Arbor, where all samples are finally processed. Key instrumentation from the University of Wisconsin is sufficiently portable for use at the Okemos, Michigan field site, including a semi-mobile anaerobic hood for anammox testing. The Raskin lab at the University of Michigan has approximately 2,000 ft^2 of laboratory space with the necessary equipment for the proposed microbiological and molecular studies. For example, the labs are equipped with several fume hoods, a laminar flow hood, PCR hoods, autoclaves, a Zeiss Axioskop microscope with a CCD camera, nucleic acid extraction, and hybridization equipment, including microarray hybridizations, thermal cyclers for PCR, including a real time PCR setup, electrophoresis equipment, including a DGGE setup, gel imaging system, two ultra-low temperature freezers, an anaerobic glove box, a floor model high-speed refrigerated centrifuge, two microcentrifuges, various orbital shaker baths and shaker tables, incubators, a luminescence spectrophotometer, and two nanodrop systems. Access is available to a variety of analytical equipment such as gas chromatographs (equipped with FID, TCD, ECD, and reduction gas detectors), UV/Vis spectrophotometers, a scintillation counter, ion chromatographs, high performance liquid chromatography systems, TOC analyzers, an atomic absorption analyzer, and ICP/MS. Additionally, Prof. Jim Diana's lab houses recently renovated aquatic labs in which lab-scale shrimp RAS tanks, which are intended for experimental use in a related project, will be set up. While the focus of this project will be on the large-scale systems in Milwaukee and Okemos, the availability of these lab-scale systems will potentially be very useful to prove hypotheses generated from initial studies.

REFERENCES

Amann, R.I., L. Krumholz, and D.A. Stahl. 1990. Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. Journal of Bacteriology 172:762-70.

Amann, R. and W. Ludwig. 2000. Ribosomal RNA-targeted nucleic acid probes for studies in microbial ecology. FEMS Microbiology Reviews 24:555-565.

APHA (American Public Health Association). 1996. Standard methods for the examination of water and wastewater, 18th edition. American Public Health Association, Washington, D.C.

Arquitt, S., H. Xu, and R. Johnstone. 2005. A system dynamics analysis of boom and bust in the shrimp aquaculture industry. System Dynamics Review 21:305-324.

Bayer, K., S. Schmitt, and U. Hentschel. 2008. Physiology, phylogeny and *in situ* evidence for bacterial and archaeal nitrifiers in the marine sponge *Aplysina aerophoba*. Environmental Microbiology 10:2942-2955.

Beman, J.M., K.J. Roberts, L. Wegley, F. Rohwer, and C.A. Francis. 2007. Distribution and diversity of archaeal ammonia monooxygenase genes associated with corals. Applied Environmental Microbiology 73:5642-5647.

Briones, A.M., S. Okabe, Y. Umemiya, N.B. Ramsing, W. Reichardt, and H. Okuyama. 2003. Ammonia-oxidizing bacteria on root biofilms and their possible contribution to N use efficiency of different rice cultivars. Plant and Soil 250:335-348.

Briones, A.M., J. Shililu, J. Githure, R. Novak, and L. Raskin. 2007a. *Thorsellia anophelis* is the dominant bacterium in a Kenyan population of adult Anopheles gambiae mosquitoes. ISME Journal 2:74-82.

Briones, A.M., B.J. Daugherty, L.T. Angenent, K.D. Rausch, M.E. Tumbleson, and L. Raskin. 2007b. Microbial diversity and dynamics in multi- and single-compartment anaerobic bioreactors processing sulfate-rich waste streams. Environmental Microbiology 9:93-106.

Chaban, B., S.Y. M. Ng, and K.F. Jarrell. 2006. Archaeal habitats - from the extreme to the ordinary. Canadian Journal of Microbiology 52(2):73-116.

Collins, M. L.P., L.A. Buchholz, and C.C. Remsen. 1991. Effect of copper on methylomonas-albus Bg8. Applied and Environmental Microbiology 57:1261-1264.

Coolen, M. J.L., B. Abbas, J. van Bleijswijk, E.C. Hopmans, M.M.M. Kuypers, S.G. Wakeham, and J.S.S. Damste. 2007. Putative ammonia-oxidizing Crenarchaeota in suboxic waters of the Black Sea: a basin-wide ecological study using 16S ribosomal and functional genes and membrane lipids. Environmental Microbiology 9:1001-1016.

Cuhel, R.L., C. Aguilar, P.D. Anderson, J.S. Maki, R.W. Paddock, C.C. Remsen, J.V. Klump, and D. Lovalvo. 2002. Underwater domains in Yellowstone Lake hydrothermal vent geochemistry and bacterial chemosynthesis. Pages 27-53 *in* R.J. Anderson and D. Harmon, editors. 6th Biennial Conference on the Greater Yellowstone Ecosystetm: Yellowstone Lake: hotbed of chaos or reservoir of resilience? October 8-10, 2001. Yellowstone Center for Resources and the George Wright Society, Hancock, Michigan; Yellowstone National Park, Wyoming.

Daims, H., P.H. Nielsen, J.L. Nielsen, S. Juretschko, and M. Wagner. 2000. Novel Nitrospira-like bacteria as dominant nitrite-oxidizers in biofilms from wastewater treatment plants: diversity and in situ physiology. Water Science and Technology 41(4-5):85-90.

Daims, H., M.W. Taylor, and M. Wagner. 2006. Wastewater treatment: a model system for microbial ecology. Trends in Biotechnology 24(11):483-489.

Francis, C.A., K.J. Roberts, J.M. Beman, A.E. Santoro, and B.B. Oakley. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proceedings of the National Academy of Sciences 102:14683-14688.

Francis, C.A., J.M. Beman, and M.M.M. Kuypers. 2007. New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. ISME Journal 1:19-27.

Grosskopf, R., P.H. Janssen, and W. Liesack. 1998. Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. Applied Environmental Microbiology 64:960-969.

Gurtler, V. and V.A. Stanisich. 1996. New approaches to typing and identification of bacteria using the 16S-23S rDNA spacer region. Microbiology-UK 142:3-16.

Gutierrez-Wing, M.T. and R.F. Malone. 2006. Biological filters in aquaculture: trends and research directions for freshwater and marine applications. Aquacultural Engineering 34(3):163-171.

Hall, P.O. and R.C. Aller. 1992. Rapid, small-volume, flow-injection analysis for sigma-CO_2 and NH_4+ in marine and fresh-waters. Limnology and Oceanography 37:1113-1119.

Harvey, D.J. 2006. Aquaculture Outlook. USDA Economic Research Service.

Hoffmann, F., H. Roy, K. Bayer, U. Hentschel, M. Pfannkuchen, F. Bruemmer, and D. De Beer. 2008. Oxygen dynamics and transport in the Mediterranean sponge *Aplysina aerophoba*. Marine Biology 153:1257-1264.

Joye, S.B. and J.T. Hollibaugh. 1995. Influence of sulfide inhibition of nitrification on nitrogen regeneration in sediments. Science 270(5236):623-625.

Kindaichi, T., Y. Kawano, T. Ito, H. Satoh, and S. Okabe. 2006. Population dynamics and in situ kinetics of nitrifying bacteria in autotrophic nitrifying biofilms as determined by real-time quantitative PCR. Biotechnology and Bioengineering 94:1111-1121.

Konneke, M., A.E. Bernhard, J.R. de la Torre, C.B. Walker, J.B. Waterbury, and D.A. Stahl. 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437(7058):543-546.

Lam, P., M.M. Jensen, G. Lavik, D.F. McGinnis, B. Muller, C.J. Schubert, R. Amann, B. Thamdrup, and M.M.M. Kuypers. 2007. Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea. Proceedings of the National Academy of Science 104(17):7104-7109.

Leininger, S., T. Urich, M. Schloter, L. Schwark, J. Qi, G.W. Nicol, J.I. Prosser, S.C. Schuster, and C. Schleper. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442(7104):806-809.

Li, X.-Z., L. Zhang, and H. Nikaido. 2004. Efflux pump-mediated intrinsic drug resistance in mycobacterium smegmatis. Antimicrobial. Agents and Chemotherapy 48:2415-2423.

Ling, J. and S.L. Chen. 2005. Impact of organic carbon on nitrification performance of different biofilters. Aquacultural Engineering 33(2):150-162.

Mankin, A.S. and R.A. Garrett. 1991. Chloramphenicol resistance mutations in the single 23s ribosomal-Rna gene of the Archaeon Halobacterium-Halobium. Journal of Bacteriology 173:3559-3563.

Marsh, T.L. 1999. Terminal restriction fragment length polymorphism (T-RFLP): an emerging method for characterizing diversity among homologous populations of amplification products. Current Opinion in Microbiology 2(3):323-327.

Masser, M.P., J. Rakocy, and T.M. Losordo. 1999. Recirculating aquaculture tank production systems - management of recirculating systems. Southern Regional Aquaculture Center, Stoneville, Mississippi.

Michaud, L., J.P. Blancheton, V. Bruni, and R. Piedrahita. 2006. Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. Aquacultural Engineering 34(3):224-233.

Mincer, T.J., M.J. Church, L.T. Taylor, C. Preston, D.M. Kar, and E.F. DeLong. 2007. Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. Environmental Microbiology 9:1162-1175.

Mobarry, B.K., M. Wagner, V. Urbain, B.E. Rittmann, and D.A. Stahl. 1996. Phylogenetic probes for analyzing abundance and spatial organization of nitrifying bacteria. Applied and Environmental Microbiology 62:2156-2162.

Mulder, A., A.A. Vandegraaf, L.A. Robertson, and J.G. Kuenen. 1995. Anaerobic ammonium oxidation discovered in a denitrifying fluidized-bed reactor. Fems Microbiology Ecology 16(3):177-183.

Muyzer, G. and K. Smalla. 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 73:127-141.

Nieto, J.J., R. Fernandezcastillo, M.T. Garcia, E. Mellado, and A. Ventosa. 1993. Survey of antimicrobial susceptibility of moderately halophilic eubacteria and extremely halophilic aerobic archaeobacteria - utilization of antimicrobial resistance as a genetic-marker. Systematic and Applied Microbiology 16(3):352-360.

Padmasiri, S.I., J.Z. Zhang, M. Fitch, B. Norddahl, E. Morgenroth, and L. Raskin. 2007. Methanogenic population dynamics and performance of an anaerobic membrane bioreactor (AnMBR) treating swine manure under high shear conditions. Water Research 41:134-144.

Park, H.-D., G.F. Wells, H. Bae, C.S. Criddle, and C.A. Francis. 2006. Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors. Applied Environmental Microbiology 72:5643-5647.

Prosser, J.I. 1989. Autotrophic nitrification in bacteria. Advances in Microbial Physiology 30:125-181.

Purkhold, U., A. Pommerening-Roser, S. Juretschko, M.C. Schmid, H.-P. Koops, and M. Wagner. 2000. Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and amoA sequence analysis: implications for molecular diversity surveys. Applied and Environmental Microbiology 66:5368-5382.

Rotthauwe, J.H., K.P. Witzel, and W. Liesack. 1997. The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Applied and Environmental Microbiology 63:4704-4712.

Santoro, A.E., C.A. Francis, N.R. de Sieyes, and A.B. Boehm. 2008. Shifts in the relative abundance of ammonia-oxidizing bacteria and archaea across physicochemical gradients in a subterranean estuary. Environmental Microbiology 10:1068-1079.

Schloss, P.D., B.R. Larget, and J. Handelsman. 2004. Integration of microbial ecology and statistics: a test to compare gene libraries. Applied and Environmental Microbiology 70:5485-5492.

Schloss, P.D. and J. Handelsman. 2005. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Applied and Environmental Microbiology 71:1501-1506.

Steger, D., P. Ettinger-Epstein, S. Whalan, U. Hentschel, R. de Nys, M. Wagner, and M.W. Taylor. 2008. Diversity and mode of transmission of ammonia-oxidizing archaea in marine sponges. Environmental Microbiology 10:1087-1094.

Thies, J.E. 2007. Soil microbial community analysis using terminal restriction fragment length Polymorphisms. Soil Science Society of America Journal 71):579-591.

Treusch, A.H., S. Leininger, A. Kletzin, S.C. Schuster, H.P. Klenk, and C. Schleper. 2005. Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. Environmental Microbiology 7:1985-1995.

Van Wyk, P. 1999. Economics of shrimp culture in recirculating aquaculture systems. Florida Department of Agriculture and Consumer Services, Tallahassee.

Venter, J.C., K. Remington, J.F. Heidelberg, A.L. Halpern, D. Rusch, J.A. Eisen, D.Y. Wu, I. Paulsen, K.E. Nelson, W. Nelson, D.E. Fouts, S. Levy, A.H. Knap, M.W. Lomas, K. Nealson, O. White, J. Peterson, J. Hoffman, R. Parsons, H. Baden-Tillson, C. Pfannkoch, Y.H. Rogers, and H.O. Smith. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. Science 304(5667):66-74.

Verstraete, W., L. Wittelbolle, K. Heylen, B. Vanparys, P. de Vos, T. van de Wiele, and N. Boon. 2007. Microbial resource management: the road to go for environmental biotechnology. Engineering in Life Sciences 7(2):117-126.

Wertz, S., F. Poly, X. Le Roux, and E. Degrange. 2008. Development and application of a PCR-denaturing gradient gel electrophoresis tool to study the diversity of Nitrobacter-like nxrA sequences in soil. Fems Microbiology Ecology 63(2):261-271.

Yonath, A. 2005. Antibiotics targeting ribosomes: resistance, selectivity, synergism, and cellular regulation. Annual Review of Biochemistry 74:649-679.

PROJECT LEADERS

State	Name/Institution	Area of Specialization
Michigan	James S. Diana University of Michigan	Aquaculture
	Lutgarde M. Raskin University of Michigan	Environmental Biotechnology
Wisconsin	Carmen Aguilar University of Wisconsin-Milwaukee	Microbial Physiology
	Russell L. Cuhel University of Wisconsin-Milwaukee	Microbial Physiology

PARTICIPATING INSTITUTIONS AND PRINCIPAL INVESTIGATORS

University of Michigan
James S. Diana
Lutgarde M. Raskin

University of Wisconsin-Milwaukee Great Lakes WATER Institute
Carmen Aguilar
Russell L. Cuhel

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS
The Regents of the University of Michigan
3003 S. State Street, 1050 Wolverine Tower, Ann Arbor MI 48109-1274

PROJECT DIRECTOR(S)
Lutgarde Raskin and James Diana

USDA AWARD NO. Year 1: Objective 1 & 3

	CSREES FUNDED WORK MONTHS			Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages	Calendar	Academic	Summer				
1. No. of Senior Personnel							
a. 2 (Co)-PD(s)	.5			$8,510			
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. ___ Research Associates-Postdoctorates							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students				$7,875			
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. 1 Technical, Shop and Other (Technician, 2 months)							
Total Salaries and Wages →				$16,385			
B. Fringe Benefits (If charged as Direct Costs)				$4,915			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$21,300			
D. Nonexpendable Equipment (Attach supporting data.)							
E. Materials and Supplies				$8,717			
F. Travel				$2,500			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I) →				$32,517			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K). →							
N. Other →							
O. Total Amount of This Request →				$32,517			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)
Cash (both Applicant and Third Party) .....→
Non-Cash Contributions (both Applicant and Third Party) →

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0524-0039. The time required to complete this information collection is estimated to average 1.00 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing the reviewing the collection of information.

FORM CSREES-2004 (12/2000)

BUDGET EXPLNATION FOR UNIVERSITY OF MICHIGAN

(Raskin and Diana)

Objective 1

A. Salaries and Wages. Two weeks of summer salary is requested for Dr. Raskin. Nine months of her salary will be paid by the Department of Civil and Environmental Engineering at the University of Michigan. Funding from other research projects will be used to pay the rest of her summer salary. Salary is requested for a graduate student (Mr. Andrew Colby) ($12.62 per hour) for approximately 12 hours per week. The rest of Mr. Colby's salary will be paid for by discretionary funds provided by Dr. Raskin.

B. Fringe Benefits. Fringe benefits are budgeted at a rate of 30% of salary funding requested ($4,915).

E. Materials and Supplies. Materials and supplies include $2,261 for 2000 reactions of real time PCR; $2,420 for 1,920 DNA sequencing reactions; $1,164 for 1000 units of high fidelity Taq DNA polymerase; $940 for FISH probes and PCR primers; $316 for PCR plates; $310 for PCR strip tubes; $653 for disposable pipet tips and heat sealing film; and $653 for chemicals and glassware. All expenditures are of direct benefit to the successful completion of the project goals.

F. Travel. Transportation, lodging, and meal expenses for an 8-day trip to the yellow perch system in Wisconsin to sample for microbial analyses and interact with collaborators ($1,160). Funding is also requested for transportation, lodging, and meal expenses for one domestic trip for the project personnel to participate in an aquaculture meeting to present results at a location to be determined ($1,340).

THE UNIVERSITY OF MICHIGAN
DIVISION OF RESEARCH DEVELOPMENT AND ADMINISTRATION
www.research.umich.edu

3003 South State Street
Ann Arbor, MI 48109-1274

August 29, 2008

NORTH CENTRAL REGIONAL AQUACULTURE CENTER
Michigan State University
13 Natural Resources Building
East Lansing, MI 48824-1222
Attn: Ted R. Batterson, Director

RE: University of Michigan proposal entitled *Comparison, Identification and Role of Microbial Communities in Recirculating Systems in the Central Region (DRDA no. 09-0900)*

Dear Mr. Batterson,

The University of Michigan is pleased to submit the enclosed Proposal and associated budget for your consideration as a project at the University of Michigan. The University's effort, as described in the Proposal, will be under the direction of Lutgarde Raskin, Ph.D. in the Department of Civil and Environmental Engineering of the College of Engineering. It is proposed for the period beginning on or about September 1, 2009 through August 31, 2010 at a cost of $65,415.

If this Proposal and associated cost are acceptable, please contact me so that we can determine the most efficient way to establish an agreement to fund this additional effort. If you have any questions or comments of a technical or scientific nature regarding the enclosed materials, please direct them to Dr. Raskin at (734) 647-9620. If you have questions or comments of an administrative or contractual nature, kindly direct them to me. Thank you for your courtesy and consideration.

Very truly yours,

Thomas W. Zdeba
Senior Project Representative
(734) 936-1292
(734) 763-4053 fax
tzdeba@umich.edu

Enclosure

Cc: Lutgarde Raskin, Ph.D.

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS
University of Wisconsin-Milwaukee
P.O. Box 340, Milwaukee WI 53201-0340

PROJECT DIRECTOR(S)
Russell Cuhel and Carmen Aguilar

USDA AWARD NO. Year 1: Objective 2

	CSREES FUNDED WORK MONTHS			Duration Proposed Months: 12 Funds Requested by Proposer	Duration Proposed Months: ____ Funds Approved by CSREES (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages							
1. No. of Senior Personnel	Calendar	Academic	Summer				
a. 2 (Co)-PD(s)	0.5			$8,077			
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. ___ Research Associates-Postdoctorates							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. 1 Technical, Shop and Other				$7,000			
Total Salaries and Wages →				$15,077			
B. Fringe Benefits (If charged as Direct Costs)				$5,126			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$20,203			
D. Nonexpendable Equipment (Attach supporting data.)							
E. Materials and Supplies				$8,000			
F. Travel				$3,030			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)				$1,250			
K. Total Direct Costs (C through I) →				$32,483			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M..... Total Direct and F&A/Indirect Costs (J plus K) →							
N. Other →							
O. Total Amount of This Request →				$32,483			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)		
Cash (both Applicant and Third Party) ..... →		
Non-Cash Contributions (both Applicant and Third Party) →		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director Russell Cuhel, Senior Scientist		
Authorized Organizational Representative		
Signature (for optional use)		

Form CSREES-2004 (12/2000)

BUDGET EXPLANATION FOR UNIVERSITY OF WISCONSIN-MILWAUKEE

(Cuhel and Aguilar)

Objective 2

A. Salaries and Wages. The project will involve one short trip (8-10 days) to the field site at Seafood Systems, Inc. in Okemos MI during which the process measurements will be made. A similar effort for the Yellow Perch system is at home base. Current technical staff is dedicated to existing projects but can be available for limited analytical work. To hire and train for the short time frame of actual sampling and analysis is not feasible, and the Principal Investigators (9 month appointments) will do most of the work for this pilot project. They are requesting 0.5 month each for academic year support. Two months of technician time can be made available for the intensive analytical phase following the sampling programs, and these are likewise requested. Should the successful pilot lead to further work, dedicated hiring will be undertaken.

B. Fringe Benefits. Fringe benefits are budgeted at a rate of 34% of salary funding requested ($5,126).

E. Materials and Supplies. The majority of costs are involved in sample collection, incubation, and analytical chemistry. Reagents and chemicals for incubations and nutrient analysis ($1,000); isotope supplies for carbon dioxide fixation studies ($1,750); roller bottles and 2-tier mechanical mixing platform ($3,500); syringe filters for sample preparation ($1,000); incidental components for analysis and incubation ($750).

F. Travel. The Principal Investigators and technician will need to go to the Seafood Systems, Inc. site in Michigan to make the actual measurements of nitrogen processing. This includes round-trip ferry for truck and trailer with three people ($710), 2 rooms for 8 days at $60 ($960), and per diem expenses for 3 people x 8 days x $40/day = $960, totaling $2,630 plus mileage and daily charges for the truck (8 x $50 = $400). Unidentified costs will be borne by the PIs.

I. All Other Direct Costs. Chloride analysis for Cl:N ratios for stoichiometry (unavailable at the host Institution) are estimated to cost $1,250 (50@$25).

UNIVERSITY of WISCONSIN
UWMILWAUKEE

Graduate School

Research Services and Administration

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/research/rsa

April 21, 2009

Dr. Ted R. Batterson, Director
North Central Regional Aquaculture Center
Michigan State University
13 Natural Resources Building
East Lansing, Michigan 48842

SUBJECT: Project entitled "Comparison, Identification, and Role of Microbial Communities in Recirculating Systems in the North Central Region (DRDA no. 09-0900)

Dear Dr. Batterson:

As the Authorized Organizational Representative (AOR) I would like to inform you that the University of Wisconsin-Milwaukee Great Lakes WATER Institute (UWM-WATER) wishes to participate in the above referenced project as a subcontractor to Michigan State University. Drs. Russell Cuhel and Carmen Aguilar will serve as the Principal Investigators of the subcontract and they have access to all of the necessary equipment, laboratory, and office space to successfully undertake this project. I also approve the budget as submitted for Drs. Russell Cuhel and Carmen Aguilar involvement in this project. Upon issuance of approval to the North Central Regional Aquaculture Center for this project, the University of Wisconsin-Milwaukee Great Lakes WATER Institute will enter into a formal agreement with your institution.

Sincerely,

Peggy M. Vanco

Peggy M. Vanco
Director

BUDGET SUMMARY FOR EACH PARTICIPATING INSTITUTION

	University of Michigan	UW-Milwaukee	TOTALS
Salaries and Wages	$16,385	$15,077	$31,462
Fringe Benefits	$4,915	$5,126	$10,041
Total Salaries, Wages, and Fringe Benefits	$21,300	$20,203	$41,503
Nonexpendable Equipment			
Materials and Supplies	$8,717	$9,250	$17,967
Travel	$2,500	$3,030	$5,530
All Other Direct Costs			
TOTAL PROJECT COSTS	**$32,517**	**$32,483**	**$65,000**

SCHEDULE FOR COMPLETION OF OBJECTIVES

Objective 1: Completed in Year 1.

Objective 2: Completed in Year 1.

Objective 3: Initiated in Year 1 and will continue through the publication phase.

LIST OF PRINCIPAL INVESTIGATORS

Carmen Aguilar, University of Wisconsin-Milwaukee
Russell L. Cuhel, University of Wisconsin-Milwaukee
James S. Diana, University of Michigan
Lutgarde M. Raskin, University of Michigan

VITA

Carmen Aguilar
Great Lakes WATER Institute
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EDUCATION

B.S. Universidad Nacional Autónoma de México, Facultad de Ciencias and Instituto de Ciencias Nucleares (UNAM), 1983, Biology
Ph.D. University of Wisconsin-Milwaukee, 1992, Biology; Trace metal biogeochemistry

POSITIONS

Associate Scientist (2005-present) and Assistant Scientist (1998-2005), Great Lakes WATER Institute; Adjunct Associate Professor (2000), Department of Biological Sciences, University of Wisconsin-Milwaukee
Science Education Coordinator (1998-2000), National Institutes of Health and Environmental Science Biomedical Core Center
Research Associate (1997) and Postdoctoral Research Associate (1996), Great Lakes WATER Institute, University of Wisconsin-Milwaukee
Postdoctoral Research Associate (1993-1996), Carnegie Institution of Washington, Geophysical Laboratory and the University of North Carolina at Chapel Hill, Institute of Marine Sciences
Research Assistant (1989-1992), Center for Great Lakes Studies

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Geophysical Union
American Society for Microbiology
American Society of Limnology and Oceanography
International Association for great Lakes Research
Phycological Society of America
Society for Advancement of Chicanos and Native Americans in Science
The Oceanographic Society

SELECTED PUBLICATIONS

Cuhel, R.L. and C. Aguilar. 2003. CISNet: environmental monitoring of coastal waters of southwestern Lake Michigan 1998-2002. Technical Report to the Coastal Ocean Program of the National Oceanic and Atmospheric Administration, Silver Spring, Maryland. 84pp.

Beard, B.L., C.M. Johnson, L Cox, H. Sun, K.H. Nealson, and C. Aguilar, 1999. Iron isotope biosignatures. Science 285:1889-1892.

Aguilar, C., M.L. Fogel, and H.W. Paerl, 1999. Dynamics of atmospheric inorganic nitrogen utilization in the coastal waters of North Carolina. Marine Ecology Progress Series 180:65-79.

Fogel, M.L., C. Aguilar, R.L. Cuhel, D.J. Hollander, J.D. Willey, and H.W. Paerl. 1999. Biological and isotopic changes in coastal waters induced by Hurricane Gordon. Limnology and Oceanography 44:1359-1369.

Little, B., P. Wagner, K. Hart, R. Ray, D. Lavoie, K.H. Nealson, and C. Aguilar. 1996. The role of metal-reducing bacteria in microbiologically influenced corrosion. Corrosion. 97:Paper 215.

VITA

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EDUCATION

B.S. University of California San Diego, 1975, Biology/Chemistry Dual
Ph.D. Massachusetts Institute of Technology and Woods Hole Oceanographic Institution, 1981, Biology and Marine Biology Dual; Marine Microbiology and Biochemistry topic

POSITIONS

Senior Scientist (2001-present), Associate Scientist (1989-2001), and Great Lakes WATER Institute, University of Wisconsin-Milwaukee
Program Director (1990-present), Research Experience for Undergraduates Site (National Science Foundation)
Research Associate Professor (1987-1988), Division of Biology and Living Resources, Rosenstiel School of Marine and Atmospheric Science, University of Miami
Shaw Visiting Professor in Aquatic Microbiology (1987-1988), Center for Great Lakes Studies, University of Wisconsin-Milwaukee
Research Assistant Professor (1982-1987) and Postdoctoral Research Associate (1981-1982), Division of Biology and Living Resources, Rosenstiel School of Marine and Atmospheric Science, University of Miami
Graduate Research Fellow (1975-1981), Woods Hole Oceanographic Institution

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Geophysical Union
American Society for Microbiology
American Society of Limnology and Oceanography
International Association for great Lakes Research
Phycological Society of America

SELECTED PUBLICATIONS

Cuhel, R.L. and C. Aguilar. 2003. CISNet: environmental monitoring of coastal waters of southwestern Lake Michigan 1998-2002. Technical Report to the Coastal Ocean Program of the National Oceanic and Atmospheric Administration, Silver Spring, Maryland. 84pp.

Fogel, M.L., C. Aguilar, R.L. Cuhel, D.J. Hollander, J.D. Willey, and H.W. Paerl. 1999. Biological and isotopic changes in coastal waters induced by Hurricane Gordon. Limnology and Oceanography 44:1359-1369.

Mayer, P., Cuhel, R. L., and N. Nyholm. 1997. A simple *in vitro* fluorescence method for biomass measurements in algal growth inhibition tests. Water Research 31:2525-2531.

Bates, T.S., R.P. Kiene, G.V. Wolfe, P.M. Matrai, F.P. Chavez, K.R. Buck, B.W. Blomquist, and R. L. Cuhel. 1994. The cycling of sulfur in surface seawater of the northeast Pacific. Journal of Geophysical Research 99:7835-7843.

Cuhel, R.L. 1993. Sulfate assimilation by aquatic microorganisms. Pages 611-620 *in*: P.F. Kemp, B.F.Sherr, E.B. Sherr, and J.J. Cole, editors. Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, Florida.

VITA

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University of Michigan
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EDUCATION

B.S. California State University, Long Beach, 1974, Marine Biology
M.A. California State University, Long Beach, 1975, Biology
Ph.D. University of Alberta, 1979, Zoology

POSITIONS

Associate Dean and Professor (1999-present), Associate Professor (1984-1996), and Assistant Professor (1979-1984), School of Natural Resources, University of Michigan
Professor of Natural Resources and Associate Research Scientist (1996-present), Center for Great Lakes and Aquatic Sciences
Fisheries Biologist (1975), California Department of Fish and Game
Fisheries Biologist (1`974), Inyo National Forest

SELECTED PUBLICATIONS

Diana, J.S. 2009. Aquaculture production and biodiversity conservation. BioScience 59:27-38.

Schwantes, V.S., J.S. Diana, and Yang Yi. 2009. Social, economic, and production characteristics of giant river prawn *Macrobrachium rosenbergii* culture in Thailand. Aquaculture 287:120-127.

Tain, F.H. and J.S. Diana. 2007. Impacts of extension practice: lessons from small farm-based aquaculture of Nile tilapia in Northeastern Thailand. Society and Natural Resources 20:583-595.

Diana, J.S. 2004. Biology and ecology of fishes, Second Edition. Biological Sciences Press, Carmel, Indiana.

Yang Yi, C.K. Lin, and J.S. Diana. 2002. Techniques to mitigate clay turbidity problems in fertilized earthen fish ponds. Aquacultural Engineering 27:39-51.

Yang Yi, C.K. Lin, and J.S. Diana. 2002. Recycling pond mud nutrients in integrated lotus-fish culture. Aquaculture 212:213-226.

Diana, J.S. and C.K. Lin. 1998. The effects of fertilization on growth and production of Nile tilapia in rain-fed ponds. Journal of the World Aquaculture Society 29:405-413.

Diana, J.S. 1997. Feeding strategies. Pages 245-263 *in* H. Egna and C. Boyd, editors. Dynamics of pond aquaculture. CRC Press, Boca Raton, Florida.

Diana, J.S., J.P. Szyper, T.R. Batterson, C.E. Boyd, and P.H. Piedrahita. 1997. Water quality in ponds. Pages 53-71 *in* H. Egna and C. Boyd, editors. Dynamics of pond aquaculture. CRC Press, Boca Raton, Florida.

Diana, J.S., C.K. Lin, and Y. Yi. 1996. Timing of supplemental feeding for tilapia production. Journal of the World Aquaculture Society 27:410-419.

Diana, J.S. and C.K. Lin. 1995. Supplemental feeding of tilapia in fertilized ponds. Journal of the World Aquaculture Society 25:497-506.

VITA

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EDUCATION

B.S.+ M.S.	Katholieke Universiteit Leuven, Belgium, 1987, Bioengineering
B.S.	Katholieke Universiteit Leuven, 1988, Economic Sciences
Ph.D.	University of Illinois at Urbana-Champaign, 1993, Environmental Engineering

POSITIONS

Professor (2005-present), Dept. of Civil and Environmental Engineering, University of Michigan
Professor (2004-2005), Institute for Genomic Biology (IGB); Professor (2003-2005), Dept. of Civil and Environmental Engineering, University of Illinois-Urbana-Champaign
Professor-Associate Professor (2000-2005), Beckman Institute, Institute Affiliate, University of Illinois-Urbana-Champaign.
Visiting Research Professor (2002-2003), Laboratory for Microbial Ecology and Technology (LabMET), University of Gent, Belgium
Associate Professor (1999-2003), Assistant Professor (1993-1999), and Graduate Research Assistant (1988-1993), Dept. of Civil and Environmental Engineering; Fellow (1996-1997), Center for Advanced Study University of Illinois-Urbana-Champaign

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Society for Microbiology
American Water Works Association
Association of Environmental Engineering and Science Professors
International Water Association
Water Environment Federation

SELECTED PUBLICATIONS

Briones, A.M., J. Shililu, J. Githure, R. Novak, and L. Raskin. 2008. Thorsellia anophelis is the dominant bacterium in a Kenyan population of adult Anopheles gambiae mosquitoes. ISME Journal 2:74-82.

Briones, A.M., B.J. Daugherty, L.T. Angenent, K.D. Rausch, M.E. Tumbleson, and L. Raskin. 2007. Microbial diversity and dynamics in multi- and single-compartment anaerobic bioreactors processing sulfate-rich waste streams. Environmental Microbiology 9:93-106.

Padmasiri, S.I., J. Zhang, M. Fitch, B. Norddahl, E. Morgenroth, and L. Raskin. 2007. Methanogenic population dynamics and performance of an anaerobic membrane bioreactor (AnMBR) treating swine manure under high shear condition. Water Research 41:134-144.

Jindal, A., S. Kocherginskaya, A. Mehboob, M. Robert, R.I. Mackie, L. Raskin, and J.L. Zilles. 2006. Antimicrobial use and resistance in swine waste treatment systems. Applied and Environmental Microbiology. 72:7813-7820.

Amin, M.M., J.L. Zilles, J. Greiner, S. Charbonneau, L. Raskin, and E. Morgenroth. 2006. Influence of the antibiotic erythromycin on anaerobic treatment of a pharmaceutical wastewater. Environmental Science & Technology 40:3971-3977.