Redox Control Bioreactor for Enhanced Nitrogen Removal from Septic Tank Effluent Project Title

A Final Report Submitted to

The NOAA/UNH Cooperative Institute for Coastal and Estuarine Environmental Technology (CICEET)

Submitted by

Dr. Jay L. Garland[1]
Dr. Daniel P. Smith[2]

[1]Dynamac Corporation
Mail Code DYN-3 Kennedy Space Center, FL
[2]Applied Environmental Technology
10809 Cedar Cove Drive, Tampa, FL 33592

February 28, 2009

This project was funded by a grant from NOAA/UNH Cooperative Institute for Coastal and Estuarine Environmental Technology, NOAA Grant Number(s) NA04NOS190109

1. Expanded Executive Summary and Key Findings

a) What is the coastal resource issue the project sought to address? Nitrogen is generally considered the "limiting nutrient" to primary productivity on estuarine ecosystems. This project addresses nitrogen loading to coastal systems from distributed domestic sanitation water treatment systems.

b) What is the tool? Hollow fiber membrane bioreactors (HFMBR) that deliver gaseous electron acceptors (i.e. O_2) and gaseous electron donors (i.e. H_2) through the lumen of hollow fibers to microorganisms in attached external biofilms, in order to transform nitrogen from the reduced form that predominates in septic tank effluent to N_2. The project evaluated two different types of hollow fiber membrane bioreactors. The first was an air-only module that treats septic tank effluent (STE). In this system, air is delivered through hollow fibers, to affect C-BOD reduction and nitrification; denitrification can also be accomplished. The second type of HFMBR was a *Redox Control Bioreactor* (RCB). The RCB receives either air or O_2 in one set of membranes and H_2 in another set of membranes, thereby enabling completely autotrophic removal of nitrogen through nitrification and denitrification.

c) How does it address the problem? A dual reactor system can reduce Total Nitrogen through relatively passive addition of gaseous substrates that are required for biological treatment. Hollow fiber module-type bioreactors can be configured for onsite deployment in distributed infrastructure systems for effect substantial nitrogen reduction.

d) How is it an improvement over existing tools? Existing "conventional" onsite technology removed only a small fraction of total nitrogen. Enhanced nitrogen removal technologies often achieve TN reductions of only 50 to 60% and include pumps and aerators that require energy, operator attention and maintenance. A dual reactor system has the potential to achieve high percentage reductions and low TN levels in final effluent, while providing a resilient technology requiring limited operator attention.

e) What is the current stage of development (bench, lab, field, prototype)? Current state of development is bench scale testing using actual septic tank effluent.

f) Describe any regulatory barriers to application? The technology will have to be permitted by regulatory agencies, which is a function that fall under state jurisdiction. The approached of different states differ. Some states permit limited technology application as innovative technology.

g) Who are the end user groups for this tool? The current end users are individual homeowners and homeowner associations, who are served by engineers. The evolution of water infrastructure in large developed areas may create a demand for this technology in areas served by centralized infrastructure, whereby deployments at small to individual household scale will enhance recycling and reduce net water import requirements.

Key Findings

Compare the performance of the tool to existing methods in terms of the following:

a) Cost. A rational cost comparison requires a Life Cycle Cost Analysis (LCCA) for all alternatives. The systems have not been costed as of this point, and LCCA analyses are also generally not available for other onsite nitrogen alternatives that meet the same treatment goals. There are still configuration modifications that could substantially reduce final costs. As with any emerging technology, further research and development, and deployment of full scale field units are needed to develop defensible costs.
b) Maintenance requirements. Maintenance requirements are primarily associated with biomass management, i.e. the periodic removal of accumulated biomass. For the modules tested, sustainable operation of the air only reactor was achieved with a cleaning interval of 14 days, while the RCB cleaning interval was not determined.
c) Accuracy. N/A
d) Speed. The first stage reactor established operation relatively rapidly, within 4 weeks.
e) Ease of use. The systems were easy to use. Little maintenance or adjustment were required, with the notable exception of H_2 delivery/control in the RCB.
f) End user capacity requirements (supplies, skills, hardware). The dual reactor system would be supplied to the site in fully assembled modules. Installation would require skills and abilities that are commonly employed in the onsite industry, including basis plumbing, air supply, and electrical control panel installation. Supplying H_2 gas is beyond current practice, and would require further technology development.
g) If the tool is knowledge, describe the advancement of science over current level.

2. Project Development

a) Abstract

Nitrogen pollution of coastal waters from on-site septic systems is a widespread problem. Advanced on-site treatment based on traditional nitrification/denitrification approaches are inherently limited; the low carbon/nitrogen ratio of the waste stream limits denitrification, leading to significant levels of residual nitrate which can readily contaminate ground water. Introducing hydrogen to on-site waste treatment represents an inexpensive, clean means of reducing residual nitrate via the stimulation of hydrogenotrophic denitrification, a microbial process which converts nitrate to N_2 without the need for organic carbon (i.e., autotrophic process). Hollow fiber membranes are an effective means of delivering relatively insoluble gaseous substrates to biological reactors, relying on diffusion rather than sparging to provide gases to biofilms growing directly on the fiber surfaces. This project develop and tested a 2 reactor approach employing an air only hollow fiber system for initial treatment and subsequent dual fiber system (Redox Control Bioreactor, or RCB) using O_2 or H_2 delivery for aerobic (i.e., nitrification) or hydrogenotrophic processes. Operation of the first stage reactor was optimized, including development of an automated cleaning procedure for maintaining biofilm levels. In subsequent testing with actual septic tank effluent (STE), the reactor performed very well in reducing C-BOD_5 and ammonia nitrogen (30 and 25 mg/L, respectively) and in providing a bioreactive environment for denitrification. This technology appears to have a potential to be integrated into existing onsite systems in a number of ways that contribute to reduction in nitrogen loading in coastal zones. This level of STE treatment prior to application to the soil would alleviate many problems associated with marginal or failing "drainfield" or "leachfield" systems. In many applications, the effluent quality achieved by the BRR, when coupled with treatment capabilities of the receiving soil, would go a long way towards achieving total nitrogen removal objectives. A module scaled to the STE flow from a single household could readily be accommodated in a similar spatial scale as the primary treatment tank (i.e. septic tank), as a treatment insert before effluent dispersal in a soil leachfield. Superior treatment (i.e. lower effluent C-BOD_5 and ammonia nitrogen) could be achieved using a larger reactor with a lower loading rate. This type of module can also be envisioned as a unit operation producing effluent suitable for water recycling or preceding further nitrogen reduction treatment prior to soil dispersal, such as by biofiltration or additional HFMBR. The *Redox Control Bioreactor* (RCB) was evaluated on both analog BRR effluent and effluent from BRR treating actual STE. The completely autotrophic process has the potential to reduce oxidized nitrogen to quiet low levels and would be applicable for coastal zone locations where lower TN levels than those produced by the BRR are required. The overall success of trial runs of the prototype RCB was mixed for both analog feed and actual effluent from STE-supplied 1st stage reactor. Substantial difficulties were encountered with maintaining nitrification, and achieving ammonia nitrogen levels less than 20 mg/L. The level and of H_2 additions seemed to influence ammonia oxidation in the RCB, and repeated attempts to overcome this apparent limitation did not solve or resolve this issue. Further RCB research and development is needed to more fully address the suitability of RCB technology for onsite application.

b) Introduction

As population growth continues in coastal areas and estuarine watersheds, so do the potential threats to surface and groundwater quality. The U.S. population residing in coastal counties is projected to increase to 165 million by 2015, with a population density of 300 persons per square mile (Crossett, et. al., 2004). Currently, more than 1,540 single-family housing units are permitted for construction every day in coastal counties. Continued population growth in coastal watersheds will lead to greater stress and potential impacts to the water quality and environmental health of estuarine systems. Nitrogen is often considered the "limiting nutrient" in coastal and estuarine waters, and on-site wastewater treatment systems (OWTS) are one prominent nitrogen loading source. Over 60 million Americans, or 23% of households nationwide, are served by on-site systems (U.S Environmental Protection Agency, 2002b), with many systems currently operating in coastal zones. In addition, the on-site infrastructure is aging and many systems may no longer be functioning as intended. According to census data cited by the U.S. Environmental Protection Agency, over half of the on-site wastewater treatment systems in the U.S. are over 30 years old (U.S. E.P.A., 2003).

On-site wastewater systems represent a threat to estuarine quality. The initial concentrations of nitrogen in septic system effluent are 100 to 1000 fold greater than in coastal receiving waters and have a high potential to result in elevated anthropomorphic nutrient loadings (Welkel and Howes, 1992). Conventional septic tank and soil adsorption systems rely on biological reactions in a layer of porous media and in the receiving groundwater to attenuate nitrogen loading before it enters surface water. I a study at Big Pine Key, Florida, the dissolved inorganic nitrogen (DIN) levels in groundwater contiguous to drainfields were 400 fold greater than DIN levels at a control location (Lapointe, et al., 1990). Groundwater NH_3-N levels at Big Pine Key reached 2.75 mM, which approximates complete breakthrough of ammonia through the on-site treatment system. In another study, conducted on a sandy Florida aquifer system, groundwater levels of both total and ammonia nitrogen were elevated above background levels at a distance of 50 meters from a conventional soil adsorption drainfield (Corbett, 2002). Available setback distances in coastal areas may often be quite limited, and complete in-tank nitrogen removal obviates the need for lengthy setback distances that are often needed to attenuate nitrogen to background levels. Groundwater nitrate concentrations have been shown to exceed drinking water standards by factors of three or greater at distances on the order of several meters from soil adsorption systems (Postma et al., 1992).

While relatively few studies are available that quantitatively link nitrogen loadings from on-site wastewater treatment systems to coastal water quality, definite evidence does appear to exist to support this connection. One of the more comprehensive studies linking on-site wastewater systems to coastal surface water quality was reported for Buttermilk Bay, Massachusetts, where 74% of nitrogen loading to the bay originated from on-site wastewater systems (Harris, 1995). A nitrogen loading model was developed for the Waquoit Bay National Estuarine Research Reserve in Massachusetts, and 39% of total nitrogen loading was attributed to on-site wastewater treatment systems (Valiela, et al., 1997). In the Florida Keys, rainfall events cause episodic discharges of groundwater contaminated with septic tank effluent into near shore

waters, leading to enhanced eutrophication processes in sensitive coral reef communities (Lapointe and Matzie, 1996).

Recently, a survey was conducted of coastal zone resource managers across the United States to gain their perspectives on significant management issues and needs (Coastal States Organization, 2004). Over 70% of the respondents considered nutrient enrichment and environmental contamination as important to very important issues. The same survey reported that 57% of respondents considered improved treatment and removal technologies to be the top-ranked technology need for environmental contamination (Coastal States Organization, 2004).

In summary, nitrogen removal in on-site wastewater treatment systems is of critical significance in coastal and estuarine watersheds.

This project evaluated the use of hollow fiber membrane aeration biofilm reactors (HFMBR) for improved on-site wastewater treatment. Membrane aeration reactors based on supplying gases by diffusion through hollow fiber membranes have been studied as an efficient means of delivering gaseous substrates for wastewater treatment (Hibiya et al. 2002; Lee and Rittman, 2002, Ergas and Rheinheimer 2004). Reliance on diffusion eliminates the energy costs associated with turbulent mixing, and the biofilms which develop directly on the membrane surfaces allow for near 100% utilization of gaseous reactants (i.e. O_2, H_2). Membrane aeration reactors based on O_2 or H_2 delivery have been tested for aerobic nitrification and hydrogenotrophic denitrification, respectively. Nitrification is the initial required nitrogen removal step in STE. Hydrogenotrophic denitrification is an attractive means for achieving more complete denitrification and thereby reducing groundwater contaminants in low C/N waste streams such as septic tank effluent in which organotrophic denitrification is limited by available carbon. Nitrogen in STE effluent is dominated by organic and ammonia nitrogen, with little nitrate or nitrite. Complete nitrogen removal systems must include both oxidation (nitrification) and reduction (denitrification) to completely remove nitrogen.

We have developed and tested a novel membrane aeration reactor, the RCB, in which both oxidizing (O_2) and reducing gases (H_2) are introduced into a single reactor to achieve complete removal of influent ammonia nitrogen (Smith et al. 2007). The use of adjacent, juxtaposed hollow fibers enables complete NH_3 removal in a single reactor through redox gradients that support simultaneous nitrification and autotrophic denitrification. A prototype RCB has been operated for several months on an analog urine wastewater (78% of nitrogen in on-site wastewater originates from toilet flushing, the majority from urine). Initially, only O_2 was supplied to establish nitrification, with progressively higher flow rates and accumulation of nitrite and nitrate. When H_2 introduction began on day 69, dramatic decreases in nitrate and nitrite quickly followed, and 70% total nitrogen removal was achieved within 11 days. The rapid response to H_2 suggests that the RCB has a resiliency to varying loadings, which is important to on-site application. Areal nitrification rates have been 5 to 6 g m^{-2} day^{-1} consistently, and are the highest rates reported for hollow fiber bioreactors. The total nitrogen removal rates are 4 g/m^2 day, and indicate that the treatment potential of the RCB is quite high.

c) Objectives

The proposed research will provide a proof of concept of a novel reactor module design for nitrogen removal from Septic Tank effluent, specifically addressed Research

Focus Area 2a. in the original Request for Proposals, to *"Develop novel and cost-effective technologies or methods to reduce or eliminate nutrient inputs to watersheds from wastewater…"* The goal of this project is to develop and test membrane aeration biofilm reactors (MABR) for advanced treatment of septic tank effluent before discharge to a soil adsorption field or other appropriate system. Specific objective include:

1) Design and construct a prototype hollow fiber membrane reactor module for direct, primary treatment of septic tank effluent (STE).
2) Design and construct a dual hollow fiber reactor module involving the delivery of both O_2 and H_2 for secondary treatment of effluent from the stage 1 reactor, allowing for enhanced nitrogen removal.
3) Continuously operate the linked reactors using actual septic tank effluent.

d) Methods

Description of Reactors

The reactors, picture in Figure 1, were comprised of 1) gas delivery, 2) liquid delivery, 3) and monitoring/control systems.

Room air (via the facility compressed gas system) was used to deliver O_2 to all tests of the first stage reactor (HFMBR1) and to oxic fibers of the RCB during some tests Air flow was controlled using an local regulator set at 35 psi then connected to the Mass Gas Flow Controller (GFC) with a range of 0 – 10 sccm (ml/min). Breathing air flows through the bioreactor and then exits to a back pressure valve followed by a mass gas flowmeter (GFM). In certain tests with the RCB, pure O_2 was delivered via a compressed gas cylinder connected to the GFC. Hydrogen gas produced by the H_2 generator has a regulated pressure of 35 psi. Hydrogen gas flows through the GFC to the bioreactor and exits to a back pressure valve followed by a GFM. The outflow is routed to a fume hood for safety.

A larger peristaltic pump (Master Flex, Model 7550-30) recirculates liquid in the reactor to promote mixing while a smaller pump (Master Flex, Model 7523-70) adds the influent. HFMBR1 contained a set of 5 spray nozzles installed on the outer shell of the reactor. Spray nozzles are used for the recirculation of the fluid in a cross flow pattern and for periodic cleaning of biofilms from the membranes with DI water. In the RCB, recirculation and feed are added in a single port at the end of the reactor. The pH probe (Omega) and DO probe (Sensorex) reside in an enclosed box (sensor cell) that is included in the recirculation loop.

All sensors (including pH probes, DO probes, mass gas flow in/out, gas pressure transducers in/out, CO_2, temperature, liquid pressure transducer) are connected to Opto22 input and output modules. Opto22 ioControl is software designed to set up programmable logic control and monitoring data. Opto22 ioDisplay software is designed to create graphical representations of the data in real time and historically stored files

Reactor performance was monitored through analysis of influent and effluent chemistry. Samples for N ion ((NH_4^+, NO_3^-, NO_2^-) analysis were filtered using a 0.2 um Millipore syringe filter (Cole Parmer, A-02915-90) and immediately stored at -20°C until analysis using a Dionex-120 Ion Chromatograph (Sunnyvale, CA) linked to Chromeleon software (PeakNet version 6.4, Sunnyvale, CA). Dionex certified anion (Inorganic Ventures, Inc., DYNCO-ICAL-1, ICNO21-1) and cation (Inorganic Ventures, Inc., DC-

ICAL-1) standards were prepared from frozen stock aliquots each week and used at the beginning of each IC run. A set of check standards (blank, anion, cation, and another blank) were run every 15 samples for verification that the retention times have not changed during the analysis process.

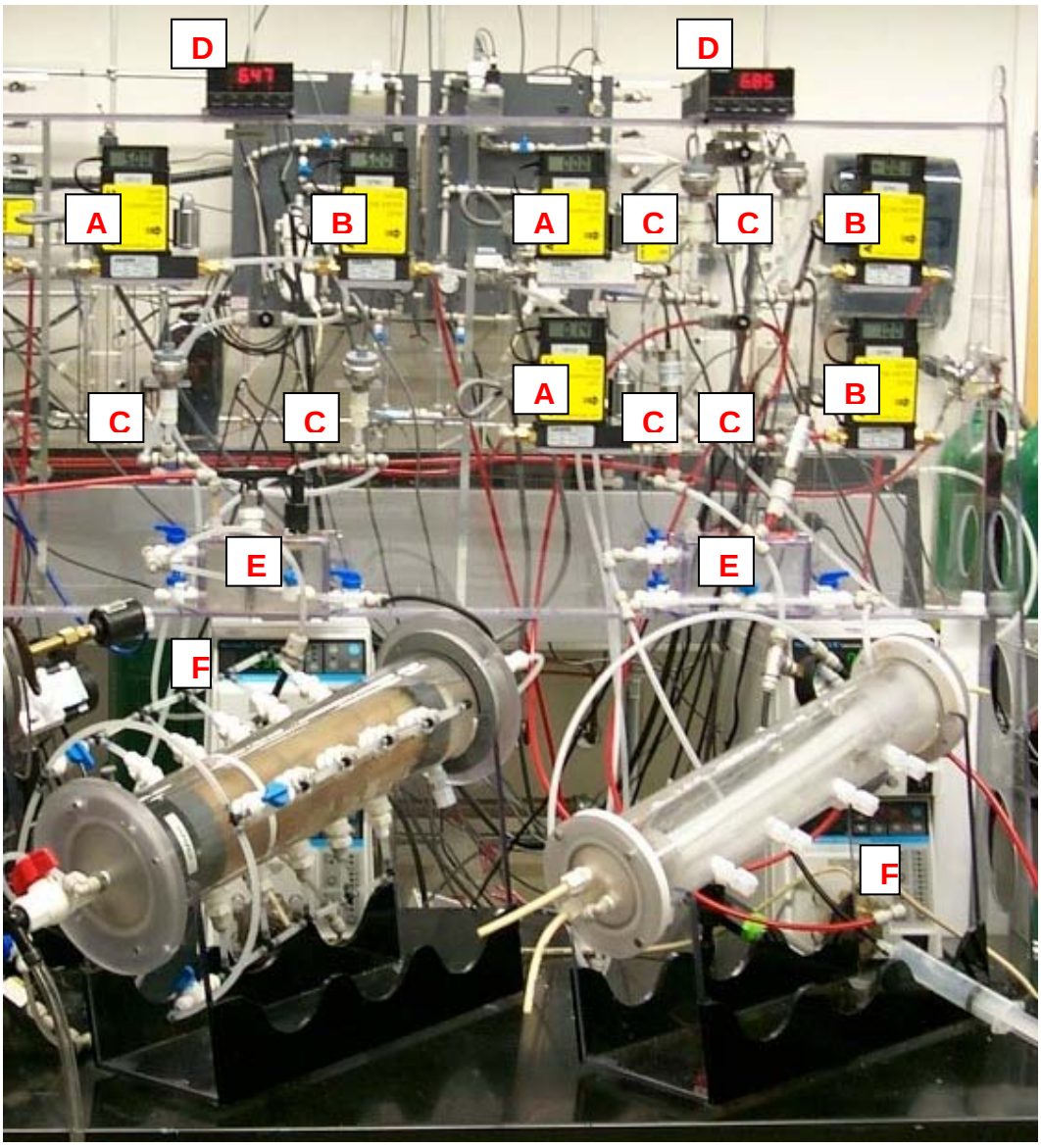

Figure 1. Overall diagram of the HFMBR1(left) and RCB (right) reactors.

A. Mass Gas Flow Controllers – Aalborg, Model GFC17, Accuracy +/- 1.5%
B. Mass Gas Flowmeters – Aalborg, Model GFM17, Accuracy +/- 1.5%
C. Pressure Transducers – Setra, Model 209, Accuracy +/- 1%
D. pH Meters – Omega, Model PHCN-37, Accuracy +/- 0.5%
E. DO Meters – Sensorex, Model DO1200, Accuracy +/- 2%
F Peristaltic Pumps – Master Flex, Model 7550-30, Model 7523-70

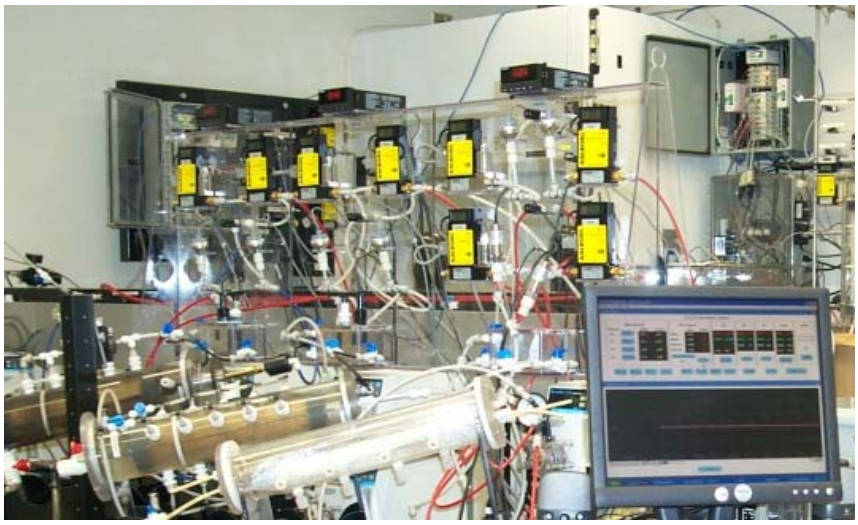

Figure 2. Photograph of the overall reactor assembly showing the Opto22 ioDisplay Software (lower right)

For COD analysis (Clesceri et al., 2003), samples were collected in glass vials thoroughly rinsed with 20% H_2SO_4, or ignited in a muffle furnace at 500°C for 1 hour. Samples were preserved at 4°C with the addition of sulfuric acid for a final pH < 2. Potassium acid phthalate stock was diluted to a standard range of 20 to 900 mg/L. Samples (run in triplicate), standards, and blanks were sealed into tubes after the addition of dichromate, and heated in a block digester (CPI International, Mod Block) at 150°C for two hours. Once cool, the samples were measured on a spectrophotometer (Beckman Coulter, Du 800) at 600 nm. Any oxidation of inorganic species was taken into account and the COD value adjusted. Tri-weekly sampling will occur during all phases of the laboratory and field testing.

A WTW OxiTop system (OxiTop OC100 and OxiTop-C) was be used to evaluate the C-BOD_5 removal efficiency of the reactor. Triplicate effluent and feed samples were tested using twelve amber bottles, set up on a continuous magnetic stir plate within an incubator set at 20°C. The samples were diluted according to the target measuring range as described in the OxiTop manual. Sodium hydroxide tablets were placed in the seal cup at the top of each sample bottle and then tightly capped by the measuring head. Each measuring head recorded 360 values per day (approximately every 4 minutes) during the nominal BOD test lasting 5.25 days.

Total alkalinity was monitored following the standard method ((Clesceri et al., 2003). Addition of 0.1N hydrochloric acid reacted with the hydroxyl ions in the samples and the titration end-point pH depended on the total carbonate species originally present in the samples. The 0.1N HCL acid solution was standardized against a 40 mL 0.05N Na_2CO_3 solution and 60 mL water titrated to pH 5, and then boiled for 3 to 5 minutes. This solution was titrated to the pH inflection point and normality was calculated. The standard 0.1N solution equated to 5.0 mg of $CaCO_3$.

Analysis of the biofilms from the HFMBR1 was conducted at the end of the experiment to verify the presence of nitrifying bacteria. The HF module was removed from the reactor and several individual HF were excised from both the outer and inner portions of the module. Biological oxygen consumption by the biofilms in response to addition of ammonia, nitrite, or organics was assessed using a microtiter-based oxygen sensor system (Garland et al., 2003; Smith et al., 2007). Separate 20 mm pieces of intact tubing (three from both the inner and outer fibers) were placed in sterile deionized water containing glass beads and the biofilm was disrupted by shaking, Resulting biofilm suspensions were added to BDoxy microtiter plates (BD Biosciences, Bedford MA), along with one of the following energy sources (final concentration): NH_4-N (100 mg L^{-1}), NO^2–N (100 mg L^{-1}), and propionic acid (100 ppm). Oxygen consumption in the plates was monitored by measuring the fluorescence of an oxygen quenched, ruthenium-based dye embedded into a gel layer on the bottom of the plates. Fluorescence was measured every 30 min for 48 h at 258C using a Wallac Victor2 Fluorometer (Perkin Elmer, Wellesly, MA) with 485 nm excitation/590 nm emission filters. Biofilm samples were also analyzed using fluorescent in situ hybridization (FISH). Intact biofilm sections fixed, sectioned, and hybridized as described in Smith et alk., 2007.

Results

HFMBR 1 Development and Testing

Initial testing of the HFMBR1 reactor indicated that long term continuous operation of the systems lead to decreased performance (Fig. 3 and 4), most likely due to the observed "overgrowth" of biofilm on the fibers and concomitant reduction in mass transfer of material through the fiber bundle. this was a generally expected result. We addressed this issue by periodic cleaning of fibers using spray nozzles attached to the reactor shell. The cleaning process involved 4 spray nozzles and rotation of the fiber module to provide more uniform exposure of the fibers to the spray. The cleaning process readily removed the majority of the biofilms, with the solids drained from the bottom of the reactor. We conducted seven separate tests of the effects of cleaning with durations of 14 to 42 days. Overall, the data (Fig. 5) indicated that performance was enhanced (BOD and filtered COD less than 30 mg/L compared to 250 mg/L in the feed) during the first 3 weeks after cleaning, and that performance declined with longer operation. The COD and BOD data have some discrepancies (i.e., BOD is high in run 1 and COD is high in run 4 during the first few weeks), but the general trend of superior post-cleaning performance held for the first 4 runs. However, later runs (5, 6, and 7) exhibited a more rapid deterioration in performance, with BOD and filtered COD levels exceeding 40-50 mg/L within 14 days. Decreased performance with time is most likely caused by increasing amounts of residual biomass left on the fibers on areas not directly exposed to the spray nozzles and subsequent higher rates of biofilm regrowth. We tested the possible effects of fiber density on the cleaning effectiv4ness by comparing the performance of the original module (263 fibers) with a new lower density (108 fiber) design. The effluent quality of the lower fiber density module, based on both filtered and unfiltered COD levels, was consistently better than that observed in the previous tests at the higher fiber density (Figure 6). Levels of filtered COD were existentially zero (i.e., below the minimum detection limit of 10 mg/L for most samplings up to 14 days after cleaning. Overall, the results suggest both an operating procedure (i.e., regular cleaning at

2 week intervals) and reactor configuration (i.e., lower fiber density) which will produce effluent of sufficient quality to not cause excessive heterotrophic growth in the RCB modules.

The HFMBR1 feed was switched to actual septic tank effluent (STE) beginning in 2007, and operated continuously with these feed for nearly 250 days. The HFMBR1 effectively treated the STE, with reactor effluent quality similar to previous testing with the septic tank analog (Fig 7.) Removal of organic material remained relatively consistent throughout the study, with BOD levels averaging 20-30 ppm (Fig. 8). The hydraulic loading rate was doubled on day 139, increasing the COD loading rate from ~ 2 g m^{-2} d^{-1} to ~4 g m^{-2} d^{-1}. COD removal rate, therefore increased from ~1.5 g m^{-2} d^{-1} before day 139 to ~3 g m^{-2} d^{-1} after day 139 (Fig 8).

Evidence of nitrification was observed soon after initiation of STE flow, with NH_4-N levels steadily dropping to levels below 10 ppm by day 130 (Fig. 8). NO_3-N levels increased in the effluent during this period (data not shown), increasing to 40-50 ppm. The increased loading rate at day 139 caused a slight increased in effluent NH_4-N levels, which averaged 25 ppm for the remainder of the study. The areal rate of nitrification increased from ~0.8 g m^{-2} d^{-1} to 1.5 g m^{-2} d^{-1} in response to the increased loading rate. NO_3-N levels decreased sharply after day 139, averaging ~10 ppm, suggesting that the increasing loading rate enhanced areal denitrification.

In order to verify the presence of nitrifying organisms within biofilms of the HFMBR1 reactor, physiological and molecular profiling was performed at the end of the experiment. Results indicated that 1) nitrifying activity (Fig. 9) and biomass (Fig. 10) was presented in the biofilm both before and after the cleaning, and 2) cleaning reduced overall biofilm thickness and seemed to preferentially remove heterotrophic organisms. Thickness of the biofilm was obviously effected by the washing and measurements showed approximately a 31 % decrease (n=20) after washing in the sections of biofilm measured. Before washing the average thickness was 141.8 μm $\pm$ 30.5 ranging from 68 um to 242.5 μm. After washing biofilm average thickness was 98.4 μm $\pm$ 25.1 ranging from 54.2 to 152.75 μm. FISH shows no difference in the presence of AOB/NOB in the biofilm after washing of the fibers. (Fig 10).

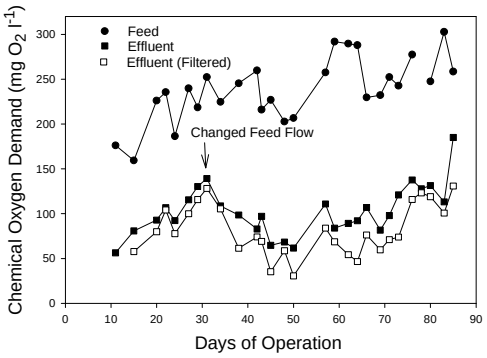

Figure 3. Performance of the HFMBR1 before and changing the feed from a longitudinal to cross flow mode.

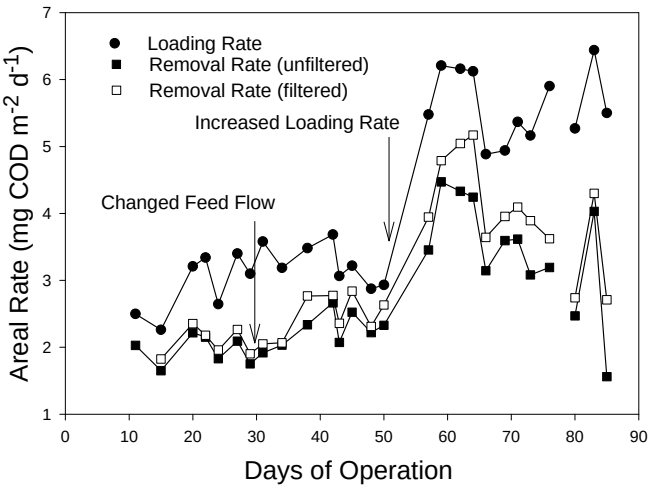

Figure 4. Performance of the HFMBR1 (expressed as a COD removal rate per m^{-2} of membrane area) before and changing the feed from a longitudinal to cross flow mode

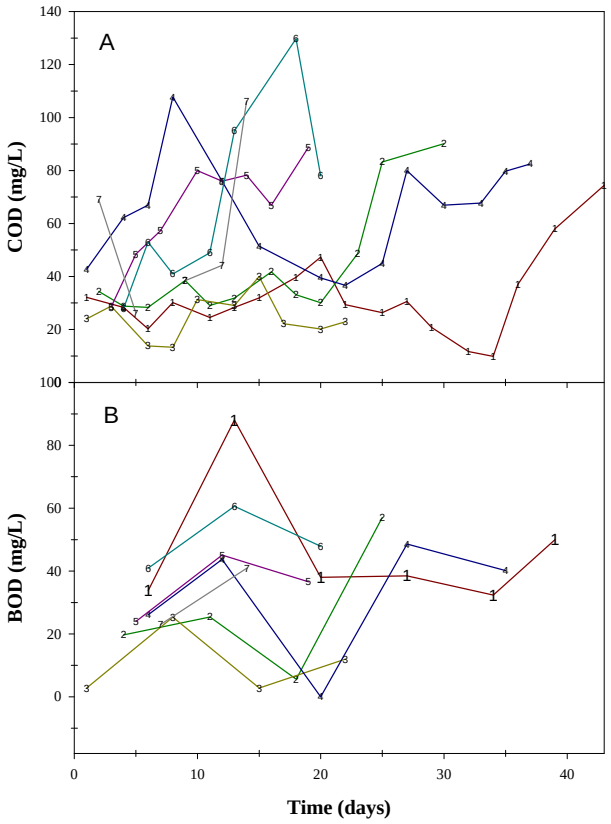

Figure 5. Effects of periodic cleaning of the fibers via spray washing on performance of the HFMBR1 reactor. X-axis represents time from cleaning of 7 consecutive trials.

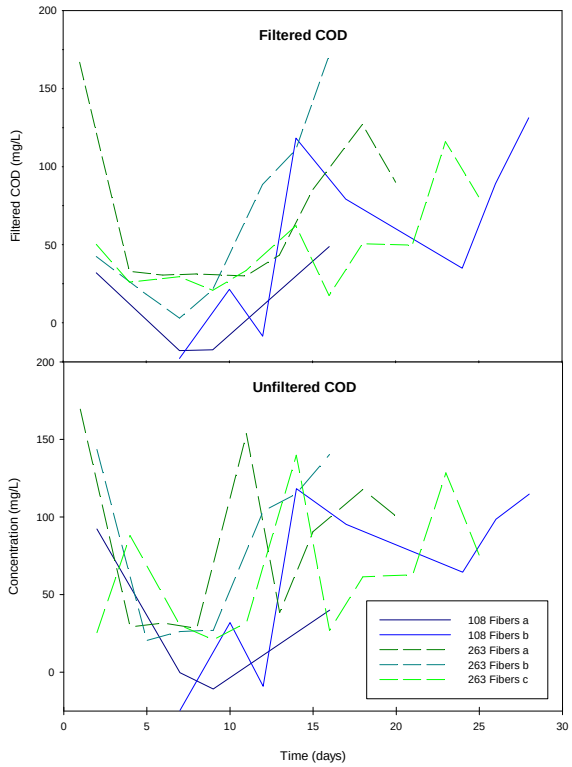

Figure 6. Comparison of performance between HFMBR1 containing a high and low fiber density. Lines represent separate trials performed sequentially, with the x-axis representing time from module cleaning.

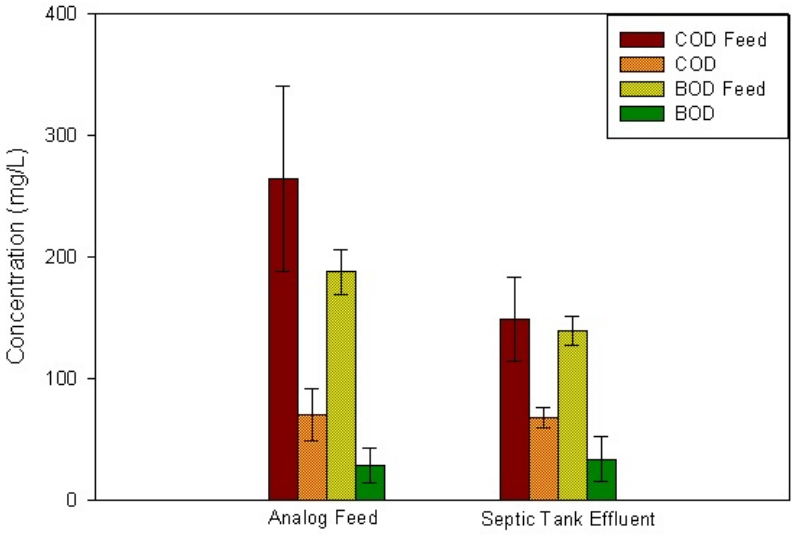

Figure 7. Comparison of HFMBR1 performance when feed analog or actual septic tank effluent.

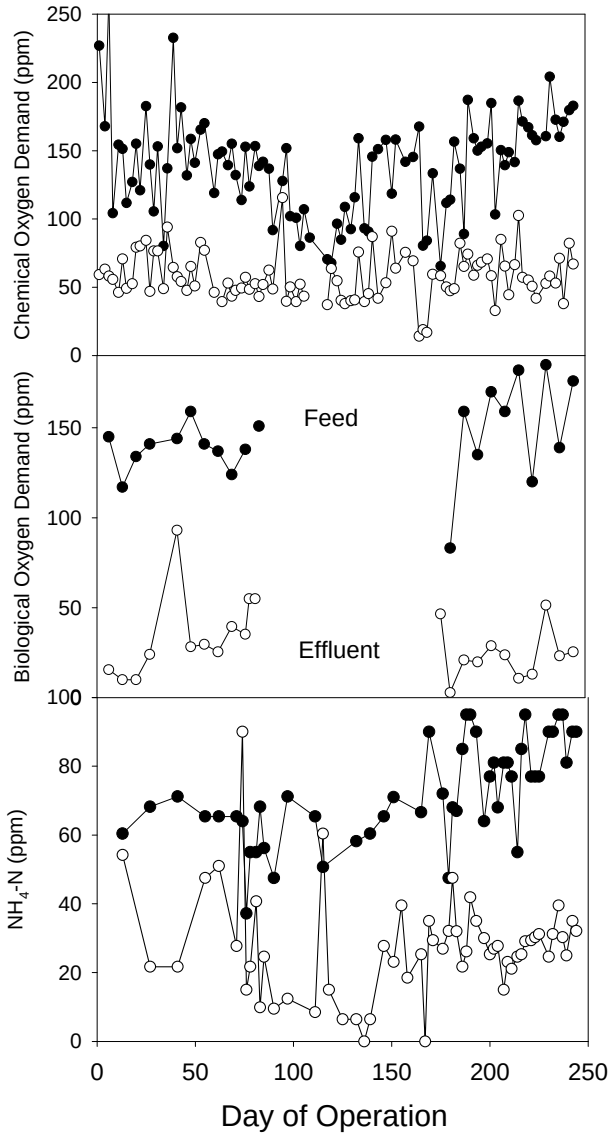

Figure 8. Performance of HFMBR1 during continuous operation with actual STE as feed. Contaminant levels reported for effluent (open symbols) and feed (filled symbols).

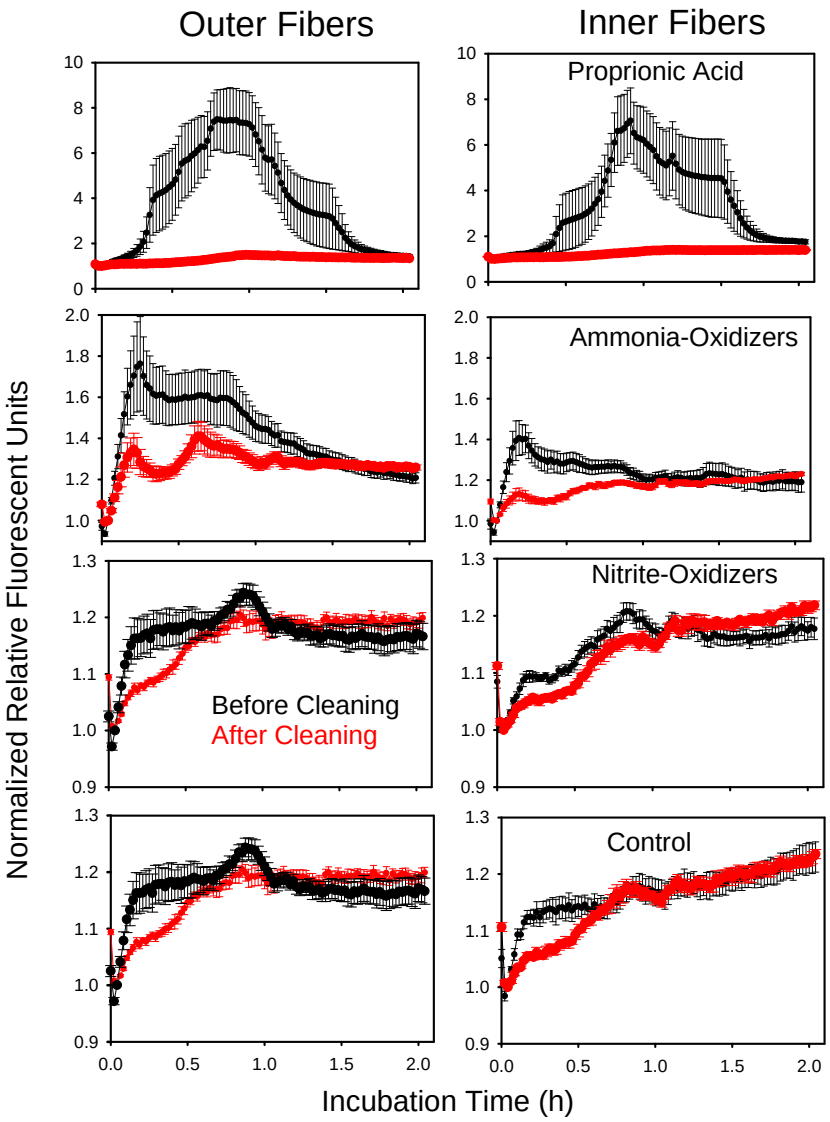

Fig. 9. Results from physiological testing of biofilm samples from the harvest of the HFMBR1 reactor both before and after cleaning of the fiber module. Graphs represent fluorescence of an O_2 sensitive dye, with larger values indicating greater oxygen use. Lines represent mean and standard deviation of three samples. Data show a dramatic decline in heterotrophic activity with cleaning, and slightly lower rates on the inner versus outer fibers. Ammonia oxidizing activity is clearly detectable (greater than the background response, or control without any carbon or nitrogen addition), and also decreases (but less dramatically) with cleaning. Nitrite oxidizing activity was not greater than background response.

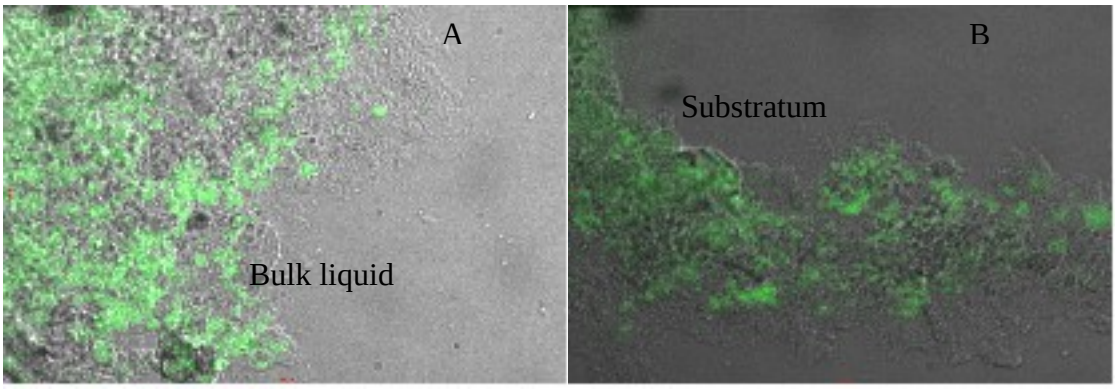

Figure 10a. Confocal laser scanning micrographs of cross sections of biofilm removed from silastic tubing before (A) and after (B) washing. The dominant nitrite oxidizing species present in both biofilms was *Nitrospira* (shown in green). Probes used for the detection of NOB were Ntspa 685, NSR 826 and NSR 1156. *Nitrobacter* (detected by Nit 3 probe) was hardly detectable. This is consistent with findings that *Nitrospira* is the dominating NOB in wwtp. In controlled experiments it has been shown that *Nitrobacter* grows rapidly when substrate (nitrite) is readily available but as this resource is depleted, the slower growing *Nitrospira* will be the dominant NOB.

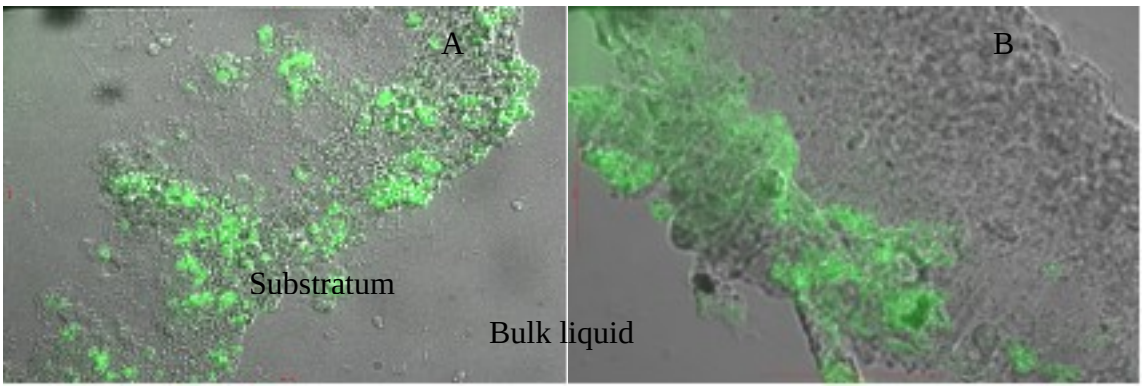

Figure 10b. Confocal laser scanning micrographs of cross sections of biofilm removed from silastic tubing before (A) and after (B) washing. These images show the presence of the ammonia oxidizing bacteria, *Nitrosomonas* (in green). All images are 600X.
Nitrosomonas is detected by the probe NSM 156. It has been hypothesized and demonstrated in controlled studies that in systems with higher ammonia concentrations, Nitrosomonas dominates over Nitrosospira, which is found in systems with lower ammonia concentrations. Both AOB species were detected in the before and after washing biofilm samples.

RCB Development and Testing

The RCB reactor was initiated with an air-only mode. Nitrification steadily increased for the first 40 days of operation, reaching an areal rate of 1-1.25 g NH_4-N converted per m^2 of membrane area per day (Fig. 11). At this time, effluent NH_4-N was

10 to 15 mg/L. The back pressure of the lumen gas was increased stepwise over the first month of operation to 5 psi in order to maximize gas transfer across the membrane. Back pressure was not further increased to ensure the integrity of the membrane module. The areal nitrification rate was considerably less that that observed in our previous proof-of-concept testing with the RCB (i.e., ~5 g m^{-2} d^{-1}), but the lower rate was consistent with the use of air (as opposed to pure O_2) in the present study and a concomitantly lower flux of O_2. H_2 flow was initiated through the juxtaposed, second set of fibers on Day 44. Denitrification developed rapidly, with NO_3-N levels reaching near zero within 7 days. The rapid development of denitrification was consistent with our previous tests with the RC B. Starting one week after H_2 initiation, however, a reduction in nitrification rate was observed, with near complete inhibition of nitrification reached by day 59. On day 59, H_2 flow was reduced from 2 sccm to 1 sccm to see if the apparent inhibitory effect of H_2 could be reversed, but no such effect was observed. The recycle rate was reduced at day 92 in hopes that reduced liquid velocity (and mass transfer between O_2 and H_2 fibers) might improve nitrification, but no positive effect was observed.

The strong inhibitory effect of H_2 was surprising given results from our proof-of-concept testing in which stable nitrification and hydrogenptrophic denitrification occurred for over 100 days. The exact mechanism of the inhibition was not clear, but we suspect it is not a direct effect of H_2 since it was not observed immediately upon H_2 addition, but rather after about 1 week. One hypothesis is that excess H_2 leads to development of H_2 oxidizing bacteria in the reactor, leading to direct competition with the nitrifiers for oxygen resources. Given the greater energy yield from the aerobic oxidation of H_2 versus NH_4 or NO_2, it would not be surprising if nitrifiers were rapidly displaced on the aerobic fibers if H_2 was available. An alternative explanation may be that the hydrogenotrophic denitrifying biofilms on the H_2 fibers release organic material which can be a utilized as a substrate by heterotrophs, leading to competition between the nitrifiers and aerobic heterotrophs on the O_2 fibers. Either of these potential inhibitory effects may not have been observed in our previous, proof-of-concept testing due to the higher areal rates of nitrification (i.e., 4-5 g m^{-2} d^{-1} vs 1-1.5 g m^{-2} d^{-1}) in that study. The potential for excess H_2 leading to H_2-oxidixers on the O_2 would have been less in the previous study since the areal denitrification rate (and concomitant consumption of H2) was greater. In addition, the initial study featured higher areal nitrification rates on the aerobic biofilms, which would have produced thicker nitrifying biofilms better able to withstand the potential impacts of organics produced by denitrifiers. Confocal microscopic images of the biofilms on the aerobic fibers from the proof-of-concept testing showed a thick nitrifying layer directly adjacent to the fibers with a non-nitrifying outer layer in contact with liquid. The non-nitrifying layer may have been composed of either H_2-oxidizers or aerobic heterotrophs feeding on organics produced by the biofilms on the adjacent denitrifying fibers. In either case, the thicker biofilm resulting form the high rates of O_2 diffusion and higher areal nitrification rates allowed for a stable nitrifying biofilms with inner layers close to the membrane, hypothetically affording protection to nitrifier metabolism. The visually thinner biofilms in the present study may not have allowed for this spatial separation.

Several strategies for ameliorating the negative impacts of H_2 can be envisioned. The simplest involves decreasing the amount of H_2 delivered to the reactor. Decreasing H_2 flux via reducing back pressure is not possible since the back pressure valve was

completely open during these tests. Incorporating less H_2 membrane surface area is another alternative, but would involve reconfiguring the reactor design. Phasic delivery of H_2 (i.e., cycling the flow on and off) is a simpler alternative which we decided to implement. Another approach to reducing the impacts of H_2 is increasing nitrification rate by switching to pure O_2 in the lumen. We decided to evaluate the reactor performance using pure O_2 as the lumen gas prior to the introduction of phasic H_2 delivery.

The change to pure O_2 on day 101 caused an expected increased in areal nitrification rate, but the stimulation was relatively modest (i.e., doubling to ~2 g m^{-2} d^{-1}) compared to the levels observed in the proof-of concept testing. Another major difference between the proof-of-concept and current testing was the higher levels of residual NH_4-N in the proof-of concept reactor (i.e., 50-75 ppm versus 10-15 ppm). The higher residual levels in the first study, despite the higher areal conversion rates noted above, were caused by the much higher feed levels of NH_4-N used in the proof-of-concept (217 ppm) versus the current (45 ppm) tests. The higher residual levels of NH_4-N would facilitate mass transfer of substrate to the aerobic fibers, thus promoting higher areal conversion rates. This difference in results suggests that the high level of residual NH_4-N in the first study and concomitant stimulation of mass transfer from bulk liquid to biofilm is an important factor in the observed higher nitrification rates. Given our processing goals in the present study (i.e., reducing NH_4-N levels from ~50 ppm to less than 5 ppm), a high level of residual NH_4-N is not a desired operating condition. However, a possible solution to achieving low effluent levels with reasonable high nitrification rates is to increase liquid flow in order to enhance bulk liquid/biofilm mass transfer Liquid recycle was increased from 300 ml min^{-1} to 1000 ml min^{-1} Day 150. Nitrification rate increased, and NH_4-N levels dropped to <5 ppm after having been consistently between 8-12 ppm. A further increase in liquid recycle to 1500 ml min^{-1} did not cause further increases in nitrification.

Two modifications in the RCB operation were defined to help improve system performance 1) cross flow of liquid to help increase mass transfer to fibers at the interior of the fiber module, and 2) pulsed addition of H_2 to eliminate negative impacts of excess H_2 on nitrification. The necessary modifications to the reactor and associated control systems were completed, and a test with RCB (feed with he organic-free STE analog) was initiated. We operated the reactor under a higher areal loading (3.0 g m^{-2} day^{-1}) during the first 30 days during which time we observed high areal nitrification rates, although effluent NH_4-N remained above 10 ppm (Fig 12). We decreased loading to 1.5 g m^{-2} day^{-1} in an effort to achieve lower effluent levels, but NH_4-N concentration remained at an average of 10 ppm for the nearly four weeks of continuous operation. However, DO was significantly reduced inside the fiber module throughout the first 60 days of operation, suggesting that the cross flow significantly increased mass transfer/mixing to depth in the fiber module. In previous trials without cross flow, we observed high levels of Do within the fiber module, but cross flow resulted in better mixing and concomitant localized areas of DO only very near individual fibers (data not shown). This was a promising results since the excess O_2 in the fiber module was one potential case for the inhibitory effects of H_2 observed in the previous trials; excess O_2 could have led to the growth of H_2-oxidizing bacteria (rather than/ or in addition to hydrogenotrophic denitrifiers) leading to competitive interactions with the nitrifiers.

Given this result, we initiated H_2 delivery on day 58. Rapid decreases in nitrification were observed as in the previous test. We initiated pulsing of H_2 in an attempt to decrease the inhibitory effect. The initial pulsing frequency was 1 minute on followed by 9 minutes off, but we decided to increase the on time to five minutes (followed by 25 min off) in order to achieve pressurization of the module and a more constant, measurable flux of H_2. Nitrification continued to decline, with effluent level of NH_4-N reaching 30-40 ppm. Increasing off time to 55 minutes appeared to stabilize the nitrification, but we decided to terminate the test at this point based on observation of severe biofilm sloughing.

These results offer promise for our ability to stably operate the RCB under simultaneous nitrification and hydrogenotrophic denitrification. The elimination of the excess dissolved O_2 via cross-flow allows for the steep redox gradients between juxtaposed fibers critical to the simultaneous processes. The ability to pulse the H_2 flux increases the possibility of limiting the persistent problem of H_2 inhibition of nitrification. Based on our previous data, we decided on the following strategy for operation of the RCB to alleviate the potential effects of H_2 interference on nitrification. We operated the RCB under high NH_4-loading (i.e., at least 3 g m^{-2} d^{-1}) to produce thicker biofilms which may be more resistant to H_2 interference. O_2 rather than air was used as the lumen gas to stimulate areal rates of nitrificaiton. In addition, H_2 flow was initiated progressively so as not to produce large fluxes of H_2 before the denitrifying biofilms were established. This was effected by pulsing the H_2 flux, beginning at 5 min on during a 1 hour period with gradual increases over several weeks until H_2 flow was continuous. This approach was successful at establishing concurrent nitrification and denitrification on the O_2 and H_2 fibers, respectively. At that point, the feed to the RCB was switched from the analog mixture to the effluent from the HFMBR1 which was processing actual STE. The reactors were operated in a linked mode for nearly 80 days. The RCB effluent quality was excellent (i.e., combined levels of NH_4-N and NO_3-N < 10 ppm) for nearly 40 days, at which point nitrification appeared to decrease. Visible inspection of the reactor showed a nearly continuous biofilm over the fiber module, so we decided to clean the module by removing and lightly spraying with water. Performance appeared to respond positively, but only transiently, so the study was terminated after 79 days. Despite the continuous biofilm growth which seem to "bridge" across fibers, juxtaposed O_2 and H_2 fibers which clearly discernable with the former appearing white while the later were black (Fig. 13).

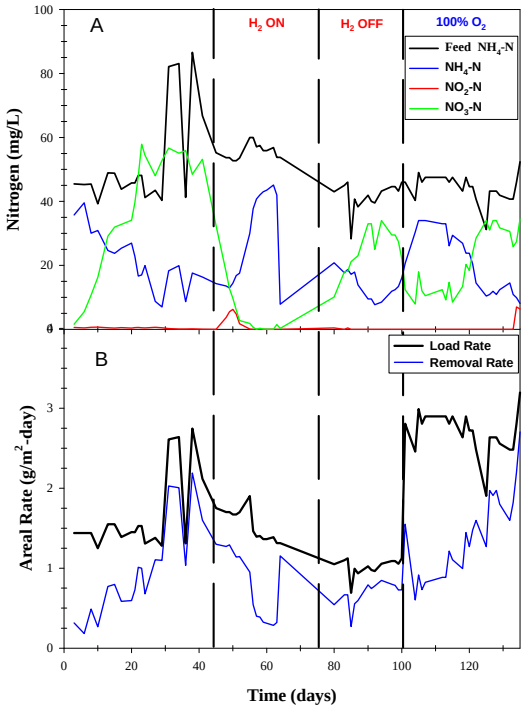

Figure 11. RCB performance in response to H_2 addition and the switch to pure O_2.

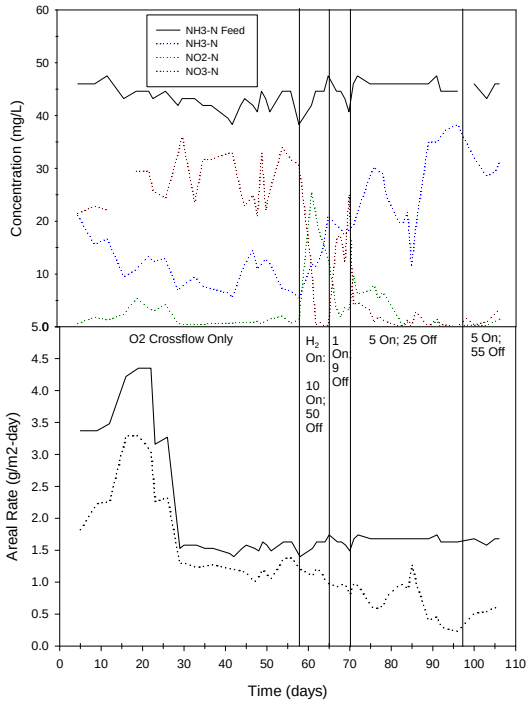

Figure 12. RCB performance under cross-flow conditions and in response to phasic H_2 delivery.

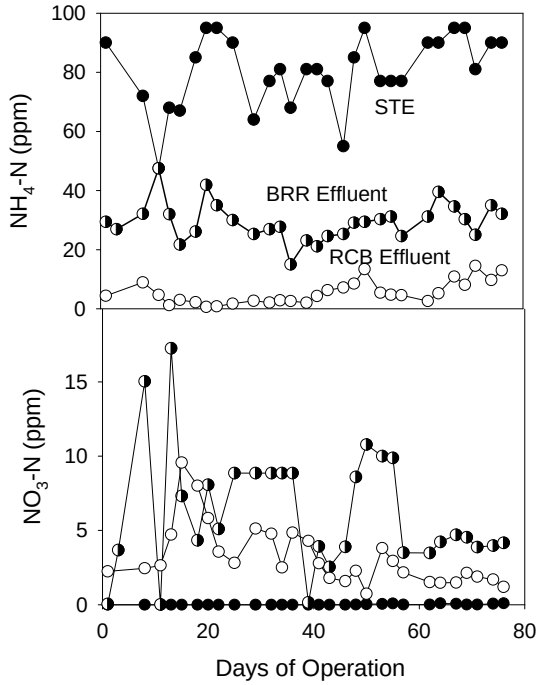

Figure 13. Performance of the linked HFMBR1(labeled BRR) and RCB reactors.

Figure 14. Picture of the RCB fiber module at harvest. Juxtaposed H_2 (black) and O_2 (white) fibers are clearly visible on the right portion of the module.

e) Discussion

Hollow fibers for aeration provide a unique physicochemical environment for biological treatment. Biofilm establish and self-organize in response to a spatial transect that extends from the hollow fiber surface to the bulk liquid. The inverse, bidirectional flow of O_2 (from the fiber) and electron donors such as NH_4-N and organics (from the liquid) provide for unique spatial differentiations of metabolic function across the biofilm. These reactions are often mediated by facultative heterotrophs. Ammonium is used in autotrophic biochemical reactions of nitrosyfication (ammonium to nitrite) and nitrification (nitrite to nitrate). The autotrophic microorganisms have relatively low yield coefficients, low growth rates, and are obligatory oxygenotrophs. As such, they are often out competed by heterotrophs in the mixed reaction systems that are characteristic of domestic sanitation water treatment. By contrast, heterotrophic organisms have much higher yield coefficients and maximum growth rates, and are metabolically more flexible.

Previous studies of membrane aerated hollow fiber reactors have shown that ammonium oxidizing bacteria accumulated near the membrane while other microorganisms (i.e. facultative heterotrophs) predominate near the biofilm/bulk water interface (Hibiya et al., 2003;Terada et al., 2003). It was found that Nitrosomonas and Nitrobacter populations were high near the fiber surface and was hypothesized to be due to less competition for oxygen at that location (Yamagiwa et al., 1998). In a hollow fiber oxygenation bioreactor treating municipal wastewater and achieving simultaneous nitrification and denitrification, the effect of water spray cleaning on activity rates of nitrification and denitrification was investigated; it was found that the cleaning procedure had minimal effect on nitrification rates while denitrification rates were substantially impacted (Suzuki et al., 2000). This result corroborates the observations of the effect of cleaning events on BRR performance.

A multi-species biofilm model was used to predict microbial species composition and differentiation in the biofilms of reactors containing gas permeable hollow fiber membrane for oxygen supply (Shanahan and Semmens 2004). The model included aerobic oxidation of organics, nitrification, and denitrification. Model simulations predicted that a nitrifying microbial population could be sustained within the biofilm of a continuously operated bioreactor and that nitrifiers would occupy a biofilm layer closer to the membrane surface than denitrification microorganisms, which would predominate at outer biofilm layers adjacent to the bulk liquid. The model prediction of sustained nitrifier populations in biofilm regions closer to the hollow fiber membrane than denitrifiers are based on basic kinetic models of microbial growth and diffusive mass transfer. One limitation of the model is that nitrosyfication (ammonium to nitrite) and nitrification (nitrite to nitrate) are modeled as a single reaction. Another perspective can be gained by considering that nitrite generated by nitrosyfication (ammonium to nitrite) can be used denitrified directly by heterotrophs. This leads to the potential for a syntrophic enhancement of ammonium oxidation at the biofilm layers closer to the membrane surface than denitrifiers, where ammonium oxidation to nitrite (i.e. nitrosyfication0 occur syntrophically with heterotrophic denitrification of nitrite. In this case, the heterotrophic utilization of nitrite in syntrophic co-culture with ammonia oxidation would locally out compete nitrite oxidation to nitrate.

Our results are thus corroborated with other experimental research and modeling efforts, which provide the supporting concepts for extension to a technological system for onsite treatment. This is the first demonstration of continuous operation of a hollow fiber oxygenation reactor for onsite treatment of STE. The ability to biofilms to self-organize into a stable nitrifying/denitrifying state without any exogenous control other than the automated cleaning procedure is particularly attractive for on-site systems in which owner management requirements should be minimal. This type of reactor has significant potential for primary treatment of septic tank effluent (or raw wastewater after some large particle separation).

Despite the better than expected N removal capabilities of the primary treatment HFMBR, the low C/N ration of on site wastewater still necessitates further treatment for enhanced nitrogen removal. Linked operation of the HFMBR1 and the RCB did provide high quality effluent, but stable operation of the RCB was difficult. The inability to reproduce our proof-of-concept results with the RCB (i.e., sustained, simultaneous nitrification and hydrogenotrophic denitrification) may have multiple causes. Our proof-of-concept testing found evidence for either direct or indirect inhibitory effects of H_2 on nitrification; this effect appeared to be exacerbated in the present study due to the thinner, less-resilient nitrifying biofilms which develop in response to lower NH_4-N loading. We were able to successfully increase nitrifying biofilm thickness throughout the fiber module by introducing cross-flow for enhanced mass transfer, switching to pure O_2 (rather air) for the lumen gas, and by increasing NH_4-N loading. Phasic delivery of H_2, particularly during development of biofilms on the H_2 fibers, appeared to diminish the inhibitory effect on nitrification. Long term operation of the RCB with a waste stream derived from actual STE was only transiently effective, potentially due to interactions with biological sulfur cycling. STE contains significant levels of sulfate derived from S-containing proteins, and we observed visual evidence for reduced sulfur on the H_2 fibers (black deposits) and oxidized sulfur on the O_2 fibers (white deposits).
Overall, our results indicate that on-site application of the RCB for removal of nitrogen requires significantly more development work before stable, effective systems can be applied.

3. Utilization

a) End User Application

The technology is still under development and has not been used for field treatment to date.]

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b) **Intellectual Property and Partnerships**

A preliminary patent on the RCB concept has been submitted

c) Knowledge Exchange

Both the PI (Garland) and Co-PI (Smith) attended the National On-site Wastewater Recycling Association (NOWRA)'s 16th Annual Technical Education and International Program March 12-14th , 2007 in Baltimore, Maryland. A poster describing the basic approach and schedule of activities for the project was presented. Dr. Smith presented a paper on the project at the following Annual NOWRA Conference in

Memphis, Tennessee (April 7-10, 2008). Dr. Smith also presented the work at the American Society of Civil Engineers World Environmental and Water Resources Congress in Tampa, Florida (May 15-19, 2006). The paper outlined the objectives of the CICEET-funded project and reported results from the preliminary testing.

Dr. Garland presented a seminar to the Texas A&M's Professional Program in Biotechnology on March 31, 2008 titled "Ecological Design in Biotechnology: Structural and Functional Approaches to Microbial Community Assembly "; the presentation included data from the CICEET project.

The PI had continual interaction the Guana Tolomato Matanzas Reserve staff during the course of the project. He visited the site in the fall of 2007 to discuss possible installation of a demonstration reactor system at the GTM Reserve education center. Four staff members from the Guana Tolomato Matanzas River NERR visited the laboratory at KSC on June 10, 2008. We toured the laboratory installation of the unit and discussed the findings to date. We developed a teaching, demonstration model of the reactor for use by the GTM NERR staff at National Estuary Day in September 2008. The GTM-NERR staff is very interested in developing an on-site system, for both advanced treatment in their sensitive barrier island and as an outreach tool on ecological design linked to their marine educational center. The research team presented potential plans related to the RCB design as well as more passive, landscape systems, and the groups agreed to pursue upcoming funding opportunities.

The Pi and co-PI are prepared two manuscripts for peer-reviewed publication based on the present work, and plan to submit them for review by summer 2009.

4. Next Steps to Application

While further testing/development of the system are needed before technology can be marketed for on-site wastewater recycling, we believe these results provide a significant justification for further work. Specifically, the self-organization of nitrifying/denitrifying biofilms in the first stage reactor with no exogenous control other than the automated cleaning system provides the basis for the basis for energy efficient, low maintenance systems fro on-site treatment. The PI and co-PI are committed to pursuing funding for field testing of these concepts.

5. End User/ Producer/ Adopter Advisor(s) Feedback:

End User Advisor: Damann Anderson, PE
Organization: Hazen and Sawyer, P.C.
Location: 10002 Princess Palm Avenue, Tampa, FL 33619
Phone number: 813 630 4498
E-mail: danderson@hazenandsawyer.com

At this stage, what are the potential applications for this research? Please discuss how you and others could potentially use the technology. *This research could lead to an innovative solution to nitrogen removal from small wastewater flows, specifically individual home scale systems. There is a demand for simple technologies to accomplish*

this, as more jurisdictions in Florida and elsewhere are requiring nitrogen removal from onsite wastewater streams to meet ecosystem or public health goals.

What are the key challenges to application of this technology? Please consider the technology itself as well as issues related to regulation, politics, socio-economic pressures, trends in the field etc.
The key challenges I see for this technology is the ability to consistently meet treatment goals for BOD and TN while keeping operation and maintenance simple and inexpensive.

Has anything changed about this project's potential applicability since the last reporting period (not applicable to the first Progress Report)?
Not really, it seems like it may be more difficult than anticipated to meet treatment goals, but if these challenges are solved, the technology would have significant application in the field.

Questions/comments/ suggestions for the researchers?
Where do you anticipate disposing of the biofilm solids from the cleaning process in application?

PI Response to End User Advisor Feedback:
It is anticipated to return biofilm solids to the primary treatment (i.e. "septic tank") where they would undergo sedimentation and anaerobic digestion.

End User Advisor: Mark Flint, PE, President
Organization: Watermark Engineering Group, Inc.
Location: 1422 Apollo Beach Blvd., Apollo Beach, FL 33572
Phone number: 813 298 4797E-mail: mflint@watermarkengineers.com

At this stage, what are the potential applications for this research? Please discuss how you and others could potentially use the technology.
I believe the technology has potential for application in special case situations where proper monitoring, knowledge of operations, and maintenance are available. It appears that a balance process operation requires attention.

What are the key challenges to application of this technology? Please consider the technology itself as well as issues related to regulation, politics, socio-economic pressures, trends in the field etc.
In Florida, there is much interest in improving septic tank performance, and in particular providing effective passive N removal systems. This technology may require more intensive operations than others being studied, and it may therefore be challenged by other technologies in this field in practical application.

Has anything changed about this project's potential applicability since the last reporting period (not applicable to the first Progress Report)?
More data is available. The BRR-RCB reactor is now processing actual STE.

Questions/comments/ suggestions for the researchers?
None at this time.

PI Response to End User Advisor Feedback:
We agree with the need for passive, autonomous systems. That's why we concluded that the first stage reactors has more near term potential then the RCB design .

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