FINAL PROJECT REPORT

Project Title: **Control of Microbial Processes for Enhanced Water Treatment using Floating Island Treatment Systems**

Project number: **#09-26**

Submitted To: Montana Board of Research and Commercialization Technology

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Project Period: July 1, 2008 through June 30, 2010

State Funds Requested: $250,316 over the two year project period
$125,158 (Year 1) $125,158 (Year 2)

Non-state matching: $84,588 over the two-year project period
$42,294 (Year 1) $42,294 (Year 2)

PROJECT SUMMARY

This project, entitled "Control of Microbial Process for Enhanced Water Treatment using Floating Island Treatment Systems" (Project # 09-26) awarded during 2008-2010, has been conducted at the Center for Biofilm Engineering (CBE) at Montana State University in collaboration with the Floating Island International Inc. (FII) research facility located near Shepherd Montana. The goal of this project is to conduct research on the dynamics of microbial communities occurring in engineered floating islands used for water treatment. Accordingly our study team has carried out basic microbial process engineering research which provides a fundamental understanding of the microbial processes at work during contaminant uptake.

A summary of important research findings is as follows: Recirculating, aerated columns have been built and operated at MSU's Center for Biofilm Engineering. These columns contain 1) matrix material, carpet fibers, gravel and as well as a control with no material. These columns have been dosed with artificial waste water on multiple occasions and the aqueous concentrations of organic carbon (COD), ammonia, nitrite, and nitrate monitored over 2 to 3 week periods between dosings. These experiments have resulted in biofilm growth in all columns which is readily capable of removing COD and converting ammonia to nitrate (nitrification) and subsequently performing denitrification. The microbial biofilm communities associated with each substrate were also examined using PCR-DGGE molecular methods and shifts in the denitrifying community members were observed when nitrate levels decreased. Earlier research results have been published in Stewart et al. 2008. Our most recent results have been submitted for publication in the *Journal of Water Science and Technology* (special edition on the use of engineered wetlands for waste water treatment scheduled for 2010). This research has not only provided the basis for improving floating island design and efficacy, but also provides comprehensive scientific observations which explain how the floating island water treatment technology works. These research results are providing much needed scientific validation of floating islands as an emerging technology for nutrient removal from waterways. We have focused on removal of key waste water constituents including organics, ammonia, and nitrate.

This research has significant commercialization potential for FII whose business model is to develop then license its inventions. FII currently has a total of 7 licensees, of whom 6 have a manufacturing facility. Major product lines include floating island products for municipal wastewater and stormwater treatment, agricultural wastewater treatment, petroleum and mining waste remediation, wildlife habitat, shoreline erosion control and wave attenuation, waterscape beautification, and boat docking. FII is surrounding its technology with extensive international patent protection. However there remains the risk of piracy, particularly in economies not noted for IP compliance. The risk of pirated floating islands hitting retail stores in the US is perhaps higher in the "general" market. The municipal market is relatively protected from this given the large scale of most municipal projects, the custom-built nature of solutions and the relatively high integrity of public servants (on the whole). Probably the greatest risk associated with FII's position is the company's ability to cash flow through an IP enforcement action.

Include budget table here…..

PROJECT REPORT

REPORT FORMAT

This project report is organized to provide a full and complete narrative which documents and summarizes the results of the entire 2-year project. The report contains a brief introduction followed by four sections which correspond to the four 6-month reporting intervals during the period July 1, 2008 through June 30, 2010. Each section discusses the work performed and the results obtained during the particular period for each of the two main project objectives. The milestones for each performance period are identified and discussed in the order they were accomplished. All 17 project milestones for this project have been successfully met.

INTRODUCTION

The goal of this project is to conduct research on the dynamics of microbial communities occurring in engineered floating islands used for water treatment. These floating islands (trade marked Biohaven™), which consist of a non-woven matrix made of 100% recycled plastic fabricated into floating mats, have been developed by Floating Island International LLC (FII), headquartered near Shepherd MT. Previous research done in test ponds at FII's outdoor research facility has provided observations of the substantial disappearance of key waste water constituents such as organic carbon, ammonia, nitrate and phosphate in the presence of floating islands. Constituent uptake in these outdoor experiments was likely due to the activity of microorganisms growing as biofilms within the island matrix (plants were not included in the experiments). While these observations are very promising, it is obvious to FII that there is a need to conduct basic microbiological research which provides a fundamental understanding of the microbial processes at work during contaminant uptake. Successful completion of this research now provides the basis for improving floating island design and efficacy, as well as providing comprehensive scientific observations which explain how the floating island water treatment technology works. Such information provides scientific validation of floating islands as an emerging technology for nutrient removal from waterways and will greatly enhance FII's ability to move this product series to market. The research and product synthesis has been accomplished by way of collaboration between FII and the Center for Biofilm Engineering (CBE) at Montana State University. CBE has conducted microbial process research which will directly support improved floating island product development by FII. CBE and FII have executed the following project objectives:

Objective 1. (CBE) *Determine the optimum operational conditions to encourage simultaneous nitrification(ammonia removal) and denitrification (nitrate removal) within a floating island environment by stimulating the appropriate microbial communities*.

Objective 2. (FII) *Optimize floating island design for aeration, circulation and energy efficiency. Conduct field experiments to test and optimize nutrient removal in wastewater systems.*
Research methods and results for each objective are presented in Sections I through IV along with discussion of appropriate project benchmarks.

SECTION I (Performance period August 1, 2008 – January 31, 2009)

Objective 1 Performance and achievement (August 1, 2008 –January 31, 2009). Specifically objective 1 is intended to examine how nitrifying microbial biofilms within the floating island matrix respond to various organic carbon loadings, the addition of ammonia, along with periods of artificial aeration. These investigations were conducted under controlled laboratory conditions using molecular biofilm community analysis methods developed by CBE for research on constructed wetlands. During this performance period our experimental system was designed, built and operated to monitor simultaneous removal rates

for ammonia, nitrite, nitrate and organic carbon. Later in the project, after the removal rates reached a "steady state condition" CBE researcher performed microbial community profile analysis to determine the spatial distribution of heterotrophs, nitrifiers, and denitrifiers (discussed in the following section). Details are presented below under discussion of performance benchmarks.

Objective 2 Performance and achievement (August 1,2008 –January 31, 2009). Objective 2 was carried out by FII at the Shepherd MT outdoor research facility. Efforts this progress period were directed at developing floating islands prototypes with improved water circulation and aeration systems, and developing an alternative-energy power supply that efficiently utilizes a combination of wind and solar energy in a portable, packaged system. Details are presented below under discussion of performance benchmarks.

Benchmarks for Performance Period 1. Performance benchmarks for this 6-month reporting period are shown in Table 1 below.

#1 (FII)	Month 3	Design prototype modular islands with improved circulation and aeration, to be used in field tests to verify laboratory results.
#2 (CBE)	Month 3	Develop and test the laboratory system for assessing microbial community shifts in floating island matrix .
#3 (FII)	Month 3	Design and construct a prototype hybrid power supply capable of logging cumulative amp-hour outputs from wind and solar sources.
# 4 (CBE)	Month 6	Conduct measurement of microbial community profile through floating island matrix.
#5 (FII)	Month 6	Construct prototype islands and conduct tests to check mechanical and biological performance (circulation, aeration, buoyancy, anchoring, nutrient removal).
#6 (FII)	Month 6	Evaluate power supply performance.

Table 1. Performance Benchmarks for six month period (August 1, 2008- January 31, 2009)

As seen in Table 1 Performance benchmarks 2 and 4 were addressed by the Center for Biofilm Engineering (CBE) while benchmarks 1,3,5, and 6 were carried out by Floating Island International (FII). We have organized reporting progress and achievement on benchmarks accordingly.

Performance and Achievement for Benchmarks 2 and 4 (CBE)

Benchmark #2 Develop and test the laboratory system for assessing microbial community shifts in floating island matrix .

This experimental system addressing benchmark #2 was successfully constructed and tested during progress period 1. This system consists of a series of 8-inch diameter PVC column, 24 inches in length, with various floating island materials packed inside as described below.

Column 1: packed with 18 inches of floating island matrix material
Column 2: duplicate of column #1
Column 3: packed with 18 inches of gravel (to simulate an artificial treatment wetland)
Column 4; Packed with 18 inches of shredded carpet fibers (carpet fibers are being considered as an alternative to matrix material)
Column 5: a control column containing no porous media

A pump reticulates water exiting the column at the bottom and returns the flow to the top of the column at which point oxygen can be added (or not) with a bubbler system. The nutrient composition added to the column is the artificial wastewater recipe shown below.

		g/L
1	C12H22O11 (sucrose)	0.2
2	Primatone (peptone)	0.22
3	NH4Cl	0.057
4	FeCl3	0.0004
5	MgSO4-7H2O	0.062
6	KH2PO4	0.044
7	H3BO4	0.01
8	CuSO4	0.0008
9	KI	0.0019
10	MnSO4	0.0078
11	NaMoO4	0.004
12	ZnSO4	0.0078
13	CaCl2	0.0019
14	NaNO3	0.091

Table 2. Recipe for synthetic wastewater

Benchmark #4. Conduct measurement of microbial community profile through floating island matrix.

During this first progress period experiments on column #1 (matrix column) had been running for over two months. During this time, water chemistry variables (pH, TOC, COD, nitrate, nitrite, ammonia, and dissolved oxygen) were monitored on a weekly basis. pH measurements were made on a Fisher Scientific Model 50 pH meter with Corning electrode, dissolved oxygen (DO) was measured using a HACH L90-HQ20 oxygen meter with an LDO dissolved oxygen probe, ammonia was measured using the HACH colorimetric method 10023 with the HACH 2000 spectrophotometer, nitrate was measured by ion chromatography using a Dionex® system with CD20 conductivity detector, and total organic carbon (TOC) was measured on a Dohrman DC-80® carbon analyzer.

These measurements indicated that, as of Juanuary31, 2009 (2 months of column operation) the microbial community in the column had not yet reached "steady state" and therefore it is premature to carry out the microbial community analysis as per benchmark # 4. This benchmark was subsequently accomplished during progress period 2 and is reported in Section II.

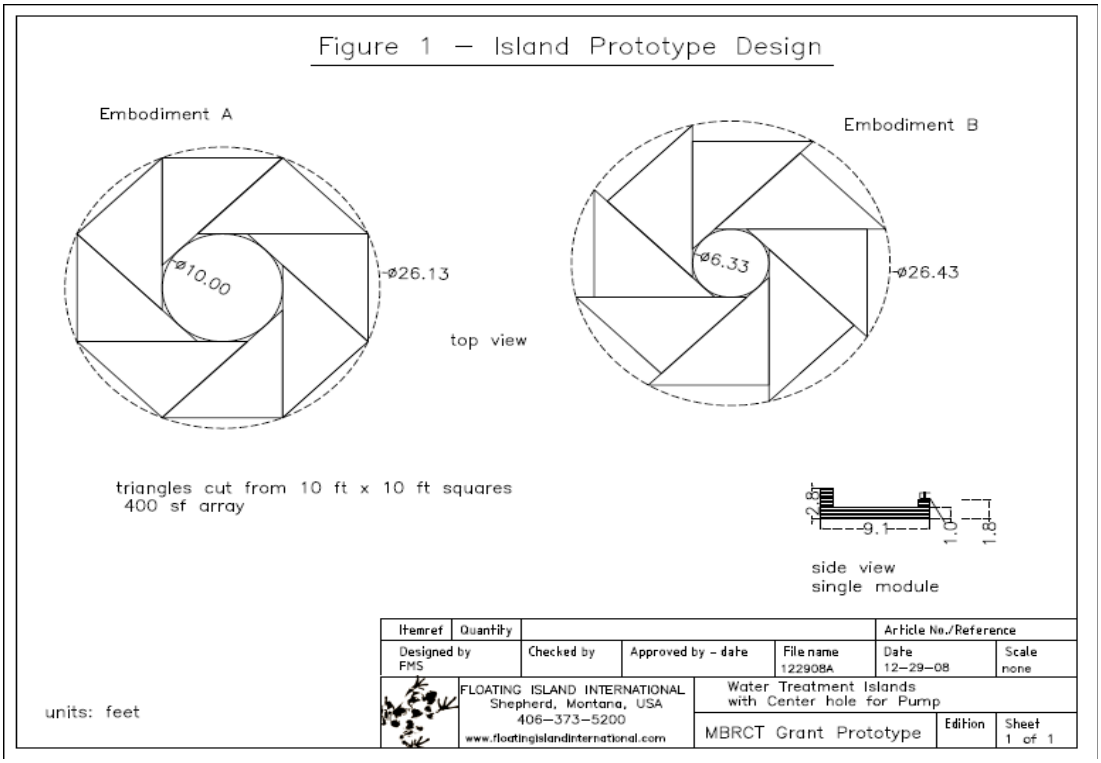
Performance and Achievement for Benchmarks 1,3,5 and 6 (FII)

Benchmark #1 Design prototype modular islands with adjustable circulation and aeration, to be used in field tests to verify laboratory results.

The prototype design is shown in Figure 1. The prototype is comprised of a circular 400-sf island with a 3-horsepower water circulator/aerator. The island is comprised of eight identical, triangular-shaped modules. The modules are constructed of stacked layers of nonwoven polyester fiber matrix. The island array is designed to float with the top surface submerged about 6 inches below water level, in order to

promote distributed vertical percolation of water through the structure for optimized microbial bioremediation. The modular design may be scaled to produce larger or smaller island arrays.

The island is designed for use in combination with a Bluefrog™ water circulator manufactured by Absolute Aeration Corporation. This circulator is a high-flow, low-head pump with a reported maximum output of 5000 gpm. Pump output is regulated with a variable-frequency controller and is continuously adjustable from zero to maximum flow. The circulator draws in water from underneath the pump and outputs the water radially a few inches above pond surface. Some aeration occurs within the pump impeller section, and additional aeration can be provided with an external compressor if required.



Benchmark #3 Design and construct a prototype hybrid power supply capable of logging cumulative amp-hour outputs from wind and solar sources.

A schematic of the prototype hybrid power supply is shown in Figure 2. The system comprises a solar panel array rated at 60 watts and a wind turbine rated at 400 watts. A data logger simultaneously records the electrical power produced by the solar panels, the power produced by the wind turbine, and the power consumed by the load. The data recording interval is adjustable, and is typically set at one hour. Data is uploaded from the logger via a notebook computer attached with a USB connector. Computer software is supplied by the logger manufacturer (Bogart Engineering of Boulder Creek, CA). Power is stored in a deep-cycle battery and consumed by either a water pump or an aerator (referred to as 'loads"). A power controller is included in the circuit in order to prevent the loads from being turned on at times when the battery is severely discharged.

Benchmark #5 Construct prototype islands and conduct tests to check mechanical performance (circulation, aeration, buoyancy, anchoring).

The first full-scale prototype island system has been constructed and was launched on November 19, 2008 in a pond at the FII Shepherd Research Center. Figures 3, and 4, are photographs of the system on the launch date. The system was operated continuously at full flow for about 55 days, after which the pump malfunctioned and became frozen into surface ice on the pond. During this first operational test, water circulated successfully through the biofilm-growing internal regions of the islands, and the water circulation of the system maintained an ice-free surface on a portion of the pond that would otherwise have been frozen.

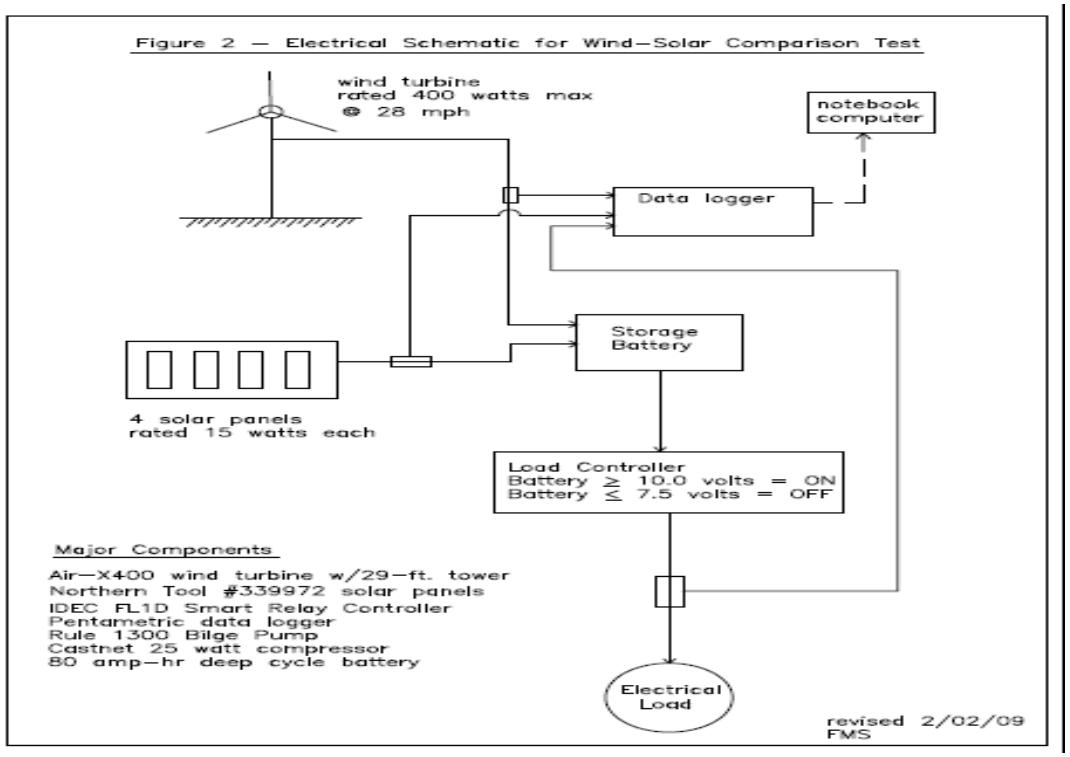


Figure 3. Radial flow Bluefrog™ pump

Figure 4. Pump mounted in floating island

Based on the initial tests, we have determined that the island's modular system generally worked well, except that the modules are too buoyant, and therefore tend to rise too far above the pond surface. The excess buoyancy is caused by polyurethane foam that is used to bond the layers of nonwoven matrix

material. We have researched and tested a replacement adhesive, and will use the new material in a future prototype.

Benchmark #6 Evaluate power supply performance.

The hybrid wind-solar power supply was tested almost continuously through the months of October, November and December 2008. All of the major components performed satisfactorily. Monthly graphs of power production and consumption have been collected on hourly intervals and are summarized in Tables 3 below.

Month	Solar (watt-hr)	Wind (watt-hr)
Oct 2008	1908	3956
Nov 2008	1436	3117
Dec 2008	749	6521

Table 3. **Electrical Power Produced**

Relative costs for producing power with the solar and wind systems are shown in the Table 4 below.

Month	Solar ($/watt-hr)	Wind ($/watt-hr)
Oct 2008	$0.21	$0.31
Nov 2008	$0.28	$0.40
Dec 2008	$0.53	$0.19

(1) Equipment Cost / Watt Hr is defined as the purchase price of the equipment divided by the monthly power output. The solar panel cost was about $400, and the wind turbine (with 30-ft. tower) cost was about $1250. The purpose of this computation is to compare the relative short-term costs of solar and wind production. As shown, solar was the better economical performer in Oct and November, but wind was much better in December.

Table 4. **Equipment Cost / Watt-Hr**[(1)]

SECTION II (February 1, 2009 –July 31, 2009)

Research methods and results for each objective accomplished during this progress period are presented in this section along with discussion of appropriate project benchmarks.

Objective 1 Performance and achievement (February 1, 2009 –July 31, 2009). During this progress period removal rates for ammonia, nitrite, nitrate and organic carbon reached steady state conditions. CBE researchers subsequently performed the microbial community profile analysis to determine the spatial distribution of heterotrophs, nitrifiers, and denitrifiers. Details are presented below under discussion of performance benchmarks.

Objective 2 Performance and achievement (February 1 ,2009 –July 31,2009). Objective 2 is being carried out by FII at the Shepherd MT outdoor research facility. Efforts during this reporting period were directed at developing floating islands prototypes with improved water circulation and aeration systems, and developing an alternative-energy power supply that efficiently utilizes a combination of wind and solar energy in a portable, packaged system. Details are presented below under discussion of performance benchmarks.

Benchmarks for Performance Period 2. Performance benchmarks for this 6-month reporting period are shown in Table 5 below.

# 4 (CBE)	Month 6	Conduct measurement of microbial community profile through floating island matrix.
#7 (CBE)	Month 9	Identify microbial community response to variations in laboratory system operating conditions.
#8 (FII)	Month 9	Continue monitoring mechanical and biological performance of island prototypes.
#9 (FII)	Month 9	Collect bacterial and water chemistry samples for analysis at CBE for the purpose of refining CBE laboratory experiments for objective 1.
#10 (CBE, FII)	Month 12	Submit a journal article which discusses the spatial distribution of the microbial community as measured for objective 1.
#11 (FII)	Month 12	Complete optimization of wind/solar power supply based on evaluation of summer and winter performance.

Table 5. Performance Benchmarks for six month period (February 1, 2009—August 1, 2009)

As seen in Table 5 Performance benchmarks 4, 7 and 10 are being addressed by the Center for Biofilm Engineering (CBE) while benchmarks 8, 9 and 11 are being carried out by Floating Island International (FII). We have organized reporting progress and achievement on benchmarks accordingly.

Performance and Achievement for Benchmarks 4, 7 and 10 (CBE)

As discussed in Section I it was necessary for the microbial community to reach a steady state condition before conducting an analysis of the microbial community profile throughout the matrix column using PCR and DGGE. The methods and results discussed below demonstrate the successful completion of both benchmarks #4 and #7.

Benchmark #4. Conduct measurement of microbial community profile through floating island matrix.

Benchmark #7 Identify microbial community response to variations in laboratory system operating conditions.

Microbial Community profile variation with depth and operating conditions

Biofilm collection and DNA Extraction. Biofilm samples were collected during the third batch run (by which time columns were well conditioned) and again during the fifth batch run (14 days after the second molasses dose) from three depths within each treatment: top (upper 5 cm of material), center (middle 5 cm of material), and bottom (lower 5 cm of material). To clearly distinguish between depths sampled, a 2.5 cm zone was left undisturbed between each of the locations. The open water column was sampled by vacuum filtering 250 mL of effluent through a 0.2 μm polycarbonate membrane. Field samples from unplanted FTW, operated by Floating Island International, were also provided and analyzed for comparison with our laboratory samples. Materials collected from each treatment were placed directly into MO BIO PowerBead Tubes (MO BIO PowerSoil™ DNA Isolation Kit). The PowerSoil™ DNA Isolation Kit was used to complete the DNA extraction as described in the manufacturer's protocol with the exception that PowerBead tubes were placed into the FastPrep® Instrument (Qbiogene, Inc.) at speed 5.5 for 45 s. DNA yield was estimated on an agarose gel with ethidium bromide staining, serial dilutions were performed for PCR, and the DNA preparations were stored at -20˚C.

PCR Targeting Functional Genes. *Ammonia monooxygenase gene.* Oligonucleotide primers were synthesized by Integrated DNA Technologies (www.idtdna.com). PCR primers RottF (5' GGGGTTTCTACTGGTGGT 3', Rotthauwe et al., 1997 - *amoA*-1F) and RottR (5' CCCCTCKGSAAAGCCTTCTTC 3', Rotthauwe et al., 1997 - *amoA*-2R) target the ammonia monooxygenase gene (*amoA*, required for ammonia oxidation to nitrite). Primer RottR was synthesized with a 5' 40-bp GC clamp (5' CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCC 3', Ferris et al., 1996) and was paired with primer RottF for amplifying fragments to be analyzed by DGGE. Presumptive presence of the *amoA* gene was indicated on an agarose gel by a 531 bp PCR product. PCR reactions (20 µL) were performed using 2X GoTaq® Green Master Mix (www.promega.com). The PCR reaction mixture consisted of 10 µL 2X GoTaq® Green Master Mix, 0.5 µL Ultrapure BSA (50 mg/mL, Ambion), 2.5 µL DEPC-treated water, 1 µL 12.5 µM forward and reverse primer, and 5 µL 1:10 diluted (unquantified) template DNA. PCR amplifications were performed on an Eppendorf Mastercycler® ep thermal cycler (Eppendorf North America, www.eppendorfna.com) using the following program. An initial denaturation for 60 s at 94°C was followed by a total of 35 cycles of amplification consisting of denaturation at 94°C for 60 s, annealing at 54°C for 60 s, and extension at 72°C for 3 min. The program ended with an extension step at 72°C for 10 min (Bahr et al., 2005). PCR products were confirmed by agarose gel electrophoresis and staining with ethidium bromide and were used for DGGE.

Nitrite reductase gene. Oligonucleotide primers were synthesized by Integrated DNA Technologies (www.idtdna.com). PCR primers NirS cd3aF (5' GTSAACGTSAAGGARACSGG 3', Michotey et al., 2000) and NirS R3cdR (5' GASTTCGGRTGSGTCTTGA 3', Throback et al., 2004) along with NirK F1aCuF (5' ATCATGGTSCTGCCGCG 3', Hallin and Lindgren, 1999) and NirK R3CuR (5' GCCTCGATCAGRTTGTGGTT 3', Hallin and Lindgren, 1999) target the two forms of the nitrite reductase gene (*nir*, required for nitrite reduction to nitric oxide). Primers NirS R3cdR and NirK R3CuR were synthesized with a 5' 40-bp GC clamp (described above) and were paired with their respective forward primers for amplifying fragments to be analyzed by DGGE. Presumptive presence of the *nirS* and *nirK* genes was indicated on an agarose gel by a 465 bp or a 502 bp PCR product, respectively. PCR amplifications were performed on an Eppendorf Mastercycler® ep thermal cycler (Eppendorf North America, www.eppendorfna.com) using the following program. An initial denaturation for 2 min at 94°C was followed by a total of 35 cycles of amplification consisting of denaturation at 94°C for 30 s, annealing at 57°C for 60 s, and extension at 72°C for 60 s. The program ended with an extension step at 72°C for 10 min (Throback et al., 2004). PCR products were confirmed by agarose gel electrophoresis and staining with ethidium bromide and were used for DGGE.

Denaturing Gradient Gel Electrophoresis. DGGE was performed on PCR products from community DNA using a DCode™ system (www.biorad.com) and reagents from Sigma-Aldrich (www.sigmaaldrich.com). Gels had a gradient of denaturant concentrations from 40% at the top of the gel to 70% at the bottom, where 100% denaturant is defined as 7 M urea and 40% formamide. Gels also contained an 8 to 12% polyacrylamide gradient from top to bottom (Girvan et al., 2003). Electrophoresis was at 60 V for 16 h. Gels were stained with Sybr®Gold (www.invitrogen.com) and documented using a FluorChem™ 8800 fluorescence imager (www.alphainnotech.com). Three marker lanes (generated from five pooled clones) were included in each DGGE gel so that cross-comparison would be possible. Bands in DGGE images were identified visually on a presence–absence basis. Band intensities were not physically measured, but visually prominent bands were considered to represent numerically significant members of the community.

An example of the DGGE image of the amoA gene appears in Figure 5. This gene is indicative of an autotrophic nitrifying community. This image indicates that nitrifying bacteria (nitrifiers) have been identified in all of the systems being investigated (carpet, gravel, and matrix) and at all levels (top, center, and bottom).

This DGGE image allows us to visualize the nitrifying community that has developed at the top, center and bottom of each of the columns. The darker bands in Figure 5 indicate that there are at least three organisms common to all of our columns and the depths examined. Interpretation of DGGE profiles should be

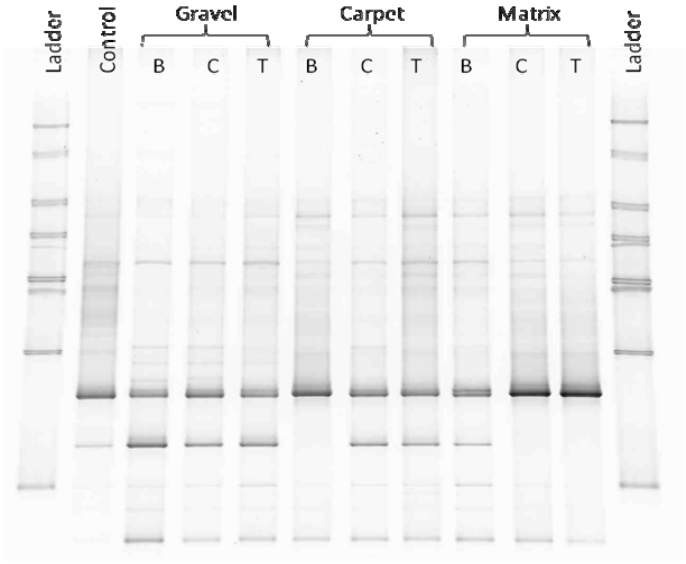


Figure 5. DGGE image of amoA gene. Location sampled: T – top, C – center, B – bottom.

done cautiously as they are invariably a mix of artifact and real diversity. Individual bands are generally assumed to represent individual organisms (genotypes), but only DNA sequencing can confirm this. The total number of bands in a profile is a rough estimate of diversity and the intensity of a band is a rough estimate of the prominence of the corresponding genotype in the microbial community (Muyzer et al., 1993). It should be noted that bands suspected to be artifact were not included in the analysis and as a result, diversity may have been underestimated.

Statistical analysis of microbial community responses and profiles. DGGE gels (like the one in Figure 5) for genes indicative of nitrification and denitrification and for biofilms taken from both the laboratory and field systems, were analyzed using Gel Compar II software (v. 6.1, Applied Maths Inc.) to visualize and compare gels. Statistical analyses were performed based on band presence/absence within each profile using the R software libraries labdsv (Roberts, 2009) and optpart (Roberts, 2010) (www.r-project.org).

Nitrifying Community. The nitrifying communities within each column were observed to have limited diversity (maximum of 12 bands observed, gravel treatment). The open water column had developed a distinct nitrifying community unlike any of the other treatment conditions (D^2=0.9944). Dosing with molasses and ending aeration did not significantly affect the structure of the nitrifying communities. The community structure of the laboratory versus field samples were distinctly different (D^2=0.9955). Field samples generally contained 5-7 bands, while laboratory samples contained 5-12 bands. It appears as though sample depth within the column was the most significant contributor to community structure ($p<0.001$, Figure 6) followed by the type of Floating Treatment Wetland (FTW) material used ($p=0.01$) with matrix and carpet communities being more similar to one another than to the gravel community.

Denitrifying Community. In order to investigate the entire denitrifying community present, both the *nirS* and *nirK* genes were characterized. Overall, the denitrifying community profiles were considerably more diverse compared to the nitrifying community profiles. As observed with the nitrifying community profile, the profiles for the open water column had developed unique communities compared to the other treatments (D^2=1, for both genes). For the *nirK* gene, the communities were highly diverse, but apparent similarities were specific to the FTW matrix material within the column (D^2=0.9996, $p=0.005$). For the *nirS* gene, the FTW matrix material were most important in determining the community that developed ($p<0.001$) with all of the gravel samples grouping onto a single branch. Adding molasses and ending

aeration also appeared to affect the *nirS* denitrifying community, though not as greatly as FTW material had (p=0.05, Figure 7). Finally, the field samples had developed significantly different denitrifying communities for the *nirS* (p=0.001) but not the *nirK* gene.

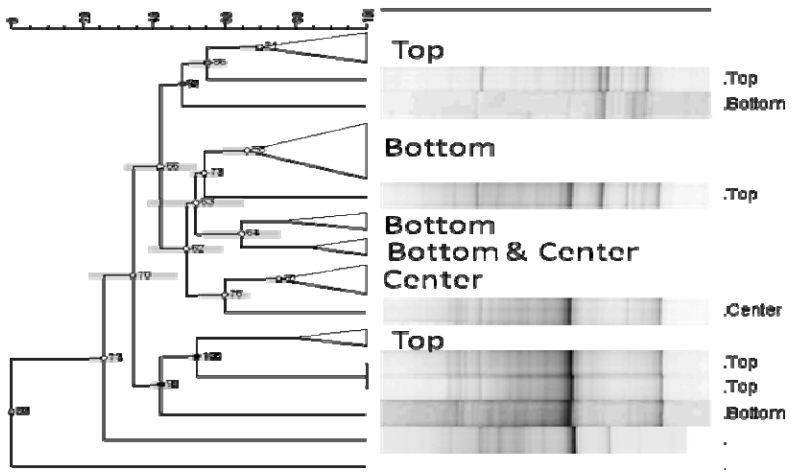
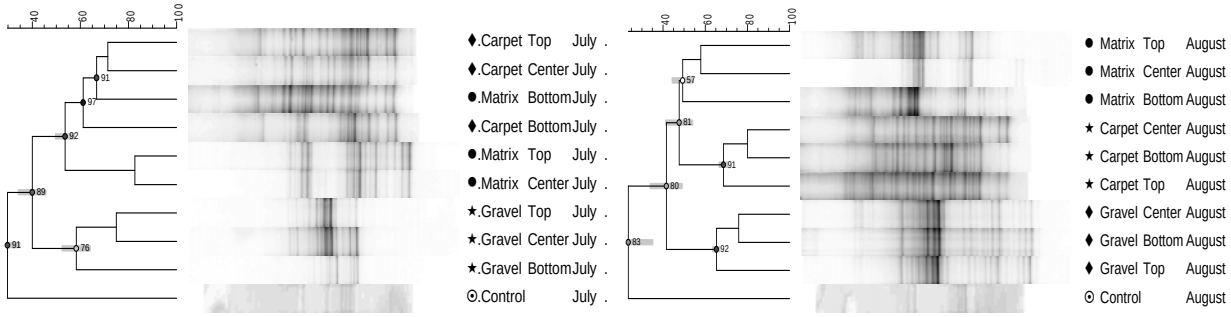


Figure 6. Shows how the fourteen major clusters separate. Depth is the main contributor for the nitrifiers. Triangles indicate that the samples within were similar enough to combine into a single cluster. Top – upper 5 cm of FTW material; Center – middle 5 cm of material; Bottom – lower 5 cm of material; unlabelled rows indicate open water column.



Pre molasses addition

Post molasses addition

Figure 7. Community profile for *nirS* pre- and post-addition of molasses. FTW material tends to dominate community profiles pre-molasses (left), however; post-addition of molasses (right) shows each material to have a distinct *nirS* community as observed with the four grouped clusters.

Summary. As expected, all treatments (regardless of material) were able to efficiently reduce the COD. Additionally, communities cultivated in the field versus laboratory conditions developed their own unique consortia for each of the genes investigated. Distance from the water surface (depth) appears to be most important to the structure of the nitrifying community followed by FTW matrix material employed. Elimination of aeration and addition of molasses did not appear to affect the established nitrifying community indicating that it may be important to first establish an efficient nitrifying community, then optimize for subsequent nitrate removal. Similarly, FTW matrix material had the largest effect on the denitrifying community present. As observed with the nitrifiers, the elimination of aeration and addition of molasses did not significantly affect the community structure, but did stimulate denitrifying activity and thus nitrate removal.

Benchmark #10. Submit a journal article which discusses the spatial distribution of the microbial community as measured for objective 1.

We have finished a manuscript entitled Floating Island Wetlands for Domestic Water Treatment (authors include J.A. Faulwetter, M. Burr, A.B. Cunningham, F.M. Stewart, A.C. Camper and O.R. Stein). The paper has been submitted to the Journal of Water Science and Technology, special edition on wetlands for water treatment. The article is scheduled for publication later in 2010.

Performance and Achievement for Benchmarks 8, 9 and 11 (FII)

Benchmark 8. Continue monitoring mechanical and biological performance of island prototypes.

During progress period 2, we repaired the defective pump on the first prototype round island, and retuned the island to service. We have also constructed a second round island equipped with a 1.5-hp aeration/circulation pump and we are currently testing it at the Shepherd Research Center (SRC). Prototype #2 is shown in the Figure 8 below. Since the biological treatment zones of the islands are set below waterline, these zones must be constructed so as to have slightly negative buoyancy. We are currently testing non-buoyant adhesives to replace the highly buoyant polyurethane foam that we have previously used as a bonding agent for the permeable matrix layers.

Figure 8– Prototype Island #2 with 1.5-hp aeration/circulation pump

Benchmark 9. Collect bacterial and water chemistry samples for analysis at CBE for the purpose of refining CBE laboratory experiments for objective 1.

We have set up a tank-scale experiment at SRC to replicate the column experiments at MSU, and we have demonstrated both nitrification and denitrification in the tanks. Molasses was added to the tanks at selected time intervals to provide the required supply of organic carbon for denitrification. We have collected a set of core samples from the tanks prior to the carbon dosing, and we will collect an additional set of cores after the denitrifying bacteria become fully established. These cores will be available for DNA testing at MSU if required. A chart of the tank experiment data is shown below in Figure 9.

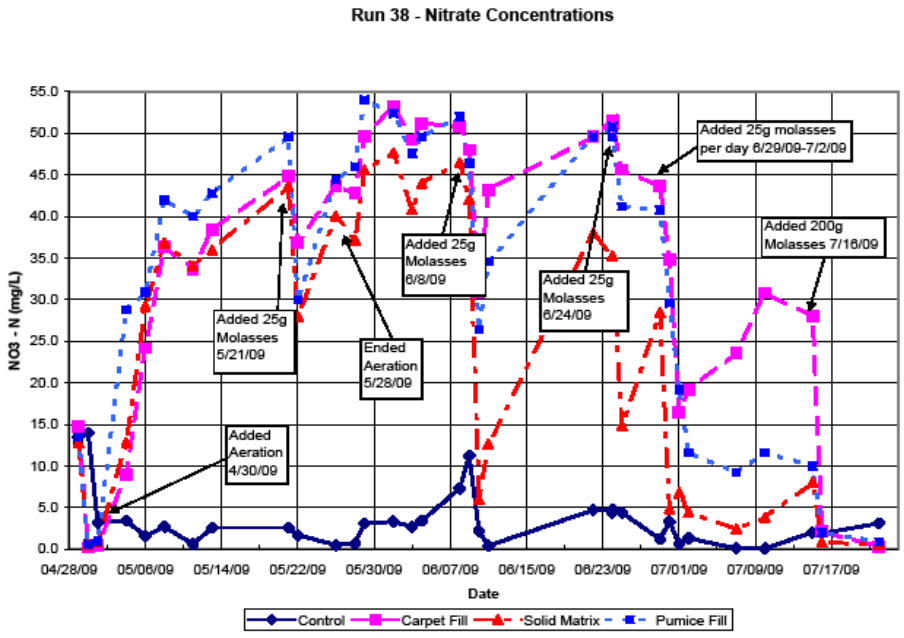


Figure 9. Nitrate Removal in the Shepherd Research Center Tank Tests

Benchmark 11. Complete optimization of wind/solar power supply based on evaluation of summer and winter performance.

We have continued the monitoring of solar and wind power production through July 2009. The power is used to run a bubbler unit that is aerating a pond adjacent to the test site. A comparison of power produced by the two systems is presented in Table 6 below. As shown, the wind power system was particularly useful for the winter months of December and January, when solar power production was very low. The wind turbine failed during May 2009, and has been returned to the manufacturer for repair. We anticipate returning it to service around September 1, 2009, and we plan to continue running the experiment during Year 2 of the study.

During this progress period, we have added a programmable controller to the system that shuts down power to the bubbler system when the storage battery voltage falls below a preset level (currently 9.0 volts). This reserves the remaining battery power for running the data logger unit, so that data is not lost during periods of no power production. In general (with the exception of the failure of the commercial wind turbine) the hybrid solar-wind power generator has been efficient and reliable.

Month	Solar (watt-hr/day)	Wind (watt-hr/day)
Oct 2008	63.6	131.9
Nov 2008	47.9	103.9
Dec 2008	25.8	224.9
Jan 2009	6.5	251.9

Feb 2009	70.6	166.4
Mar 2009	172.9 (1)	264.5
Apr 2009	166.1	246.5
May 2009	294.7	n/a (2)
Jun 2009	316.7	n/a
Jul 2009	412.9	n/a

Notes: (1) added solar panels to double the size of the array on March 20, 2009
(2) wind turbine had an electronic failure during May 2009; currently being repaired

Table 6 – Electrical Power Produced

SECTION III (August 1, 2009 –January 31, 2010)

Research methods and results for each objective accomplished during this progress period are presented in this section along with discussion of appropriate project benchmarks.

Objective 1 Performance and achievement (August , 2009 – January 31, 2010). Specifically this objective examines how nitrifying and denitrifying microbial biofilms within the floating island matrix respond to various organic carbon loadings, the addition of ammonia, along with periods of artificial aeration. These investigations are being conducted under controlled laboratory conditions using molecular biofilm community analysis methods developed by CBE for research on constructed wetlands. CBE researchers have made good progress to date. Our experimental system has been designed, built and operated for the past two months. The system is now capable of monitoring simultaneous removal rates for ammonia, nitrite, nitrate and organic carbon. Removal rates have now reached a "steady state condition" and microbial community profile analysis to determine the spatial distribution of heterotrophs, nitrifiers, and denitrifiers have been performed. Details are presented below under discussion of performance benchmarks.

Objective 2 Performance and achievement (August 1, 2009 – Jan 31, 2010). Objective 2 is being carried out by FII at the Shepherd, MT outdoor research facility, and at a wastewater treatment lagoon in Billings, MT. The primary effort during this past reporting period has been directed toward the design and construction of a pilot-scale floating treatment system and installation of the system in a residential wastewater treatment facility. Other efforts during this period include continued maintenance and monitoring of the solar/wind alternative-energy power supply, and running additional tank-scale experiments at the Shepherd facility to confirm the MSU laboratory results. Details of the achievements during the period are presented below under discussion of performance benchmarks.

Benchmarks for Performance Period 3. Performance benchmarks for this 6-month reporting period are shown in Table 7 below. As seen in Table 7 Performance benchmarks 12 and 15 are being addressed by the Center for Biofilm Engineering (CBE) while benchmarks 13 and 14 are being carried out by FII.

#12 (CBE)	Month 15	Determine how floating island thickness influences nitrification and denitrification.
#13 (FII)	Month 15	Use CBE laboratory results to modify design of modular islands (consider island shape and thickness, aeration flow and duty cycle, circulation flow and duty cycle, chemical supplements, etc.).
#14 (FII)	Month 18	Conduct field tests of modified islands; collect bacteria and water chemistry samples for analysis at CBE and FII.
#15 (CBE)	Month 18	Analyze nitrate and ammonia removal rates and microbial community profiles from deployed floating islands in the field.

Table 7. Performance Benchmarks for six month period (August 1, 2009 – February 1, 2010)

Performance and Achievement for Benchmarks 12 and 15 (CBE).

Benchmark # 12. Determine how floating island thickness influences nitrification and denitrification.

Results from microbial community profiling showed an interesting phenomena. This being that, after the biofilm reached maturity, both nitrifying and denitrifying bacteria were found to be present throughout the entire thickness of the floating island column—regardless of the composition (matrix, carpet fiber, or sand). Initially our hypothesis was that nitrification would tend to occur near the top aerated part of the column and denitrification would occur near the bottom. We assumed that species distribution via community profiling would reflect the same behavior. The observation that both nitrifying and denitrifying species occur throughout the column sheds enlightening insight into the microbial ecology of floating islands during waste water treatment. This result has been reported in the journal article cited in benchmark 10.

Benchmark #15 (CBE). Analyze nitrate and ammonia removal rates and microbial community profiles from deployed floating islands in the field.

Analysis of nitrate and ammonia was conducted at the Rehberg floating island treatment site during October 2009. Ammonia levels were non-detectable after treatment and nitrate levels were at 2.6 mg/l. These data are regarded as preliminary and additional testing will continue during 2010.

Performance and Achievement for Benchmarks 13, and 14 (FII)

Benchmark #13 - Use CBE laboratory results to modify design of modular islands (consider island shape and thickness, aeration flow and duty cycle, circulation flow and duty cycle, chemical supplements, etc.).

Based on the results of the grant-sponsored research to date, the City of Billings agreed to provide a test site and partial funding for a pilot-scale floating island treatment system. This first deployment of a pilot-scale system in a real wastewater setting is a major milestone in the commercialization plan for the floating island product line. A 2500-square foot island with a 1.5-horsepower circulation pump was constructed at the facility of a licensee of Floating Island International (Headwaters Floating Island of Billings, MT). The system was installed in a wastewater treatment lagoon at the Rehberg Ranch Subdivision in Billings during November, 2009, and is currently performing as expected. The pump system has functioned successfully through several sub-zero periods of winter weather (see Figure 10).

Rehberg Pilot-Scale Island on 11/16/09

Pilot-Scale Island during Sub-Freezing Temperatures on 11/13/09

Figure 10. A 2500-square foot island with a 1.5-horsepower circulation pump installed in a wastewater treatment lagoon at the Rehberg Ranch Subdivision in Billings MT.

Benchmark #14 - Conduct field tests of modified islands; collect bacteria and water chemistry samples for analysis at CBE.

We have collected matrix samples from the Shepherd tank experiments, and biofilms from the samples have been analyzed by MSU and compared to biofilms collected from the MSU column experiments. We have continued to do in-house water quality analyses for the experiments run at the Shepherd facility.

SECTION IV (Progress period 4, February 1, 2010 –July 31, 2010)

Research methods and results for each objective accomplished during this progress period are presented in this section along with discussion of appropriate project benchmarks.

Objective 1 Performance and achievement (January 31, 2010 – July 31, 2010)

Summarized below are the water quality analyses from the 5 experimental recirculation columns operated by CBE. This summary describes the biofilm-based removal of organic carbon, ammonia, and nitrate in response to various circulation and aeration schemes. Two types of experiments were run: Type I involved starting the columns with 20 liters of simulated domestic waste water (see Table 2) then observing the removal of various constitutes over 28 or 42 day periods (without adding any additional wastewater). Type II experiments were run the same as Type I with the exception that additional 500??? ml doses of simulated waste water were added once each week during the experiment.

Type I One-time waste water dose experiments

Experimental Methods. Experiments were conducted in laboratory scale systems consisting of 20 cm diameter columns containing matrix material 20 cm thick submerged 10 cm below the water surface. Matrix material was either a very porous commercial mat made from 100% recycled plastic or loose shredded carpet fibers contained within a porous mesh, both supplied by Floating Island International (www.floatingislandinternational.com). Additionally, two otherwise identical columns, one filled with 20 cm pea gravel, the other left as open water, were included for comparison. Columns were each filled with 20 liters of simulated domestic wastewater containing ~500 mg/L COD (mostly from sucrose), ~15 mg/L NH_4-N and ~15 mg/L NO_3-N, ~ 15 mg organic N/L from Primatone (Sigma), and other inorganic components (Taylor et al., 2010). All columns were unplanted, inoculated with soil and pond water, and operated in batch mode with continuous recirculation from the bottom of the column (at 20 mL/min) with continuous aeration (unless noted) from aquarium pumps into the 10 cm surface water above the matrix. We summarize data from five consecutive batch runs (B1-B5) that followed a conditioning period of four batches over a three month period. B1, B2, and B3 were run for 28 days. B4 and B5 were run for 42 days. During B4 and B5, columns were dosed with 10 g molasses (measured as 820 mg COD/g) on day 25 and again on day 29 in order to provide reducing equivalents for denitrification. There was no aeration during days 29-42.

Experimental Results. Because all water quality analyses were done on filtered samples (0.2 µm pore size), bacterial cells were excluded. The laboratory columns containing plastic matrix, carpet fibers, pea gravel or open water were all effective at removing COD and nitrogen (data not shown). There was relatively little difference among treatments (except as noted below). COD removal in all treatments was ~90% within the first two weeks of each batch, i.e., from ~500 mg COD/L initially to < 50 mg COD/L by day 14. Initial total dissolved nitrogen was ~60 mg N/L, consisting of ~30 mg N/L organic N (from Primatone), ~15 mg NO_3-N/L, and ~15 mg NH_4-N/L. Total dissolved N generally decreased by ~50% within the first two weeks, but leveled off after that. In the first week, removal of NO_3-N was usually ~90%, probably from denitrification. NH_4-N usually increased in the first week, probably from mineralization of organic N. An exception was the gravel column where NH_4-N decreased immediately.

This behavior may have been the result of adsorption onto the gravel matrix. By days 21-28, NO_3-N usually began to accumulate again and often accounted for most of the total N. There was usually a corresponding loss of NH_4-N during this time period, indicating that nitrification was occurring. Differences in nitrification between new and conditioned plastic matrix suggest that about three months were required to establish an effective nitrifying biofilm community. By day 28 in batches B1-B3, the columns had reached a steady state in which COD was virtually absent, and almost all of the total dissolved N was a NO_3-N (~20-30 mg N/L). To test the hypothesis that denitrification in these batches had been carbon-limited, we introduced doses of molasses (10 g molasses/column) on days 25 and 29 of B4. Dosing produced a spike in COD (to ~370 mg COD/L), but by the end of the batch two weeks later, >90% of this COD had also been removed. The molasses was effective at increasing denitrification. By the end of the batch on day 42, total dissolved N was <5 mg N/L, NO_3-N was <4 mg N/L, and NH_4-N was <2 mg N/L. COD was <100 mg/L, but this residual COD from the molasses would probably have been removed had the batch been allowed to run beyond day 42. Batch B5 produced results that were similar to B4. NO_2-N was not a component of the synthetic wastewater and was usually ≤ 1 mg N/L for all treatments and time points.

Type II Multiple waste water dose experiments

Type II experiments began with 20 liters of simulated wastewater followed by the weekly addition of 800 ml of 1x waste water. The recirculation rate was raised from 20 mL/min used in the Type I experimented to 400 ml/min. There was no aeration for the first 30 days followed by full aeration from day 30 through 46.

Removal of COD followed a similar trend for all materials tested (i.e old and new matrix, carpet fibers and gravel). The initial COD level of 440 mg/l rapidly declined to between 5 to15 mg/l after 30 days. The COD then remained steady at these levels (through day 46) after aeration was started.

The nitrogen species behavior again exhibited a similar trend for all materials tested. Initially total dissolved nitrogen was 50 mg N/l, ammonia nitrogen (NH_4-N)was 18 mg N/l, and nitrate nitrogen ((NO_3-N) was 12 mg N/l. During the first 30 days (without aeration) NH_4-N levels rose slightly then became quasi-steady in the range to 20mgN/l to 30 mg N/l, while NO_3-N fell to essentially zero during the first week. Because additional total organic nitrogen, ammonia, and nitrate were added each week, this quasi-steady state behavior demonstrates that nitrification and denitrification was occurring simultaneously in all four columns. During the aerated period from day 30 to day 46 total dissolved nitrogen rose abruptly to between 40 and 44 mg N/l, ammonia levels dropped to between 10 mg N/l and 0 mg N/l (carpet), and nitrate levels rose to approximately 25 mg N/l in all columns. In the open water control (recirculation with no aeration) total nitrogen dropped quickly from 50 mg N/l to a quasi-steady state of about 35 mg N/l. Ammonia levels rose gradually from 20 mg N/l and stabilized in the range of 28 to 30 mg N/l

Summary. Results from the Type I and II experiments suggest several important findings: 1) biofilms growing all materials tested resulted substantial removal of COD (organic carbon) with and without aeration, 2) Simultaneous nitrification and denitrification was observed during periods of no aeration (circulation only), and 3) nitrification and denitrification rates can be controlled by manipulating the aeration schedule, and 4) biofilms growing on the sides of the control column reduced COD levels from 440 mg/l to approximately 100 mg (compared to 5 to 15 mg/l in the material columns)—thereby indicating superior removal from biofilms growing in all floating Island materials tested. Total nitrogen dropped from 50 mg N/l in the control to about 35 mg N/l indicating a slightly reduced removal (about 5 mg N/l) compared to the material columns. Virtually all of the total nitrogen in the control columns was in the form of ammonia (i.e. nitrate went to zero).

Objective 2 Performance and achievement (January 31, 2010 – July 31, 2010*)*

Benchmarks for Performance Period 4. Performance benchmarks for this 6-month reporting period are shown in Table 1 below.

#16 (FII)	Month 21	Evaluate performance of modified islands under summer and winter conditions, start preparation of articles describing the field tests.
#17 (CBE, FII)	Month 24	Complete and submit peer-reviewed article and technical article for trade journals, final report for MBRCT.

Bench Mark #17 has been completed by way of the following journal submission:

Faulwetter, Jennifer L., Mark D. Burr, Anne C. Camper, Otto R. Stein. "The effect of plant species and sample location on microbial biofilms associated with constructed wetlands", will be Submitted to the International Society for Microbial Ecology Journal during the fall 2010. As with all other publications for this project support from MBRCT will be acknowledged.

COMMERCIALIZATION PLAN

Floating Island International (FII) LLC, headquartered at the Shepherd Research facility near Shepherd MT, is a privately owned Company, founded by a group of partners led by inventor Bruce Kania. The company business model is to develop then license its inventions. FII currently has a total of 7 licensees, of whom 6 have a manufacturing facility. The licensees are located in Montana, Louisiana, California, Minnesota, North Carolina, New Mexico, New Zealand and China. Some licensees have multiple international territories, ie, the China licensee holds China, Taiwan, Macao and Hong Kong, and the New Zealand licensee also holds Australia and Singapore. In addition to licensees, FII has also made agreements with several distributors, who purchase island products from licensees. FII currently has distributors in Canada, South Africa, South Korea, and Pennsylvania.

Major product lines are expected to include floating island products for municipal wastewater and stormwater treatment, agricultural wastewater treatment, petroleum and mining waste remediation, wildlife habitat, shoreline erosion control and wave attenuation, waterscape beautification, and boat docking.

FII is surrounding its technology with extensive international patent protection. However there remains the risk of piracy, particularly in economies not noted for IP compliance. The risk of pirated floating islands hitting retail stores in the US is perhaps higher in the "general" market. The municipal market is relatively protected from this given the large scale of most municipal projects, the custom-built nature of solutions and the relatively high integrity of public servants (on the whole). Probably the greatest risk associated with FII's position is the company's ability to cash flow through an IP enforcement action.

FII experienced its first profitable year in 2009, with a reported gross income of $1,585,416 and a net income of $219,078. Gross and net incomes are anticipated to grow at a rate of about 25% annually over the next five years. At the date of this report (9/2010) FII had no long-term debt.

ECONOMIC IMPACTS

Royalty revenue from the licensees and distributors is used to support the ongoing research and develop programs at FII in Montana. During 2010, FII has hired a Montana marketing firm (Kinetics of Billings) and has contracted with four additional Montana professional experts from the engineering and business sectors.

Since startup, FII has pursued an aggressive policy of intellectual property protection. At the date of this report, FII has obtained four patents related to floating islands in the U.S., as well as five patents in New Zealand, one in China, and one in Macau. In addition, there are numerous patent applications pending in the U.S. and internationally, and several new concepts under development with applications in progress.

Senator Jon Tester toured the FII facility during July 2010 and conducted an outdoor press conference at a test pond to bring attention to potential floating island applications for the Gulf of Mexico oil spill. The visit was documented on several television stations, as well as a front page article in the Billing Gazette newspaper. Senator Tester was informed of the funding provided by the MBRCT, and has indicated strong support for FII and its products. One product the "BioBarrier", has received initial federal approval for use in marine oil spill mitigation, and a test of this product is currently underway on the coast of Louisiana. There has also been an article in the Bozeman Chronicle newspaper describing the efforts of Al Cunningham (MSU-CBE) and Frank Stewart (Stewart Engineering) as Bozeman components of the floating island research and development program.

APPENDIX A Experimental Procedures and Results

Batch-Run Experiments Procedures and Result page 21

Continuous-Flow Column Experiments Procedures and Results page 23

Pilot-Scale Testing at a Residential Wastewater Treatment Lagoon Procedures and Results page 34

Batch-Run Experiments

Setup

A series of eight sequential batch-run experiments was conducted during a 130-day period. The tests were run in 8-inch diameter PVC columns, with one sample of substrate per column. Tested substrate materials included one sample each of old PET matrix, new PET matrix, post consumer carpet (PCC) fibers comprised primarily of polypropylene enclosed in a porous nylon net bag, and pea gravel. The old PET matrix and the pea gravel had been used in previous biofilm growth experiments. The new matrix had several months of active biofilm growth, the pea gravel was dried after the previous experiment, and the new PET matrix had not been preconditioned. The samples were cylindrically shaped and had dimensions of 20.3 cm diameter and 20.3 cm length (8 inches diameter by 8 inches thick). The experiment also included a control column with no substrate material.

At the beginning of each run, each column was filled with 20 L of artificial wastewater (The recipe is shown below), and was inoculated with a water sample from a wetland project. Each substrate sample was completely submerged. Water was continuously recirculated through the substrate materials from top to bottom at a rate of 20 ml min^{-1}. Aeration was accomplished by bubbling through an open tube into the water at the top of the column at a rate of 1 L min^{-1}. Length of the runs varied from 2 to 4 weeks.

Artificial Wastewater Recipe

Startup Recipe	FW	1X mg/L	1X g/L
C12H22O11 (sucrose)	342.0	200.0	0.2
Primatone (peptone)		220.0	0.22
NH4Cl	53.5	57.0	0.057
FeCl3	162.2	0.4	0.0004
MgSO4-7H2O	246.5	62.0	0.062
K2HPO4	174.2	44.0	0.044
H3BO4	61.8	10.0	0.01
CuSO4-5H2O	249.7	0.8	0.0008
KI	166.0	1.9	0.0019
MnSO4-H2O	169.0	7.8	0.0078
NaMoO4-2H2O	242.0	4.0	0.004
ZnSO4-7H2O	287.6	7.8	0.0078
CaCl2-2H2O	147.0	1.9	0.0019
NaNO3	65.0	91.0	0.091

Summary of Results

All of the four substrate materials as well as the control showed significant removal of ammonia. The carpet fiber and gravel substrates produced nearly complete removal of ammonia, while the two PET

matrix substrates and the control did not achieve complete ammonia removal. For Run 6, the ammonia removal rates (mg ft^{-2} day^{-1}) were as follows:

Carpet fiber: 57.1
Gravel: 57.1
Old matrix: 30.5
New matrix: 26.3
Control: 26.3
The pH values for the two PET samples typically (but always) dropped more than the pH values for the carpet and gravel, suggesting that some pH inhibition might be occurring for the matrix samples.

None of the samples showed significant removal of the nitrate that was produced by nitrification of ammonia, as a result of oxygen inhibition of the facultative denitrifying bacteria. This issue was addressed in the following continuous-run experiments.

Continuous-Flow Column Experiments

The purpose of the continuous-flow experiments was to calculate and compare the relative removal rates of the various substrate materials under conditions that simulated real-world wastewater lagoon conditions. Removal rates were monitored for one loading rate, two recirculation rates, and various aeration cycles.

Setup

The continuous-flow experiments were conducted using the same columns and growth substrate materials that were used previously for the batch-flow experiments. Tested materials included one sample each of "old" PET matrix, "new " PET matrix, post consumer carpet (PCC) fibers comprised primarily of polypropylene enclosed in a porous nylon net bag, and pea gravel from a previous wetland experiment. Since all of the substrate samples had been previously used in the batch-run experiments, they were all expected to comprise mature biofilm colonies at the start of the continuous-run experiments. The samples were cylindrically shaped and had dimensions of 20.3 cm diameter and 20.3 cm length (8 inches diameter by 8 inches thick). The experiment also included a control column with no substrate material.

At the beginning of the experiment, each column was filled with 20 L of 1X strength artificial wastewater. Continuous flow conditions were simulated by replacing 800 mL of column water with fresh 1X artificial wastewater each Monday, Wednesday and Friday. This resulted in a throughput rate equivalent to 0.34 L d^{-1}, or a volume replacement of about 1.7% per day within each column.

Each substrate sample was completely submerged. Water was continuously recirculated through the substrate materials from top to bottom. A first experimental run was made with a recirculation rate of 200 mL min^{-1}, and a second run was made with a recirculation rate of 400 ml min^{-1}. (0.15 and 0.30 gal min^{-1} ft^{-2} in conventional floating island units). The control was not aerated, and the recirculation rate was set to 20 mL min^{-1}, (in order to simulate a stagnant section of a treatment lagoon).

Measured parameters included total nitrogen (TN as N), ammonia (NH_3 + NH_4 as N), nitrate (NO_3 as N), chemical oxygen demand (COD), oxidation-reduction potential (ORP) and pH. The pH measurements were made on a Fisher Scientific Model 50 pH meter with Corning electrode. TN, ammonia, nitrate, and COD were measured with a Hach DR2000 spectrophotometer.

Summary of Results

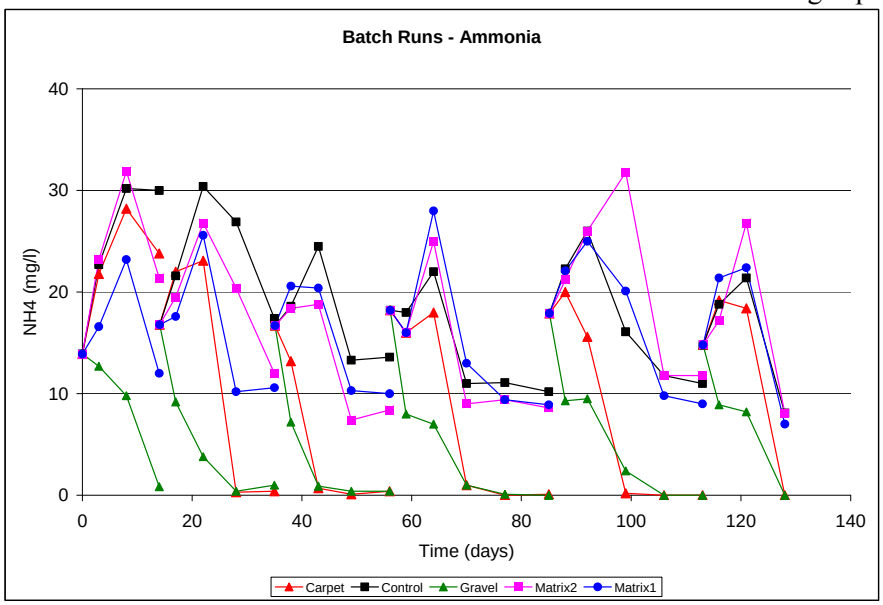
Graphical results of the removals of nitrate, ammonia, total nitrogen and chemical oxygen demand for the two continuous runs are shown on the attached graphs.

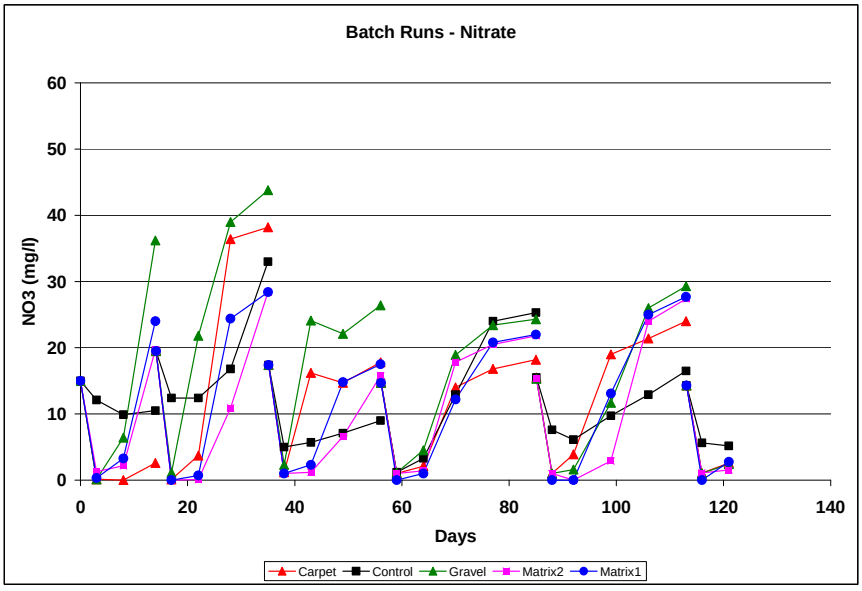
1. In general, proper aeration cycling proved to be more important than recirculation rate for nitrogen removal in these experiments. As previously discussed, aeration is necessary for ammonia removal (the nitrification process), because the autotrophic nitrifying bacteria require both oxygen and carbon dioxide for metabolism. However, aeration is detrimental to the nitrate removal process (denitrification), because the denitrifying bacteria will preferentially use oxygen over nitrate if oxygen is available. Continuous Run 1 incorporated periods of relatively rapid on and off cycling of the aeration system (i.e., several times per week), while Continuous Run 2 employed much longer cycling periods (i.e., approximately one month on followed by one month off). Although neither cycling scheme was optimal (as evidenced by the fluctuating concentrations of ammonia, nitrate and total nitrogen), an improved cycling scheme can be deduced by observing the data. For example, the graph of CR2 for the carpet fiber substrate shows nearly complete nitrate removal and poor ammonia removal for the initial period of no aeration (days 0 – 30), then nearly complete ammonia removal but poor nitrate removal for the aerated period (days 30 – 65). The best combined removal occurred during the transition period when aeration was started (day 31), which resulted in a minimum total nitrogen concentration of 20 mg L^{-1} for the one-day period, while the average total nitrogen concentration during the 65-day run was around 30 mg L^{-1}. In contrast, during the short-period aeration of CR Run 1 (days 80 – 140), total nitrogen had an average concentration of around 18 mg L^{-1}. Since the total nitrogen concentration was comprised almost completely of nitrate during this period, it seems likely that system performance could have been significantly improved by reducing the "on" periods of the aeration cycle, by turning the aeration on only when ammonia levels exceed a set threshold value. From the Run 1 data, an aeration duty cycle of approximately 50% on/50% off resulted in over-aeration and resulting incomplete denitrification. These results should be used for future full-scale installations.

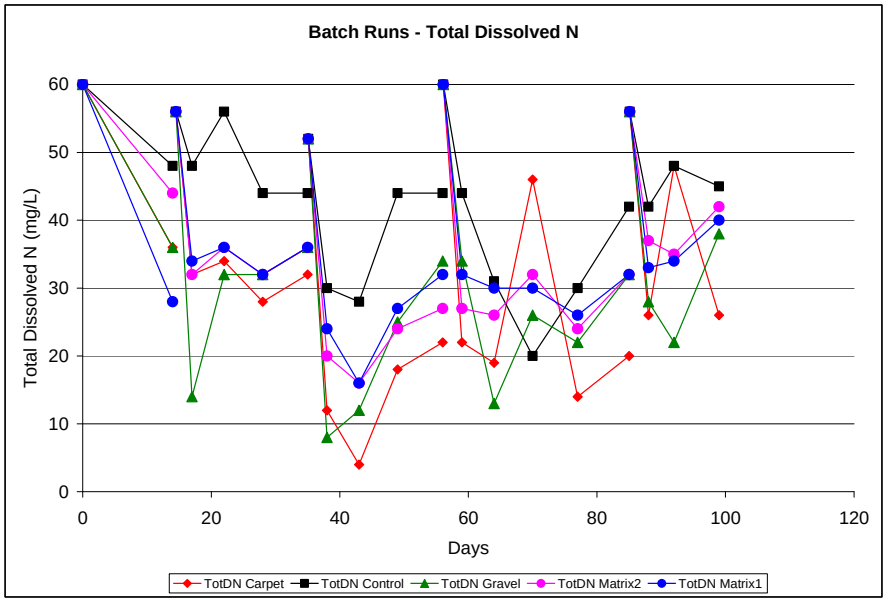
2. The carpet fiber and gravel substrates outperformed the PET matrix substrates for removing ammonia under aerated conditions. The graphs for Continuous Run 1 and Continuous Run 2 indicate that ammonia removal was almost complete for both the carpet fiber and gravel substrates during periods of pulsed or continuous aeration, but that the new and old PET matrix substrates were not able achieve this level of removal for either pulsed or continuous aeration. As shown on the attached graph, The pH values for the carpet fibers and gravel substrates remained at 5.3 or greater, while pH for the new and old PET matrix substrates fell to about 4.2 and 3.8, respectively during the runs. We hypothesize that the pH values for the two PET substrates fell below the minimum threshold for the denitrification processes to operate efficiently, while the pH levels in the carpet fiber and gravel columns remained high enough for efficient denitrification. To avoid future pH problems with scaled-up installations, pH values should be monitored and adjusted as required to maintain pH within an acceptable range of approximately 7.5 to 5.0.

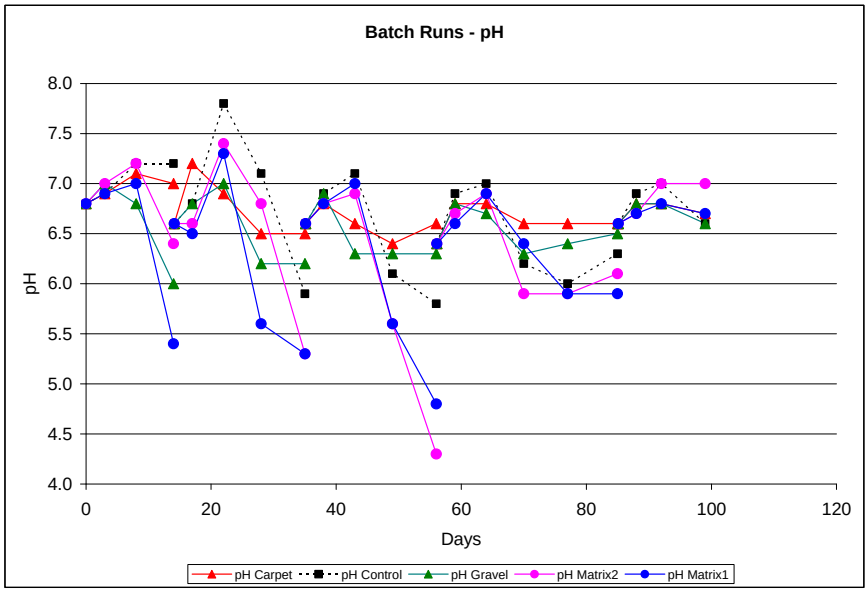
3. The pH buffering capacities varied among different substrate materials. In order to find an explanation for the variation in pH values described above, we ran a series of buffering-capacity tests on equal-volume samples collected from the columns of carpet fiber, old PET matrix, and gravel. All of these samples had been used continuously in the column experiments for a period of about 2 years,; therefore, we assumed that any additives (such as calcium carbonate in the carpet fibers) would have been depleted by the time that the buffering capacity tests were conducted. Tests were performed for column water alone, wet substrate with biofilm, and wet substrate after rinsing to remove biofilm. Two replicates were made for each condition, and the results are shown in the attached graphs. As shown on the graphs, the carpet fiber sample had

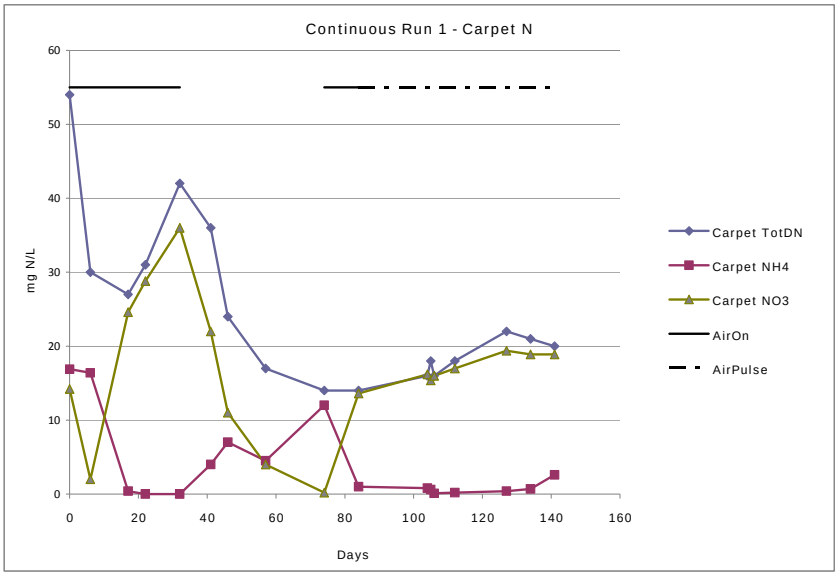
about twice the buffering capacity of gravel, and about 6 times the buffering capacity of the old PET matrix. Biofilm accounted for about half of the buffering capacity for each of the samples.

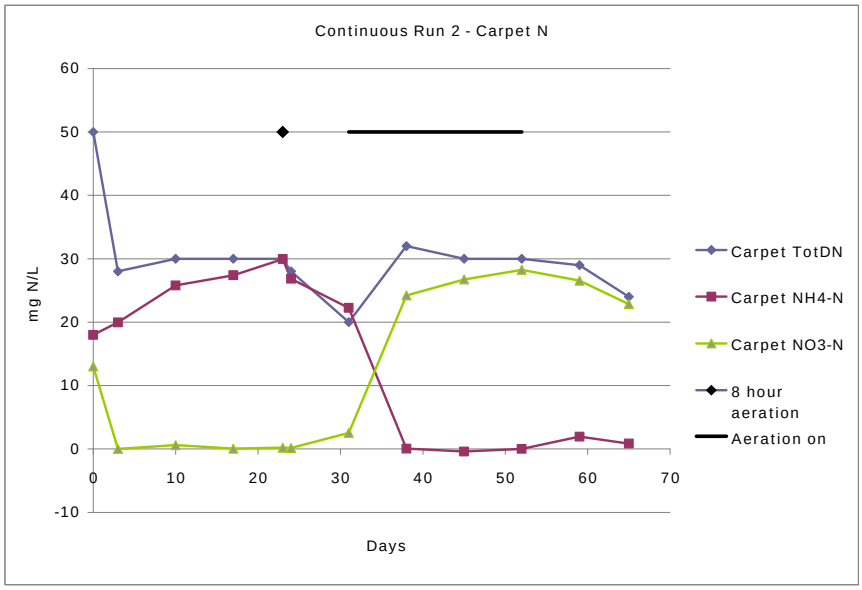


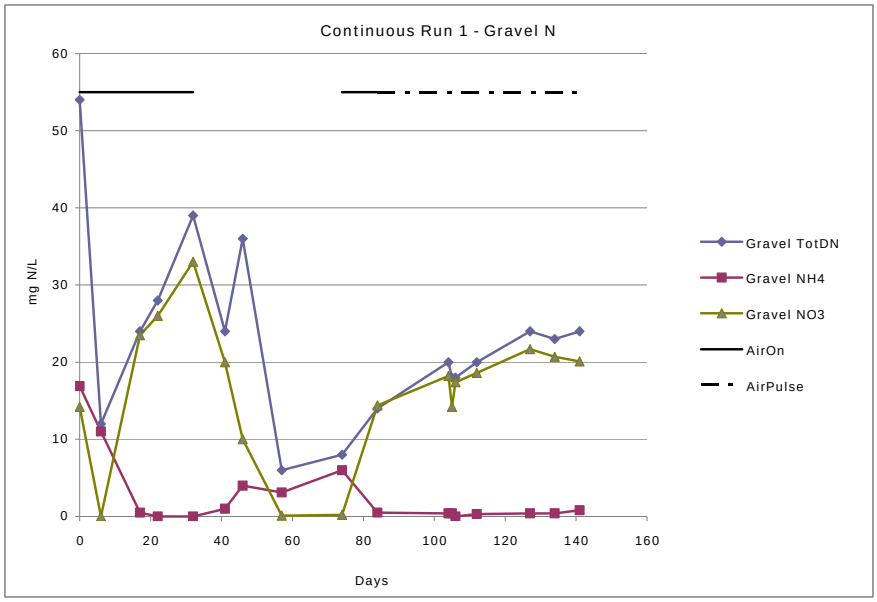



Batch Runs - Total Dissolved N
Total Dissolved N (mg/L)
60
50
40
30
20
10
0
0
20
40
60
80
100
120
Days
TotDN Carpet
TotDN Control
TotDN Gravel
TotDN Matrix2
TotDN Matrix1

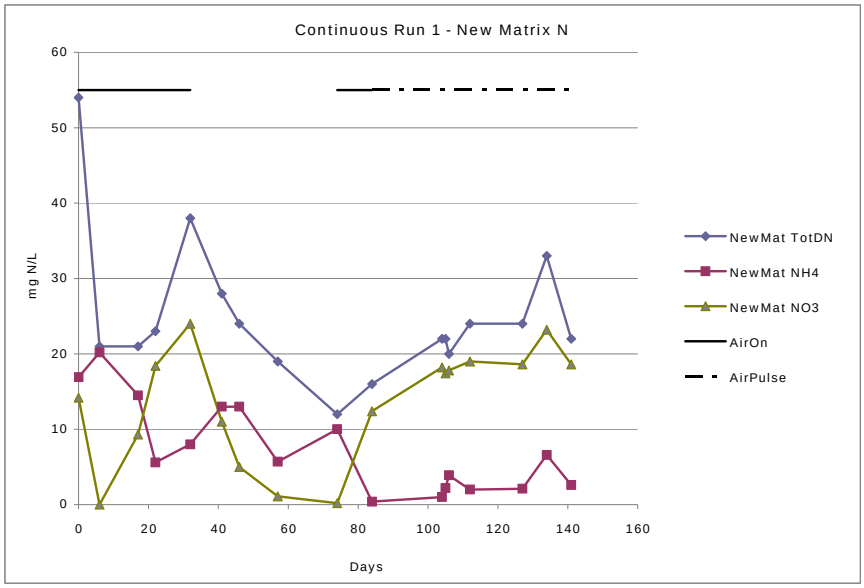

Batch Runs - pH
pH
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
0
20
40
60
80
100
120
Days
pH Carpet
pH Control
pH Gravel
pH Matrix2
pH Matrix1

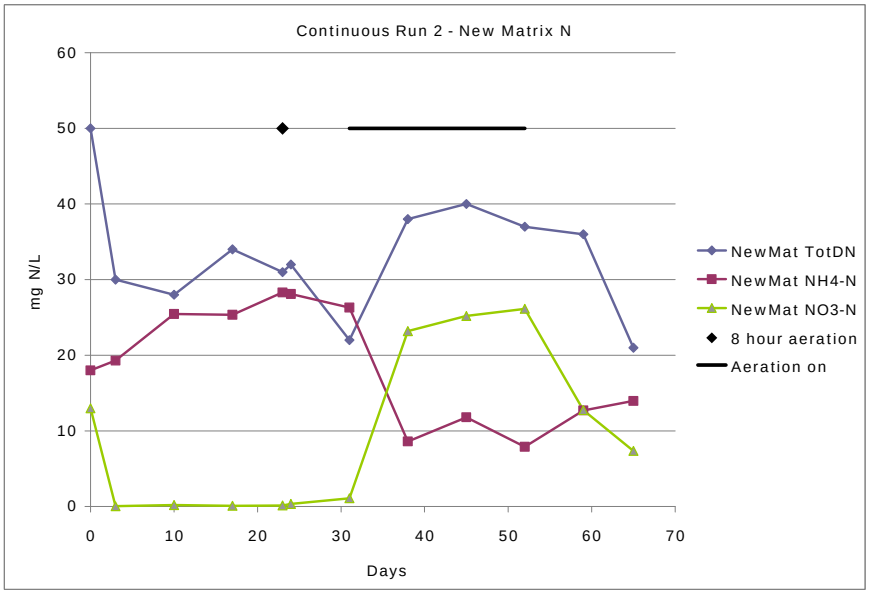

Continuous Run 1 - Carpet N
mg N/L
Days
Carpet TotDN
Carpet NH4
Carpet NO3
AirOn
AirPulse

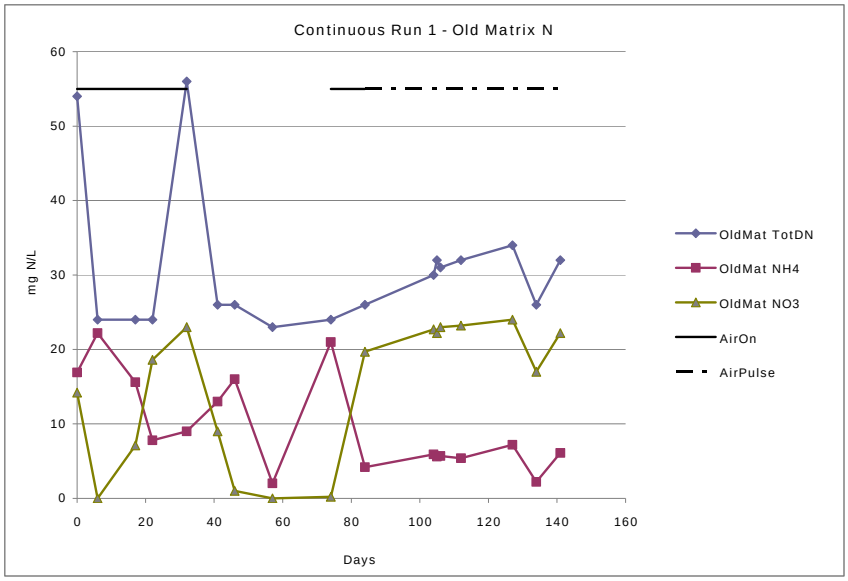

Continuous Run 2 - Carpet N
mg N/L
Days
Carpet TotDN
Carpet NH4-N
Carpet NO3-N
8 hour aeration
Aeration on

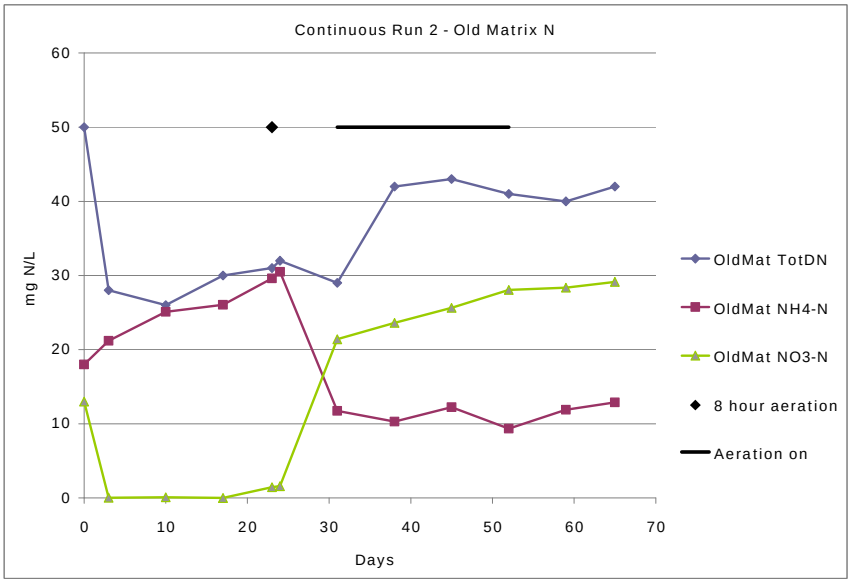

Continuous Run 1 - Gravel N
mg N/L
Days
Gravel TotDN
Gravel NH4
Gravel NO3
AirOn
AirPulse

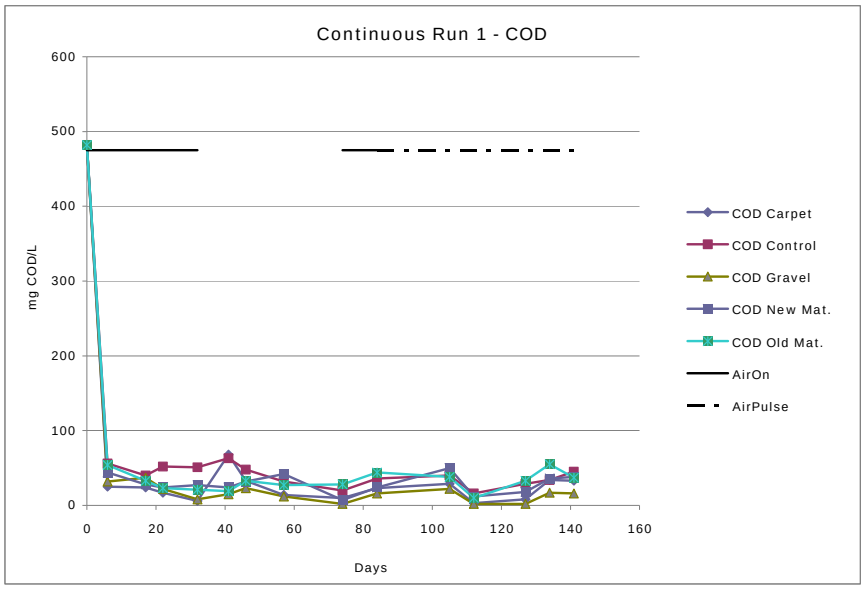

Continuous Run 2 - Gravel N
mg N/L
Days
Gravel TotDN
Gravel NH4-N
Gravel NO3-N
8 hour aeration
Aeration on

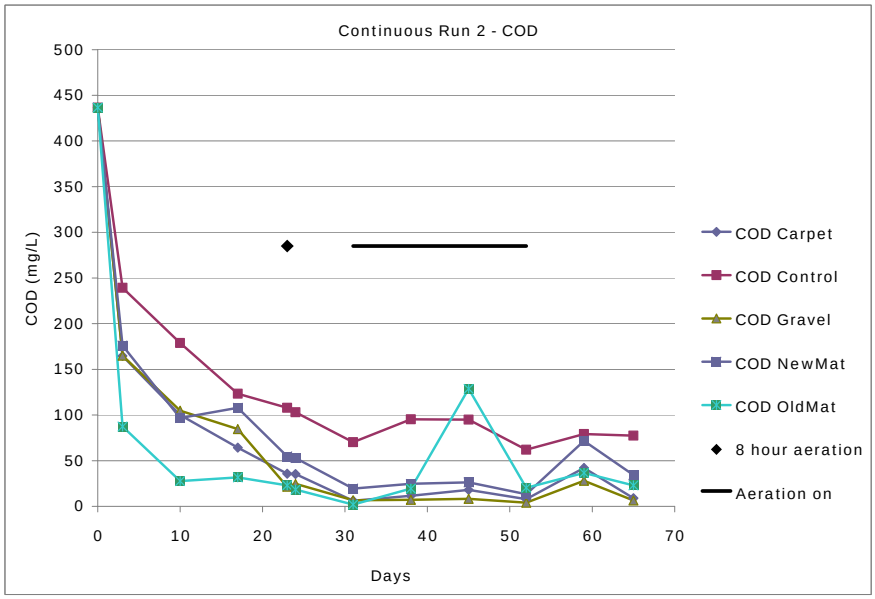

Continuous Run 1 - New Matrix N
mg N/L
Days
NewMat TotDN
NewMat NH4
NewMat NO3
AirOn
AirPulse

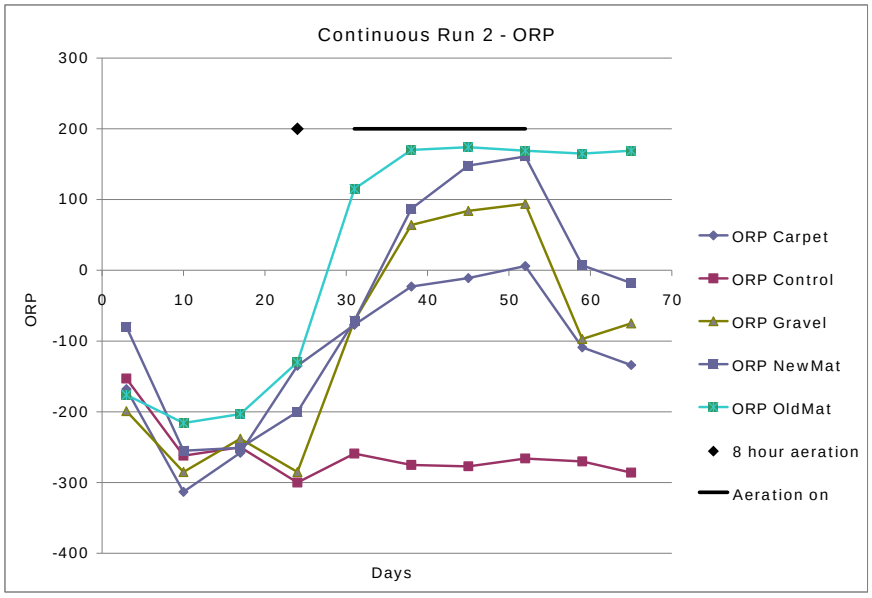

Continuous Run 2 - New Matrix N
mg N/L
Days
NewMat TotDN
NewMat NH4-N
NewMat NO3-N
8 hour aeration
Aeration on

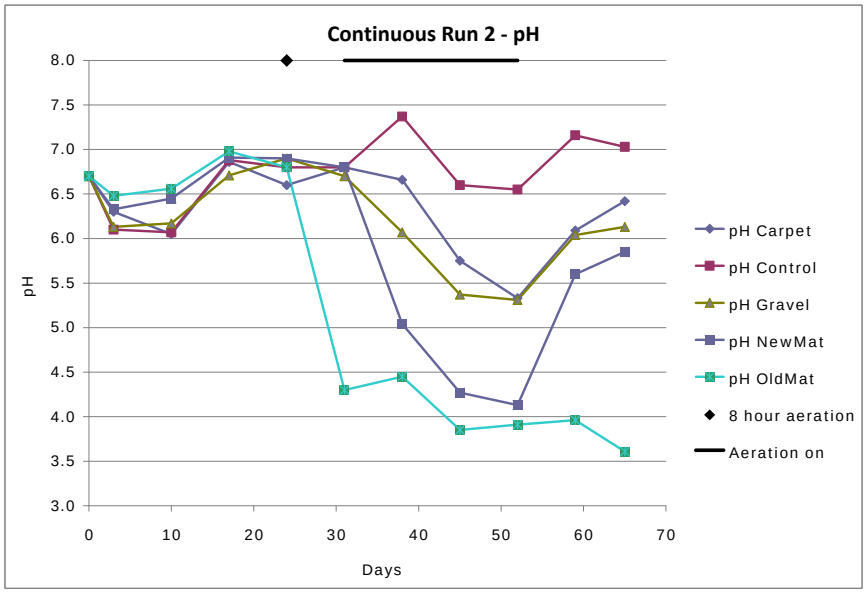

Continuous Run 1 - Old Matrix N
mg N/L
Days
OldMat TotDN
OldMat NH4
OldMat NO3
AirOn
AirPulse

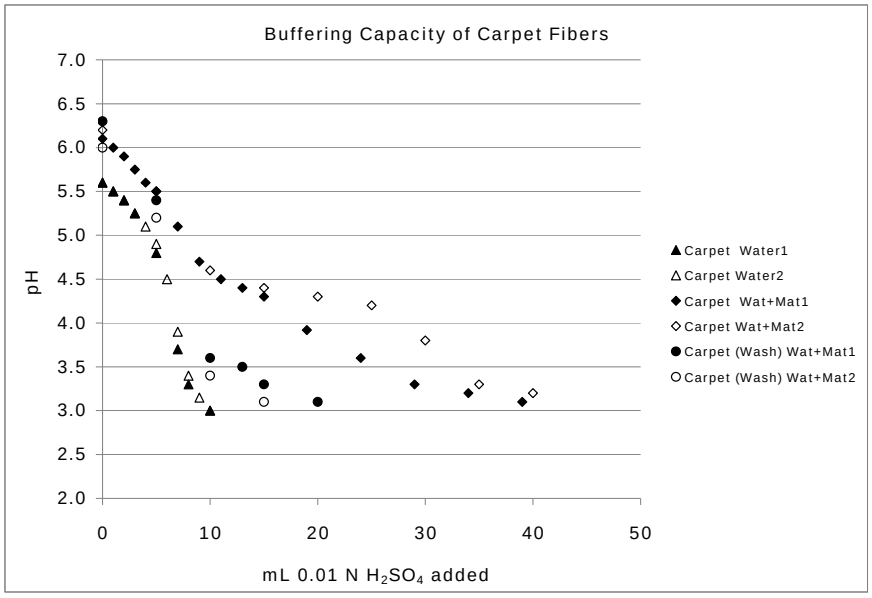

Continuous Run 2 - Old Matrix N
mg N/L
Days
OldMat TotDN
OldMat NH4-N
OldMat NO3-N
8 hour aeration
Aeration on

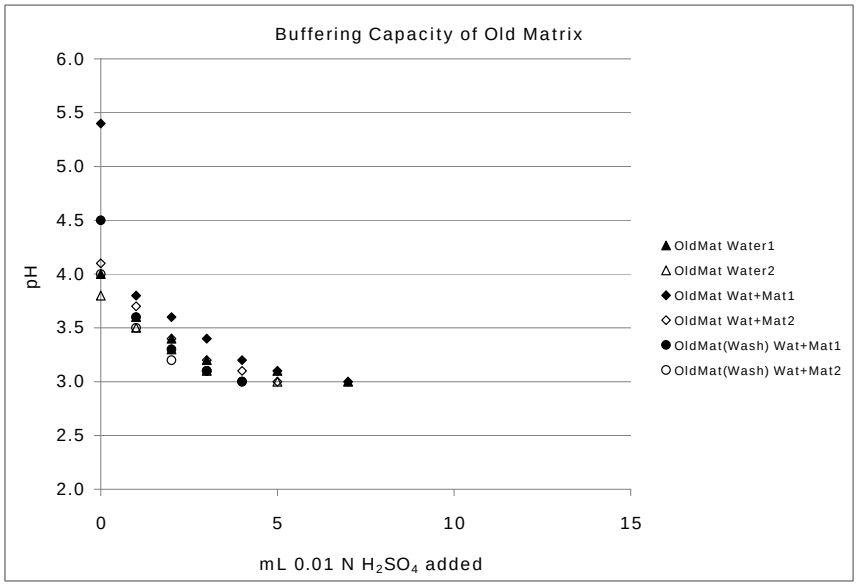

Continuous Run 1 - COD
mg COD/L
600
500
400
300
200
100
0
0
20
40
60
80
100
120
140
160
Days
COD Carpet
COD Control
COD Gravel
COD New Mat.
COD Old Mat.
AirOn
AirPulse

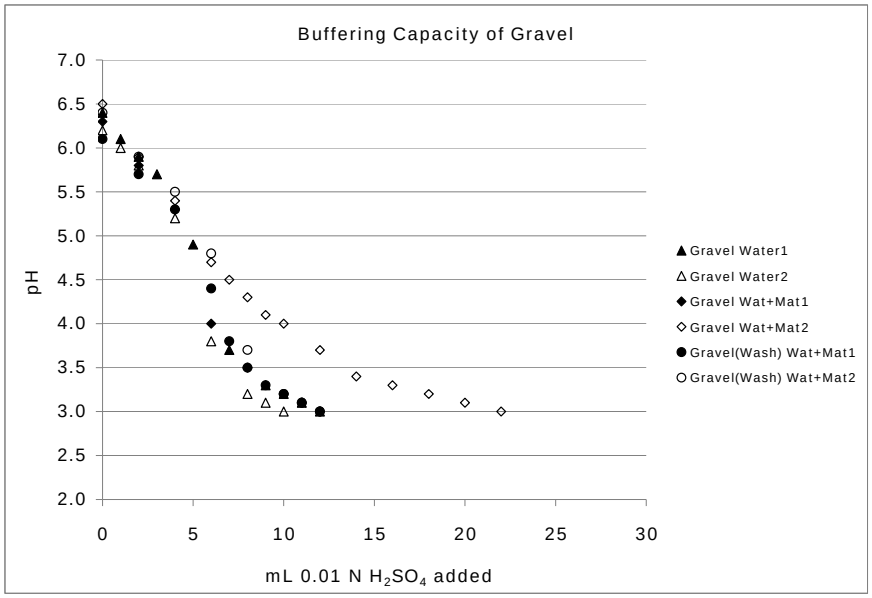

Continuous Run 2 - COD
COD (mg/L)
500
450
400
350
300
250
200
150
100
50
0
0
10
20
30
40
50
60
70
Days
COD Carpet
COD Control
COD Gravel
COD NewMat
COD OldMat
8 hour aeration
Aeration on


Continuous Run 2 - ORP
300
200
100
0
-100
-200
-300
-400
ORP
0
10
20
30
40
50
60
70
Days
ORP Carpet
ORP Control
ORP Gravel
ORP NewMat
ORP OldMat
8 hour aeration
Aeration on


Continuous Run 2 - pH
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
pH
0
10
20
30
40
50
60
70
Days
pH Carpet
pH Control
pH Gravel
pH NewMat
pH OldMat
8 hour aeration
Aeration on


Buffering Capacity of Carpet Fibers
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
pH
0
10
20
30
40
50
mL 0.01 N H2SO4 added
Carpet Water1
Carpet Water2
Carpet Wat+Mat1
Carpet Wat+Mat2
Carpet (Wash) Wat+Mat1
Carpet (Wash) Wat+Mat2


Buffering Capacity of Old Matrix
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
pH
0
5
10
15
mL 0.01 N H2SO4 added
OldMat Water1
OldMat Water2
OldMat Wat+Mat1
OldMat Wat+Mat2
OldMat(Wash) Wat+Mat1
OldMat(Wash) Wat+Mat2



Pilot-Scale Testing at a Residential Wastewater Treatment Lagoon

Site Description

A 2500-square foot circular island with a 1.5-horsepower circulation pump was installed in a wastewater treatment lagoon at the Rehberg Ranch Subdivision in Billings MT during November, 2009, and is currently operating successfully. The pilot project was funded by a joint effort of the City of Billings, the MBRCT Grant, Floating Island International, Headwaters Floating Island, and AquaMaster Inc. The system has survived several sub-zero periods of winter weather without significant damage. The island covers approximately 6% of the lagoon surface area. Vegetation growth on the elevated portions of the matrix has been prolific, while growth of aquatic plants within the submerged center of the island has been minimal. The attached photos show the island during the first year of operation.

The site comprises two similar treatment lagoons, one of which contains the island, and the other which is used as a control. Inflow to the lagoons consists of residential sewage that has been through a grinder but is otherwise raw. The inflow volume is split equally between the two lagoons, which are operated in parallel. Treated outflow from each lagoon is run through a sterilizer prior to transfer to a third storage pond, and eventual surface discharge. Evaporation exceeded inflow to both lagoons starting in early August 2010, and there was no discharge from either lagoon during the high-evaporation period.

Biweekly monitoring has been performed under Grant funding since the island was launched, and additional biweekly monitoring (on alternating weeks) has been performed by the City of Billings as part of their project support effort, resulting in weekly data from December 2009 to the present (August 25, 2010). After the Grant period, the City of Billings plans to continue monitoring through the end of 2010, and we anticipate that funding will become available that will provide for continued monitoring for the life of the island. Monitoring is conducted at the outlet of each of the lagoons and the influent to the lagoons. Efficacy is measured by comparing the increased contaminant removal in the island lagoon compared to the control lagoon. Monitored parameters include total phosphorus, phosphate, total nitrogen, ammonia, nitrate, biochemical oxygen demand (BOD), total suspended solids (TSS). City of Billings measurements also include dissolved oxygen (DO) and pH.

Results

The system required a period of several months for the two lagoons to stabilize, since they were switched from sequential flow to parallel flow at the beginning of the project; therefore, the first relevant comparative data starts around 3/13/09. In general, the island has had a significant effect on improving the rates of removal for total nitrogen and ammonia for the entire period. The island had a smaller but significant effect on removing total phosphorus and phosphate for a portion of the installed period (3/13/09 through 5/21/10), but has not shown significant removal of total phosphorus or phosphate for the period 7/01/10 through 8/12/10. The island has shown some increased removal of BOD and TSS during the entire period. Table 1 is a summary of island performance from April through June of 2010.

Table 1 – Pilot Study Summary

Location	Billings, MT
Parameters Studied	Ammonia, nitrate, total nitrogen, total phosphorus, phosphate, TSS, BOD
System Type	Aerated lagoon
Floating Island Size	2300 ft^2 (214 m^2) submerged treatment ecosystem (with 1300 ft^2 submerged treatment area and 1000 ft^2 elevated plant growth perimeter)
Water Source	Municipal wastewater (raw, post-grinder WW from a residential subdivision)
Installation Date	November 2009
Flow Rate	12 gpm (2.7 m^3/hr)
Water Body Depth	Estimated at 12 ft (3.7 m)
Water Body Area	36,000 ft^2 (3345 m^2)
Plant Species	Native plants – primarily sedges
Installed Cost	$70,000

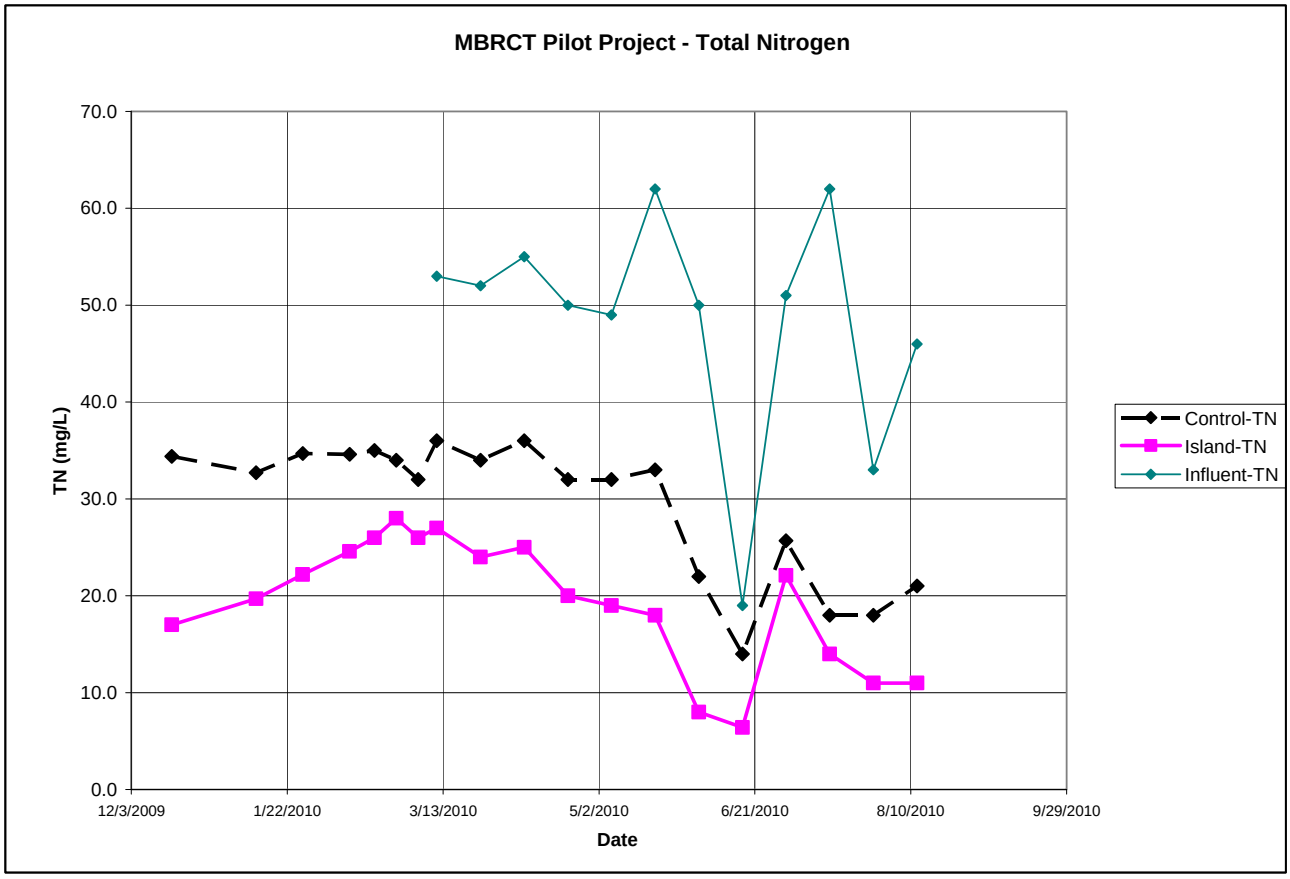
Background:
The Rehberg Ranch Residential Subdivision was built in 2005 on the outskirts of Billings, Montana USA, a city of 120,000 people. Rehberg Subdivision is located in an area beyond the reach of the City of Billings' municipal sewer system. The stand-alone wastewater treatment system for the subdivision is an aerated lagoon wastewater treatment system designed to meet USEPA secondary standards for BOD and TSS. Treated water, rather than being discharged to surface water or groundwater, is land-applied to native prairie grasses that require relatively low nutrient loads. Floating islands (floating treatment wetlands) are being used to remove contaminants so treated water can be applied to less acreage at a higher rate, which will reduce costs.

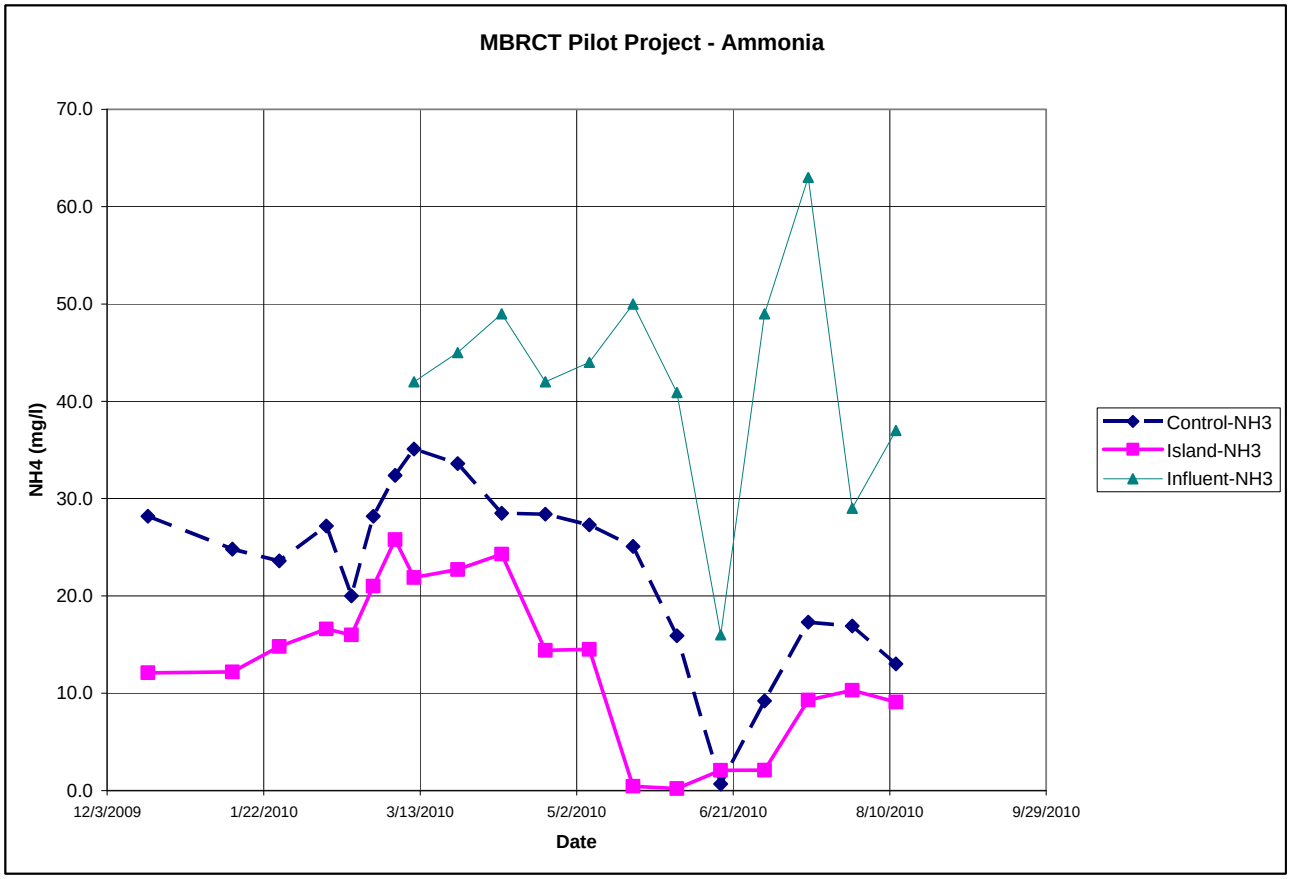
Results
(Averages April - June 2010)

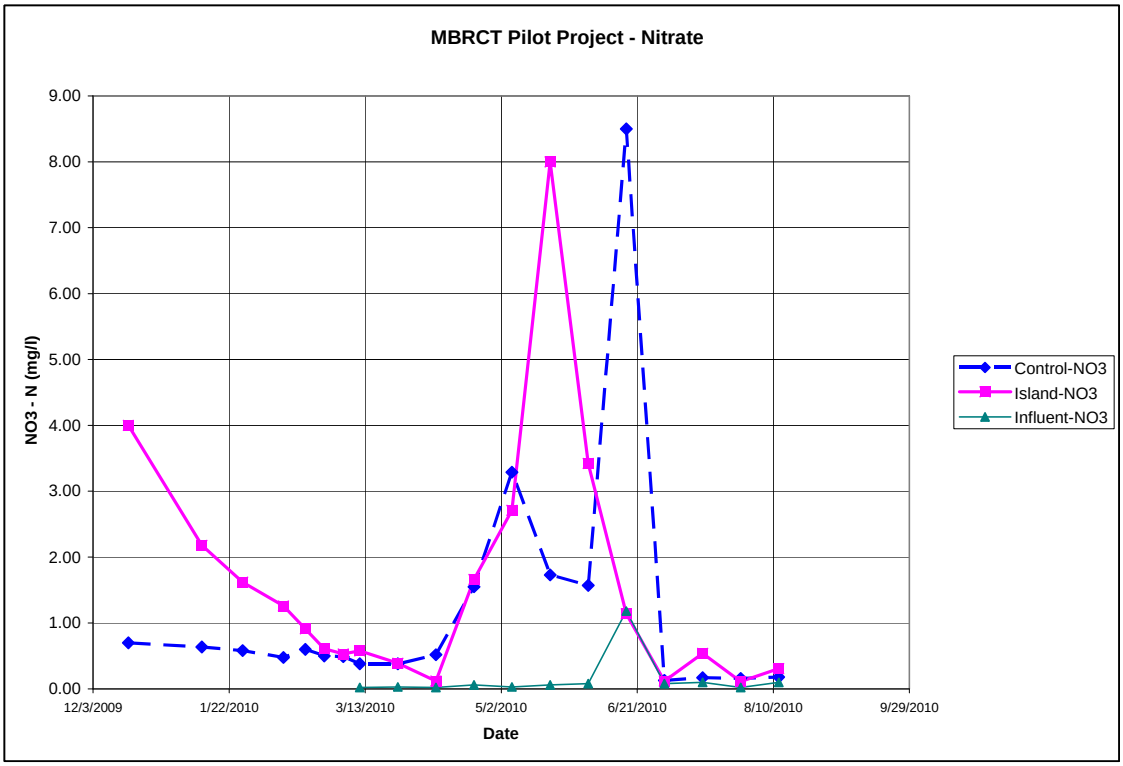
Parameters	**Floating Island Removal Rate (mg/day/ft2)**	**Improvement Compared to Control Lagoon**
Ammonia	480	38%
Total phosphorus	54	27%
TSS	200	9%
BOD	630	9%

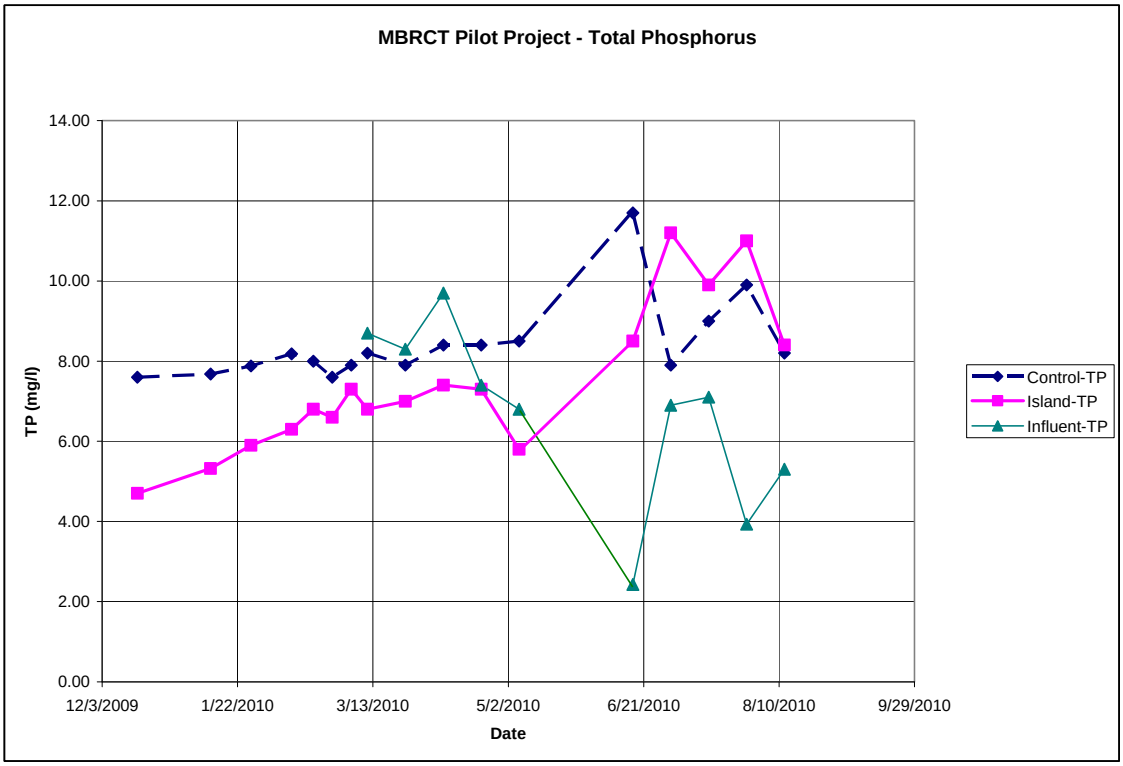
Conclusions of the Pilot Study

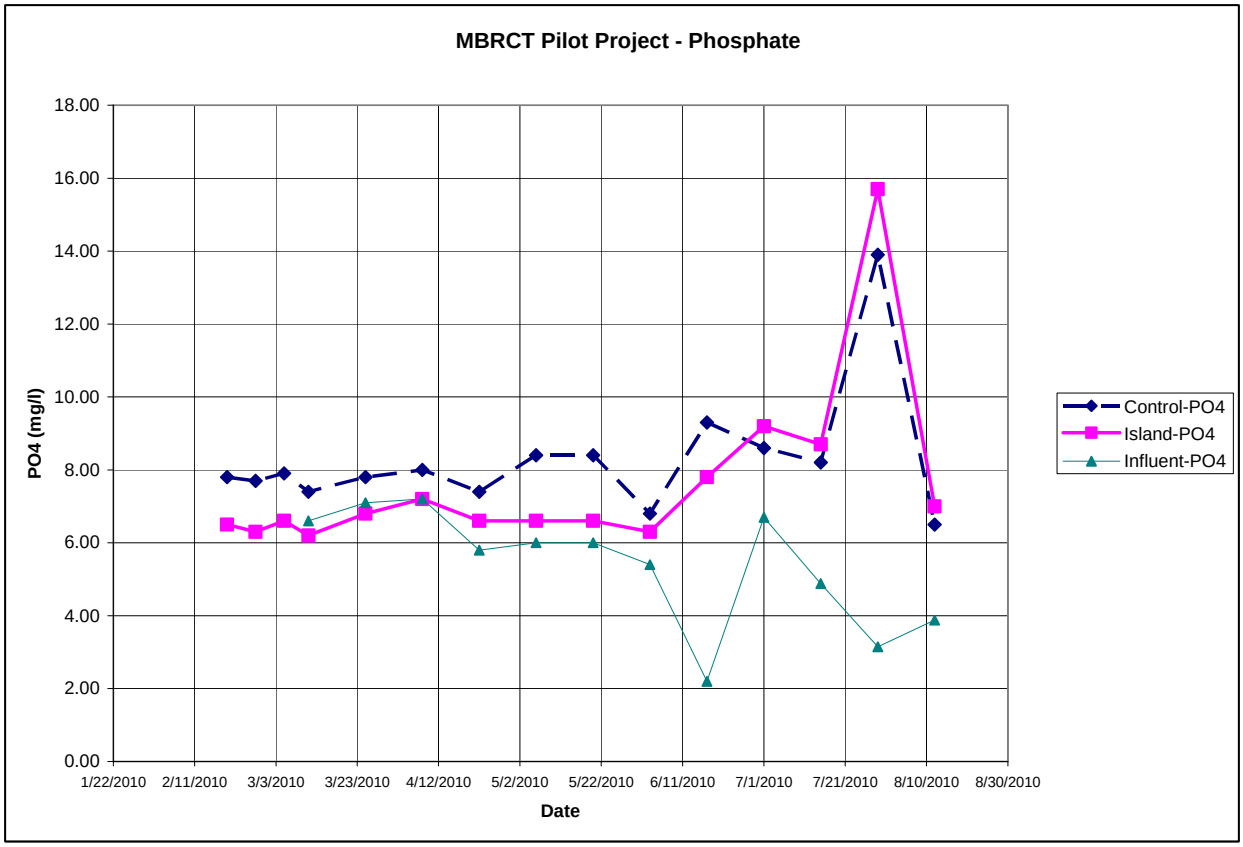
Results to date have been promising, and we expect the island performance to improve after the Grant period has ended. From the graphs, neither the test lagoon nor the control lagoon has experienced significant problems with excess nitrate buildup, while there is residual ammonia in both lagoons. Based on our experimental work with the MSU column experiments, these conditions indicate that island performance for removal of ammonia and total nitrogen can be improved by increasing aeration through the island. Based on our tank-scale work during a previous MBRCT Grant, we confirmed that phosphate removal is also strongly improved by aeration. Based on these data, we are planning to install a forced-air bubbler system under the island during September 2010, and we expect the additional aeration to improve island performance for removal of both nitrogen and phosphorus.

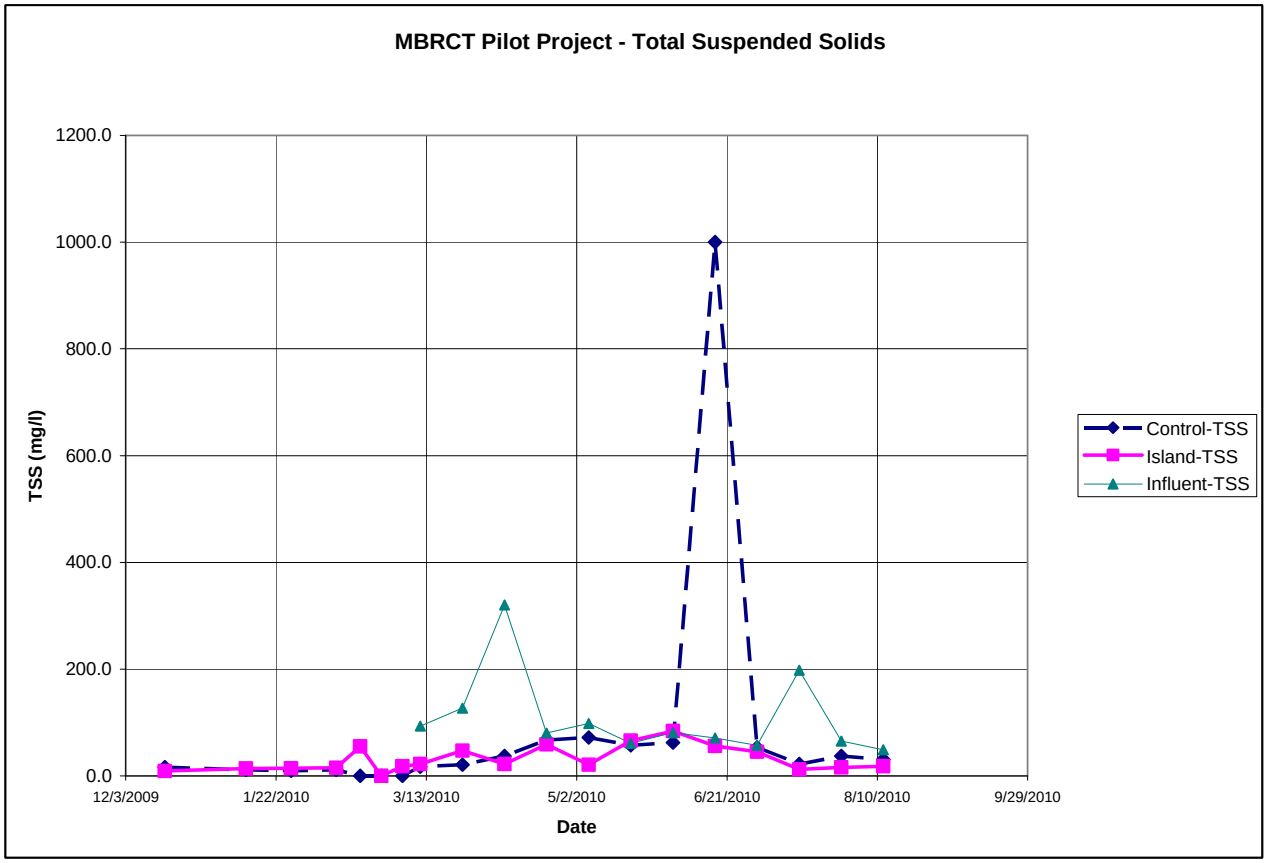

MBRCT Pilot Project - Total Nitrogen
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
TN (mg/L)
12/3/2009
1/22/2010
3/13/2010
5/2/2010
6/21/2010
8/10/2010
9/29/2010
Date
Control-TN
Island-TN
Influent-TN


MBRCT Pilot Project - Ammonia
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
NH4 (mg/l)
12/3/2009
1/22/2010
3/13/2010
5/2/2010
6/21/2010
8/10/2010
9/29/2010
Date
Control-NH3
Island-NH3
Influent-NH3


MBRCT Pilot Project - Nitrate
NO3 - N (mg/l)
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
12/3/2009
1/22/2010
3/13/2010
5/2/2010
6/21/2010
8/10/2010
9/29/2010
Date
Control-NO3
Island-NO3
Influent-NO3

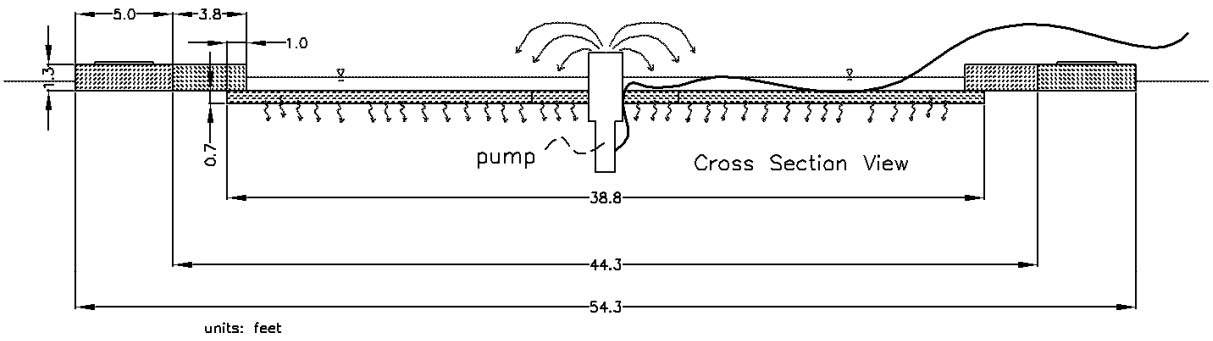

MBRCT Pilot Project - Total Phosphorus
TP (mg/l)
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
12/3/2009
1/22/2010
3/13/2010
5/2/2010
6/21/2010
8/10/2010
9/29/2010
Date
Control-TP
Island-TP
Influent-TP

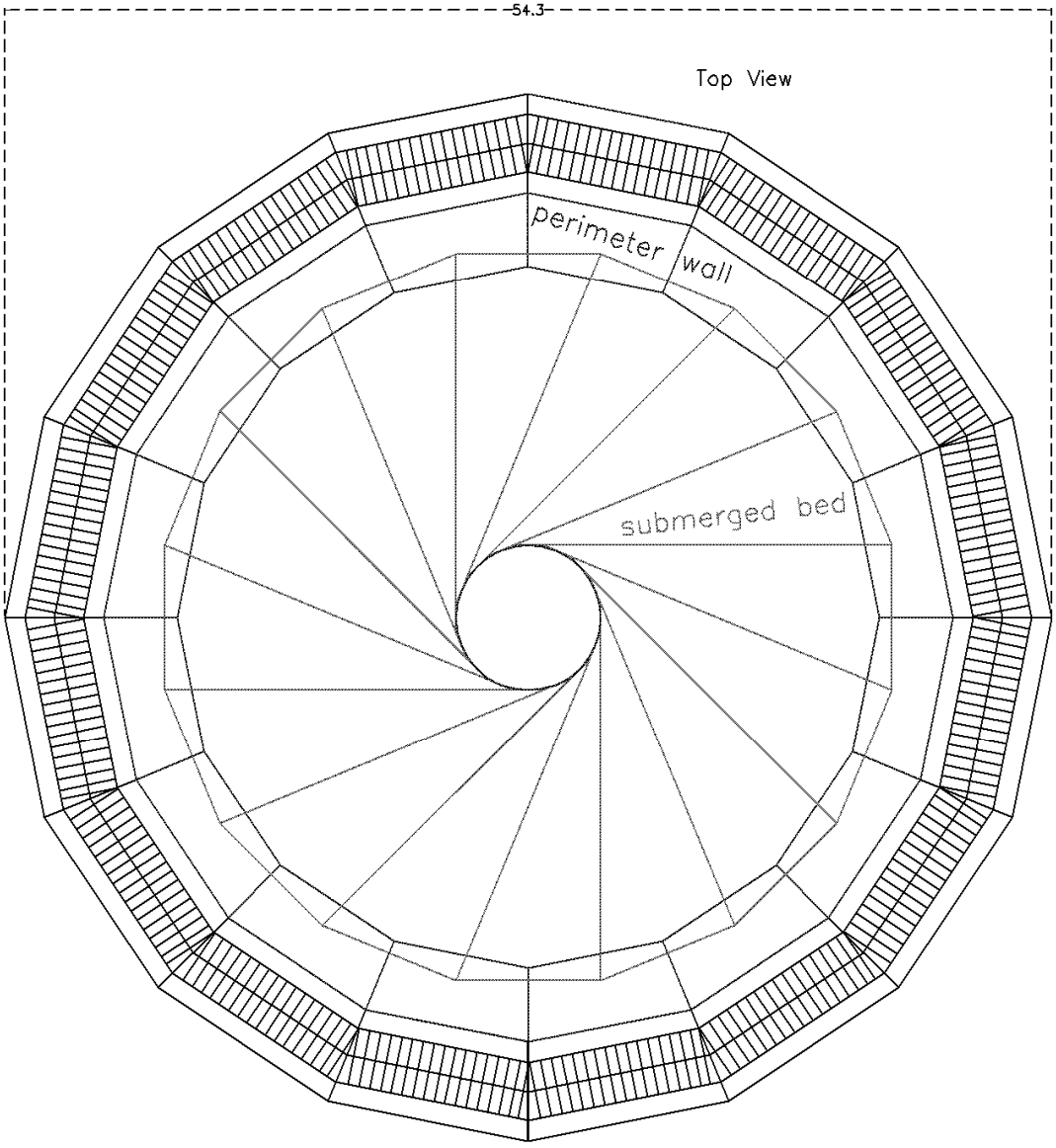

MBRCT Pilot Project - Phosphate
PO4 (mg/l)
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
1/22/2010
2/11/2010
3/3/2010
3/23/2010
4/12/2010
5/2/2010
5/22/2010
6/11/2010
7/1/2010
7/21/2010
8/10/2010
8/30/2010
Date
Control-PO4
Island-PO4
Influent-PO4


MBRCT Pilot Project - Total Suspended Solids
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
TSS (mg/l)
12/3/2009
1/22/2010
3/13/2010
5/2/2010
6/21/2010
8/10/2010
9/29/2010
Date
Control-TSS
Island-TSS
Influent-TSS

MBRCT Pilot Project – Floating Treatment Island

Billings, Montana, USA





Just After Launch – Fall 2009
Early Winter - 2009

Feb 2010
July 2010