

# Enhancing Nitrogen Removal in Stormwater Treatment Facilities for Transportation

Final Report  
January 2015

**Paul T. Imhoff**  
Professor  
University of Delaware  
Newark DE 19716

**Pei C. Chiu**  
Professor  
University of Delaware  
Newark DE 19716

**Qizhong Guo**  
Professor  
Rutgers University  
Piscataway NJ 08854

External Project Manager  
Marianne Walch  
DelDOT Stormwater Quality Program

In cooperation with  
Rutgers, The State University of New Jersey  
And  
State of Delaware  
Department of Transportation  
And  
U.S. Department of Transportation  
Federal Highway Administration

## **Disclaimer Statement**

The contents of this report reflect the views of the authors, who are responsible for the facts and the accuracy of the information presented herein. This document is disseminated under the sponsorship of the Department of Transportation, University Transportation Centers Program, in the interest of information exchange. The U.S. Government assumes no liability for the contents or use thereof.

TECHNICAL REPORT STANDARD TITLE PAGE

1. Report No. CAIT-UTC-045	2. Government Accession No.	3. Recipient's Catalog No.	
4. Title and Subtitle  Enhancing Nitrogen Removal in Stormwater Treatment Facilities for Transportation		5. Report Date January 2015	
		6. Performing Organization Code CAIT/Delaware	
7. Author(s) Paul T. Imhoff, Pei C Chiu, Qizhong Guo		8. Performing Organization Report No. CAIT-UTC-045	
9. Performing Organization, Name and Address Department of Civil and Environmental Engineering University of Delaware Newark, DE 19716		10. Work Unit No.	
		11. Contract or Grant No. DTRT12-G-UTC16	
12. Sponsoring Agency Name and Address Center for Advanced Infrastructure and Transportation Rutgers, The State University of New Jersey 100 Brett Road Piscataway, NJ 08854		13. Type of Report and Period Covered Final Report 1/01/14 - 12/31/2014	
		14. Sponsoring Agency Code	
15. Supplementary Notes U.S Department of Transportation/Research and Innovative Technology Administration 1200 New Jersey Avenue, SE Washington, DC 20590-0001			
16. Abstract Stormwater from roadways is a point source of pollution. State DOTs must comply with Total Maximum Daily Load (TMDL) regulations for nutrients such as nitrogen, which causes water quality impairment. Existing stormwater treatment technologies, such as bioretention cells, do not remove nitrogen adequately to meet water quality standards. New technologies that can more effectively remove nitrogen and reduce the footprint required for stormwater treatment will result in significant savings for State DOTs. We have hypothesized that biochar can be used in bioretention cells to promote microbial removal of nitrate from stormwater and improve bioretention cell performance. The goal of this project is to experimentally test this hypothesis and produce the data necessary to (1) develop a better understanding of biochar-enhanced denitrification and (2) secure multi-year support for a field-scale assessment of the proposed technology. We established a culture of the anaerobic bacterium <i>Geobacter metallireducens</i> and "trained" it to utilize nitrate as an electron acceptor. Batch experiments were carried out to illustrate the ability of a commercial wood biochar, which is being tested in a field detention cell in Delaware, to support nitrate removal by <i>G. metallireducens</i> . This project provided direct or indirect research support for 12 students and resulted in 1 conference paper, 1 conference presentation, 3 seminars/public lectures, and 3 awarded multi-year grants.			
17. Key Words stormwater, nitrate, biochar, microbial denitrification		18. Distributional Statement	
19. Security Classification Unclassified	20. Security Classification (of this page) Unclassified	21. No. of Pages 27	22. Price

## **Acknowledgments**

We thank Marianne Walch of Delaware Department of Transportation (DelDOT) for continually gave us input and guidance on the stormwater problem – particularly on nitrogen removal – and why our work is important to DelDOT.

## 1.0 DESCRIPTION OF THE PROBLEM

Stormwater discharge from roadways is a point source of pollution and thus subject to regulation under the NPDES permitting program. As a co-permittee along with municipalities, State DOTs must comply with Total Maximum Daily Load (TMDL) regulations for bacteria and nutrients and work towards achieving prescribed waste load allocations. TMDL is the maximum amount of a pollutant that a water body can receive without violating the water quality standards. In Delaware, nutrient loading to surface waters is one of the leading causes of water quality impairment. In order to meet water quality standards in the state's nutrient impaired waterways, TMDL regulations may require systematic reduction of point and non-point source discharges of nitrogen and phosphorus into these waterways - including those from roadways. Stormwater treatment technologies, such as detention ponds, can remove nutrients effectively. However, the increased nutrient removal required by TMDL regulations will be costly, since more real estate is required for increased treatment with existing technologies. New treatment technologies are needed that significantly reduce the footprint required for stormwater systems treating roadway runoff - which would result in significant cost reductions for State DOTs.

Through a project supported by the Delaware Department of Transportation (DelDOT), an innovative technology that utilizes biochar and zero-valent iron (ZVI) to enhance the removal of nutrients from roadway runoff is under investigation at the University of Delaware. Preliminary results show significant (up to 50%) removal of ammonium and nearly complete removal of nitrate under laboratory conditions. A pilot scale field test was conducted at the end of 2014, which is expected to substantiate the laboratory-observed removal rates. While the mechanism for ammonium removal by biochar has been explained, a fundamental understanding of how nitrate, the predominant form of nitrogen in stormwater, is removed in the treatment system is still lacking. Without such an understanding, pilot scale data cannot be rigorously interpreted and designs cannot be developed for full-scale systems.

The objective of this project is to produce the data necessary to test the hypothesis that biochar can serve as an electron donor to support the microbial reduction of nitrate in stormwater treatment cells. Such data by themselves may be insufficient to completely elucidate the mechanisms for nitrate removal, but when combined with the pilot-scale data from the field study the data may yield an improved conceptual picture of nitrogen removal and provide (1) a path forward for full-scale design and testing, and (2) the baseline information needed to secure multi-year support for a complete assessment of this promising technology.

## 2.0 APPROACH

We selected the anaerobic bacterium *Geobacter metallireducens* for this research. This microorganism is ubiquitous in anaerobic subsurface and is known to respire humic substances, which have similar redox-active functional groups as biochar. In addition, *G. metallireducens* can be acclimatized, or "trained", to utilize nitrate as an electron acceptor for growth. We chose a commercial wood-based biochar from The Biochar Company ([thebiocharcompany.com](http://thebiocharcompany.com)). The biochar is also being used for a pilot-scale field study in Newark, Delaware. A series of laboratory batch experiments were carried out to evaluate the ability of the biochar to support nitrate degradation by *G. metallireducens*.

## 3.0 METHODOLOGY

### 3.1 Culture Propagation and Maintenance

### 3.1.1 Culture Growth and Maintenance:

*G. metallireducens* was acquired from ATCC (product no. 53774) and propagated by transferring 0.5 mL of the anaerobically thawed pure seed culture to a 30 mL serum bottle containing 6 mL of Fe(III)-acetate growth medium. The serum bottle was then sealed with butyl rubber stopper and aluminum crimp cap and incubated at 30 °C until the medium color changed from amber to dark greenish (after about 12 days). The Fe(III)-acetate growth media (ATCC medium 1768, Appendix A) contained 13.7 g of Fe(III) citrate, 6.8 g of sodium acetate, 0.25 g of NH<sub>4</sub>Cl, 0.678 g of NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O, 0.1 g of KCl, 2.5 g of NaHCO<sub>3</sub>, 10 mL of Wolfe's vitamin solution (ATCC MD-VS), and 10 mL Wolfe's trace mineral solution (ATCC MD-TMS) in 1 L of distilled water. The medium was adjusted to a final pH between 6.8 and 7.0 and degassed using a N<sub>2</sub>:CO<sub>2</sub> (80:20) gas mix. Typically, 1 L of growth medium was prepared and anaerobically transferred into multiple 125-mL serum bottles, each receiving 50-100 mL. During the filling process, the headspace of serum bottles were continuously purged with 80:20 N<sub>2</sub>:CO<sub>2</sub> mix. Bottles were sealed with butyl rubber stoppers and aluminum crimps and then autoclaved for 15-20 min at 121 °C. The sterilized media in serum bottles were then stored at 4 °C if not immediately used. Before use, the sterilized media in serum bottles were purged with 80:20 N<sub>2</sub>:CO<sub>2</sub> mix using a sterile disposable needle.

For routine culture maintenance, 2 mL of liquid *G. metallireducens* culture grown on Fe(III)-acetate (iron G. culture) was transferred into 50 mL of freshly prepared Fe(III)-acetate medium, supplemented with 0.5 mL of 413 mM cysteine-HCl solution (50 g/L, a reducing agent to consume trace O<sub>2</sub> and maintain strictly anaerobic conditions). Culture transfer was carried out once every 8–10 days (Figure B1, Appendix B) at room temperature (20-22 °C) and two iron G. culture bottles were prepared in each transfer. All culture transfers were performed inside the anaerobic glove box (Coy, MI) using sterile disposable syringes and needles. Surface of rubber stoppers were sterilized with a 70% ethanol solution prior to injection. Serum bottles were wrapped with aluminum foil to minimize exposure to light during incubation.

### 3.1.2 Establishment of a Nitrate-Degrading Culture

After five transfers of the original iron G. culture, 10 mL of mature (greenish) iron G. culture was used to inoculate a 125-mL serum bottle containing 90 mL of anoxic nitrate-acetate growth medium. The bottle was then incubated at 30 °C. The nitrate-acetate medium was the same as the Fe(III)-acetate medium except that Fe(III) citrate (55.9 mM) was replaced with sodium nitrate (20 mM) as the electron acceptor. Sodium acetate (82.9 mM) remained the sole electron donor in the medium. Initial attempts of developing nitrate-acetate G. culture were not successful; subsequently the growth medium was modified with higher quantities of NH<sub>4</sub>Cl from 0.25 g (ATCC medium 1768) to 1.5 g (Lovley & Philipps, 1988), adjustment of pH to 6.8 (Chovanec et al. 2012), and addition of 1 mL of cysteine-HCl solution per 100 mL of medium. Figure B2 shows a successful transfer of iron G. culture into nitrate-acetate medium.

Succeeding transfers were carried out in nitrate-acetate growth medium with a reduced amount of sodium nitrate (5 mM) and sodium acetate (10 mM) to control the pH and to optimize the use of growth medium. To maintain the nitrate G. culture 2 mL of mature culture was transferred to 50 mL of fresh nitrate-acetate medium supplemented with 0.4 mL of cysteine solution, and 0.1 mL of 0.22 µm-filtered medium solution containing Fe(II) from completely reduced iron G. culture medium. A minimum of 50 µM supplementary Fe(II) solution was necessary because *G. metallireducens* requires multi-heme cytochrome *c* for nitrate respiration (Senko and Stolz, 2001). The nitrate G. culture was incubated at room temperature and only one bottle was transferred on a weekly basis. One-week old nitrate G. culture would receive 0.5 mL of 1 M nitrate stock solution and transferred after 2 weeks. Figure B3 shows examples of the nitrate G. culture. The changes in color and turbidity from clear to pinkish and cloudy after 5-7 days of incubation at room temperature indicate microbial growth.

### 3.1.3 Cell Suspension Preparation

To prepare cell suspensions for batch experiments, 10 serum bottles, each containing 10 mL of nitrate G. culture (i.e. 10 % culture transfer), 90 mL of modified nitrate-acetate media, 0.8 mL of cysteine stock solution and 0.2 mL of filtered Fe(II) solution, were incubated at 30–32 °C for 4 days. The cultures were periodically sampled to monitor acetate and nitrate concentrations in order to ensure that cells harvested were grown with a slight excess in nitrate (i.e. cells were in oxidized state). While inside the anaerobic glove box, the contents of the 10 serum bottles were transferred into 50 mL conical centrifuge tubes (i.e. 2 centrifuge tubes per serum bottle) and screw caps were wrapped with yellow vinyl tape (Figure C1, Appendix C). The sealed tubes were centrifuged at 8,000 rpm for 15 min. Preliminary tests using blank media with resazurin (an indicator dye for detecting the presence of oxygen) showed this protocol would keep the culture suspension anaerobic during centrifugation. Blank medium was prepared in the same way as the nitrate-acetate growth medium except it contained no acetate, nitrate, or ammonium. The cell pellets were then concentrated into 12 tubes and resuspended using blank media with 0.25 mL of cysteine solution per 50 mL of culture suspension. The 12 tubes were centrifuged and then concentrated into four tubes, and the process was repeated until the cells were concentrated in one single tube. The cell pellet in that tube was resuspended in 33 mL of blank media. Cell density of the suspension was determined by measuring the optical density of diluted suspension at 600 nm using a Cary UV/Vis spectrophotometer. The cell suspension was such that the measured absorbance at 600 nm was between 0.3 and 0.6 (within McFarland standards). Direct cell counting using a Hausser Scientific Bright-Line™ counting chamber was performed to confirm the applicability of the McFarland standard correlations between cell density and measured absorbance at 600 nm.

### 3.2 Biochar Preparation

Commercially produced biochar (Soil Reef) from hardwood chips through slow pyrolysis at 600°C was purchased from The Biochar Company, PA. The physicochemical properties of the biochar are provided in Table 1. The biochar was sieved to obtain a particle size between 250-500 µm. The biochar was then suspended in deionized (DI) water in a 1000 mL Erlenmeyer flask at a concentration of ~50 g of biochar per L of DI water. In order to oxidize the redox-labile functional groups in the biochar, the suspension was aerated with low-pressure air for days, after which it was left to settle for 4–10 hrs (Figures D1 and D2, Appendix D). During aeration, 0.2–0.5 mL of 6N H<sub>2</sub>SO<sub>4</sub> was added every 0.5–1 hr until the total volume added was 6 mL. The pH of the biochar suspension was monitored after acid addition to make sure the pH remained around 7.0. After particle settling, the suspension was decanted and water replaced with clean DI water. Colloidal particles that did not settle were removed along with the decanted water. The aeration and settlement cycle was repeated until the suspension had been aerated for a total of approximately 60–70 hours and had been washed with 2000–2500 mL of DI water. The biochar was then vacuum filtered and placed onto separate aluminum foil trays (Figure D3, Appendix D). The trays were weighed and repeatedly dried inside a vacuum oven at 55–65 °C until the biochar mass remained constant (i.e. all moisture had been removed, Figure D4, Appendix D). The dry oxidized biochar was then stored at room temperature in a glass container wrapped in aluminum foil. A total of ~70 g of dry oxidized biochar was produced from 3 flasks of biochar suspensions.

### 3.3 Sample Collection and Analysis

Samples were collected at per-determined times during the course of a batch experiment for measurement of NH<sub>4</sub><sup>+</sup> and three anions, acetate, nitrite, and nitrate. All liquid sampling from the serum bottles was done under strict protocol to prevent microbial contamination and oxygen infusion. The rubber stopper of each

bottle was always sterilized with Kimwipe soaked in 70% ethanol prior to sampling. The glass sampling syringe used had a sterile disposable 21G needle and was filled and flushed twice with an 80:20 N<sub>2</sub>:CO<sub>2</sub> gas mix. The syringe was then emptied and immediately inserted into the stopper for sample collection. A 1-mL sample was drawn from each bottle and diluted 10 folds with DI water in a 10-mL volumetric flask (Figure E1, Appendix E). After mixing, the diluted sample was immediately filtered with a 25 mm diameter syringe filter (0.22- $\mu$ m MCE, Millex-GS) and transferred into two separate plastic vials (1.5 mL for NH<sub>4</sub><sup>+</sup> analysis and 8 mL for anions analysis). Filtered liquid samples were stored at 4°C for 1-2 days if ion chromatography (IC) analysis could not be performed immediately after sampling.

Acetate, nitrite and nitrate analyses were performed using a Metrohm 850 Professional IC AnCat unit. The mobile phases were a mixture of Metrohm MPak A Supp 5 (3.2 mM sodium carbonate, 1.0 mM sodium bicarbonate) and 6.5 % v/v acetone at a flow rate of 0.7 mL/min and the column oven was set at 28°C. The concentrations of acetate, nitrite and nitrate were detected using 887 Professional UV-vis detector and quantified using MagIC Net analytical software. NH<sub>4</sub><sup>+</sup> analysis was conducted using a Dionex IC (ICS-1100) equipped with an Ion PAC CS16 (5 x 250 mm). The mobile phase was 38 mN sulfuric acid at 1 mL/min. The concentrations of NH<sub>4</sub><sup>+</sup> were detected using a conductivity detector and quantified using Chromeleon 7.0 software.

Prior to each experiment, standard calibration curves for NH<sub>4</sub><sup>+</sup>, acetate, nitrite and nitrate were established from individual stock solutions prepared in blank media (containing sodium bicarbonate buffer, vitamins, KCl, and trace minerals at pH 6.8). One mL sample from various individual stock solutions was diluted 10 folds with DI water to prepare standard solutions (between 0.1 and 1.0 mM) of ammonium chloride, sodium acetate, sodium nitrate, and sodium nitrite. Samples were taken from the standard solutions for IC analyses in order to create calibration curves (see Figures F1–F4, Appendix F). For quality control and assurance purposes, each batch of samples analyzed by IC also included 10-fold DI water-diluted samples of 2.5 mM standard solutions of ammonium chloride, sodium acetate, sodium nitrite, and nitrate.

### **3.4 Experiment 1: Biochar as Electron Acceptor to Support *G. metallireducens***

A batch experiment with two different masses of oxidized biochar (2 g and 4 g) and nitrate culture cell suspension was set-up in triplicates in 125 mL serum bottles with 100 mL of blank media plus 0.5 mL of cysteine stock solution. Additional triplicate bottles were set up to serve as controls: abiotic control, with 2 g oxidized biochar only, biotic control with cells only, and blank control with medium only. In this experiment, air-oxidized biochar served as a metabolic electron acceptor for *G. metallireducens* to utilize the added acetate as electron donor.

Prior to the experiment, the weight of each of the serum bottles with oxidized biochar (6 bottles with 2 g of biochar and 3 bottles with 4 g of biochar) was recorded. The bottles were then wrapped with aluminum foil and placed inside a vacuum chamber for 3 cycles of vacuum and flushing with N<sub>2</sub> gas. The degassed serum bottles were then sealed with rubber stoppers and aluminum crimps inside the glove box. To make sure that the biochar micro-pores were completely anaerobic, the serum bottles were individually vacuumed using a sterile needle and a vacuum pump. The degassed serum bottles were kept under vacuum condition for 60 hrs inside a vacuum chamber and then individually flushed with 80:20 N<sub>2</sub>:CO<sub>2</sub> gas mix.

The degassed serum bottles with biochar were brought inside the glove box and the crimps and stoppers were removed. One hundred mL of blank medium at pH 6.8 was added to each bottle using disposable sterile pipettes (Figure G1, Appendix G). The pH of the blank medium used for bottles with 2 g and 4 g biochar had been previously adjusted to 6.7 and 6.5, respectively using HCl. Preliminary tests were done



to determine the pH adjustments to the blank media such that the equilibrium pH with biochar would be approximately 6.8. All of the 15 serum bottles were amended with 0.5 mL of 50 g/L cysteine solution to eliminate trace amounts of oxygen. Three mL of the concentrated *G. metallireducens* cells (ca.  $4 \times 10^{10}$  cells/mL) were added to each of the biotic serum bottles using disposable sterile pipettes (Figure G2, Appendix G). All 15 serum bottles were then re-sealed with new stoppers and crimps and brought outside of the glove box (Figure G3, Appendix G). The headspace of each bottle was flushed for 30 seconds with an 80:20 N<sub>2</sub>:CO<sub>2</sub> gas mix. The bottles were transferred back inside the glove box, and 0.4 mL of 1 M sodium acetate solution was added to each bottle using a syringe, marking the start of the experiment.

Immediately after, each bottle was swirled to mix its content and then brought outside the glove box for incubation at 30–32 °C. Liquid samples were taken at 3, 8, 18, 24, 39, 48, 72, 96, 120 144 hrs following procedure described in Section 3.3. To make sure that replicate bottles had the same incubation period, the triplicate bottles in each treatment or control group was taken in and out of the incubator at the same time. Every after 3 sampling times, the head space of the serum bottles were flushed with an 80:20 N<sub>2</sub>:CO<sub>2</sub> gas mix to replace the liquid volume taken out. Liquid samples were analyzed for NH<sub>4</sub><sup>+</sup>, acetate, nitrite, and nitrate.

### **3.5 Experiment 2: Microbially Reduced Biochar as Electron Donor for Nitrate Reduction**

The second batch experiment was set-up immediately after the biochar-acetate experiment. In this biochar-nitrate experiment, microbially reduced biochar was the electron donor when *G. metallireducens* metabolically utilized the added nitrate as electron acceptor.

Biotic serum bottles with 4 g biochar from experiment 1 were carried over to the second batch experiment as biotic biologically-reduced biochar group. To replenish the liquid volume removed, these biotic serum bottles with biochar were refilled with ~9.5 mL of pH 6.8 blank medium. Additional sets of experimental bottles (abiotic 4g oxidized biochar and cells only) were set up in triplicates as described previously. Cell suspension of concentrated *G. metallireducens* cells ( $8 \times 10^9$  cells/mL) was prepared but only used for cells-only bottles. Biologically reduced biochar bottles with cells were not re-inoculated with *Geobacter* cells. All 9 serum bottles contained 100 mL blank media, 0.5 mL cysteine HCl stock solution and 0.8 mL of 588.4 mM sodium nitrate stock solution and were incubated at 30–32°C. Liquid samples were taken at 3, 8, 18, 24, 39, 48, 72, 96, 120 144 hrs to quantify NH<sub>4</sub><sup>+</sup>, acetate, nitrite, and nitrate. Sampling and analytical protocols observed in the previous batch experiment were also implemented in the second batch experiment.

## **4.0 FINDINGS**

### **4.1 Biochar as Electron Acceptor to Support *G. metallireducens***

Within the first 72 hours, 60 - 80 % of initial acetate concentrations in biotic serum bottles with oxidized biochar were removed while negligible change in acetate concentrations were observed in abiotic (media plus oxidized biochar and acetate) and biotic (media plus cells and acetate) control bottles (Figure 1). This supports the hypothesis that *G. metallireducens* can utilize oxidized biochar to metabolize acetate, and that bacterial activity alone was predominant of all possible processes that could consume/remove acetate from solution (e.g. sorption). Figure 1 also shows that *G. metallireducens* could not utilize acetate as electron donor without biochar, since acetate concentrations in biotic control bottles remained practically constant within 72 hrs. After 72 hrs, however, acetate concentrations in control bottles started to decrease while in biotic bottles containing biochar acetate concentration started to increase.

## 4.2 Microbially Reduced Biochar as Electron Donor for Nitrate Reduction

The effect of microbially reduced biochar on nitrate utilization is illustrated in Figures 2 and 3. Without addition of biochar, nitrate removal was limited, which occurred only initially and ceased upon depletion of residual electron donor(s) carried over from the inoculum. Inclusion of pre-reduced biochar sustained nitrate removal by *G. metallireducens* beyond the first two days, and complete consumption of nitrate was observed *only* when reduced biochar was present (Figure 3).

The SEM images of *G. metallireducens* used in these experiments, harvested from the acetate-nitrate medium and the acetate-iron oxide system are shown in Figure 4.

## 5.0 CONCLUSIONS

The batch experimental data clearly illustrate that the commercial biochar can act as both a reducing agent and an oxidizing agent to support the activities of anaerobic microorganisms such as *G. metallireducens*, including the oxidation of organic substrates (e.g., acetate) and the reduction of electron acceptors such as nitrate. Under similar conditions such as in saturated and anaerobic bioretention cells containing soil and biochar, microorganisms capable of reducing nitrate can do so using biochar as an electron source, which would result in faster and more complete removal of nitrate from stormwater.

In addition to the research described above, this project provided research opportunities (with or without financial support) for a number of undergraduate and high school students:

<b>Name</b>	<b>School / Program</b>	<b>Time Involved</b>	<b>Funding Source</b>
Robert Rose	UD Env. Engineering	summer 2014	CAIT (\$1,500) & NSF EPSCoR
Naomi Chang	UD Env. Engineering	spring 2014	DWRC fellowship
Chris Youngquist	UD Env. Engineering	summer + fall 2014	DWRC fellowship
Jing Jin	UD Env. Engineering	spring 2014	volunteer
Jillian Allen	UD Env. Engineering	winter + spring 2014	DENIN scholarship
Lyndsay Fagan	UD Env. Engineering	winter + spring 2014	DENIN scholarship
Sangwon Jeon	UD Bio/Chemistry	winter + spring 2014	independent study
Zelin Zhang	DTCC Chemistry/Math	summer 2014	NSF EPSCoR
Jared Saquing	Wilmington Charter	summer 2014	UD CoE HS internship
Sriram Cyr	Wilmington Charter	summer 2014	UD CoE HS internship

This project also provided indirect support (supplies and travel money) for two University of Delaware Ph.D. students funded on fellowships: Lauren S. Lechner and Jing Tian.

One conference paper resulted from this work: Tian, J., Yi, S., Imhoff, P., Chiu, P., Guo, M., Maresca, J., Beneski, V., and Cooksey, S. (2014) Biochar-Amended Media for Enhanced Nutrient Removal in Stormwater Facilities. World Environmental and Water Resources Congress 2014: pp. 197-208. doi: 10.1061/9780784413548.022.

This work was presented in an oral presentation at the ASCE World Environmental and Water Resources Congress 2014: Water Without Borders, June 2014. In addition, several seminars and public lectures have resulted from this project:

- UTC Brown Bag seminar "Enhancing Nitrogen Removal in Stormwater Facilities for Transportation" presented by a graduate student, Ms. Jing Tian, on 3/11/14;
- Presentation at Delaware Department of Transportation Forum, "Integrating Zero-Valent Iron and Biochar Amendments in Green Stormwater Management Systems for Enhanced Treatment of Roadway Runoff" on May 7, 2014;
- A public lecture in the Environmental Frontier Seminar Series, "Microbial Nitrate Removal Promoted by Zero-Valent Iron and Black Carbon (Biochar)" on December 8, 2014.

This project has involved multiple participants and collaborating organizations, including

- Dr. Qizhong (George) Guo, Department of Civil and Environmental Engineering, Rutgers, The State University of New Jersey
- Delaware Environmental Institute (DENIN), University of Delaware
- Charles H. Hegberg, reGenesis Consulting Services, LLC
- The Biochar Company (<http://thebiocharcompany.com/>)
- Dr. Marianne Walch, Environmental Scientist, Delaware Department of Transportation (DelDOT), Stormwater Quality Program

Finally, the ideas and preliminary results from this work were used to help write three research proposals submitted in 2014, all three of which were funded:

- National Fish and Wildlife Foundation, Chesapeake Bay Innovative Nutrient and Sediment Reduction 2014 Program, "Reducing Stormwater Volume and Nutrients with Biochar", \$316,349, 12/2014-11/2016;
- Transportation Research Board, NCHRP IDEA Program, "Reducing Stormwater Runoff and Pollutant Loading with Biochar Addition to Highway Greenways", \$129,000, 3/2015-2/2017;
- Mid-Atlantic Transportation Sustainability Center, "Simultaneous Removal of Nitrogen and Phosphorus from Stormwater by Zero-Valent Iron and Biochar in Bioretention Cells", \$149,999, 1/2015-6/2016.

## 6.0 RECOMMENDATIONS

In a bioretention cell, the biochar-promoted microbial reduction of nitrate is expected to be particularly pronounced if a significant saturated (anaerobic) region is included in the cell design, and if a reducing agent (e.g., zero-valent iron) is provided as an exogenous electron source. This study provides empirical data that support the proposed enhancement mechanism as well as knowledge that can guide future design and implementation of field-scale stormwater treatment systems. While this work represents an important step toward developing a robust and sustainable technology to mitigate the stormwater nutrient problem in a cost-effective manner, larger and longer-term funding will be necessary to bring this new, exciting technology to maturity and broad deployment.

**FOR PUBLICATION AND DISTRIBUTION PURPOSES, PLEASE  
EMAIL REPORT WITHIN TWO MONTHS AFTER PROJECT  
COMPLETION TO:**

**Marta Zurbriggen at [m.zurbriggen@rutgers.edu](mailto:m.zurbriggen@rutgers.edu)**

**Table 1. Physicochemical properties of the biochar.**

<b>Parameters</b>	<b>Units</b>	<b>Values<sup>a</sup></b>	<b>Method</b>
pH		8.71 <sup>b</sup>	1:20 w/v in DI water, 24 h mixing
Electrical Conductivity (EC 20 w/w)	mmhos cm <sup>-1</sup>	283	4.11 USCC:dil. Rajkovich
Total Ash	% of total mass	21.4	ASTM D-1762-84
particle density	g cm <sup>-3</sup>	1.816 <sup>b</sup>	
N <sub>2</sub> -BET surface area	m <sup>2</sup> g <sup>-1</sup>	338 <sup>c</sup>	
Organic Carbon (org-C)	% of total mass	74.2	Dry Combustion-ASTM 4373
Total Nitrogen (N)	% of total mass	0.59	Dry Combustion-ASTM 4373
Hydrogen/Carbon (H/C) Ratio		0.26	Dry Combustion-ASTM 4374
Liming (neutral value as CaCO <sub>3</sub> )	% CaCO <sub>3</sub>	14.7	Rayment & Higinson
Total Potassium(K)	mg/Kg dry mass	3566	Enders & Lehman
Available K	mg/Kg dry mass	4034	Wang after Rajan
Total Phosphorous(P)	mg/Kg dry mass	2528	Enders & Lehman
Available P	mg/Kg dry mass	1608	Wang after Rajan
Total N	mg/Kg dry mass	0.59	KjN
Ammonia (NH <sub>4</sub> -N)	mg/Kg dry mass	4.1	Rayment & Higinson
Nitrate (NO <sub>3</sub> -N)	mg/Kg dry mass	63	Rayment & Higinson
Organic (Org-N)	mg/Kg dry mass	5800	calculated
Volatile Matter	% dry mass	79	ASTM D-1762-84
Arsenic (As)	mg/Kg dry mass	1.4	EPA 3050B/EPA 6010
Cadmium (Cd)	mg/Kg dry mass	<0.01	EPA 3050B/EPA 6010
Chromium (Cr)	mg/Kg dry mass	11	EPA 3050B/EPA 6010
Cobalt (Co)	mg/Kg dry mass	1.3	EPA 3050B/EPA 6010
Copper (Cu)	mg/Kg dry mass	24	EPA 3050B/EPA 6010
Lead (Pb)	mg/Kg dry mass	3	EPA 3050B/EPA 6010
Molybdenum (Mo)	mg/Kg dry mass	0.22	EPA 3050B/EPA 6010
Mercury (Hg)	mg/Kg dry mass	<0.2	EPA 7471
Nickel (Ni)	mg/Kg dry mass	5.1	EPA 3050B/EPA 6010
Selenium (Se)	mg/Kg dry mass	<0.3	EPA 3050B/EPA 6010
Zinc (Zn)	mg/Kg dry mass	15	EPA 3050B/EPA 6010
Boron (B)	mg/Kg dry mass	40	TMECC
Chlorine (Cl)	mg/Kg dry mass	325	TMECC
Sodium (Na)	mg/Kg dry mass	465	EPA 3050B/EPA 6010

<sup>a</sup>The Biochar Company data; <sup>b</sup>measured; <sup>c</sup>Teixido et. al, 2013.

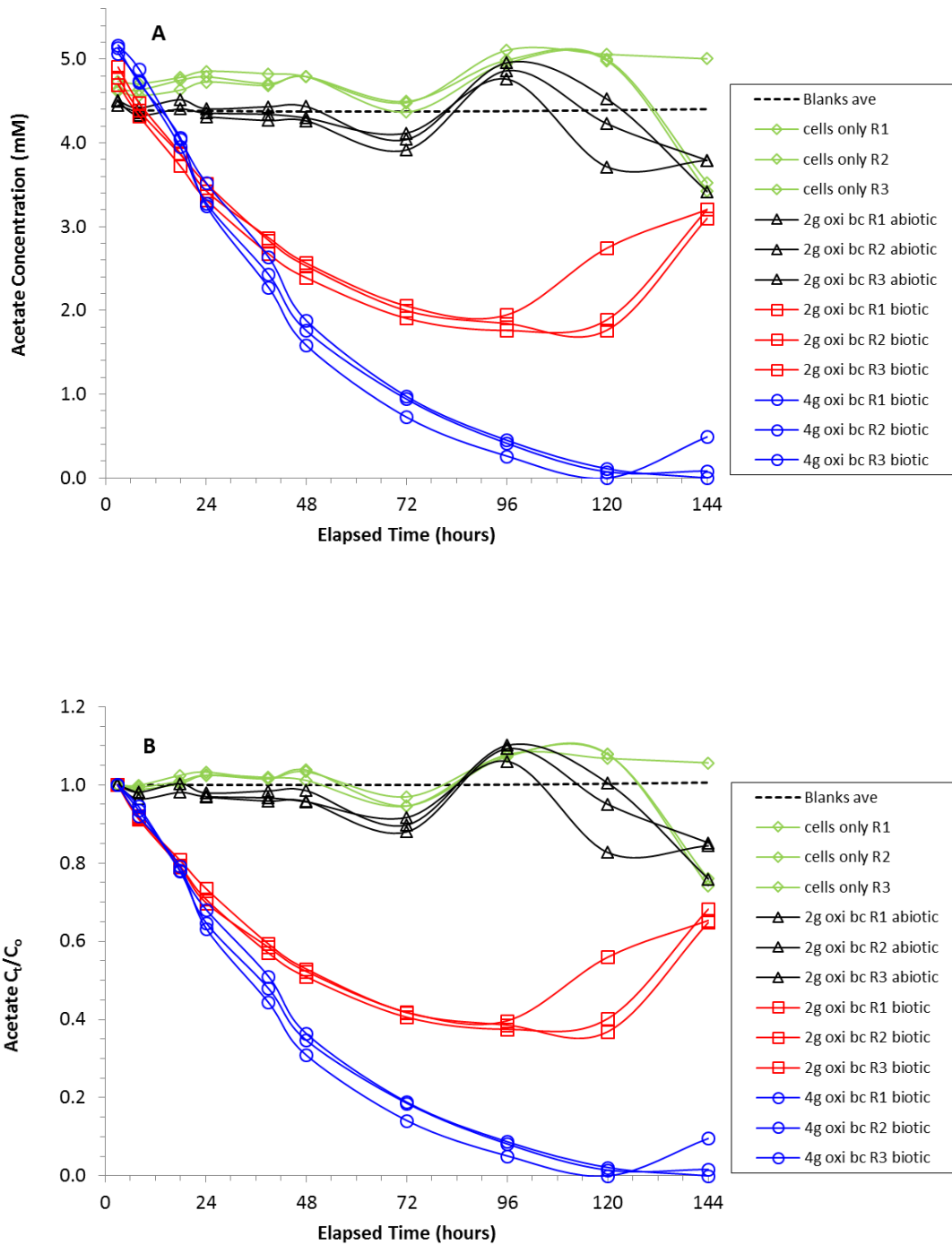


Figure 1. (A) Measured and (B) relative acetate concentrations as a function of time in serum bottles filled with media only (blanks), media plus *G.* cells, media plus 2 g of oxidized (oxi) biochar (bc) (abiotic control), media plus 2 g of oxidized biochar and *G.* cells (biotic), and media plus 4 g of oxidized biochar and *G.* cells. R1, R2, and R3 refer to triplicate bottles.

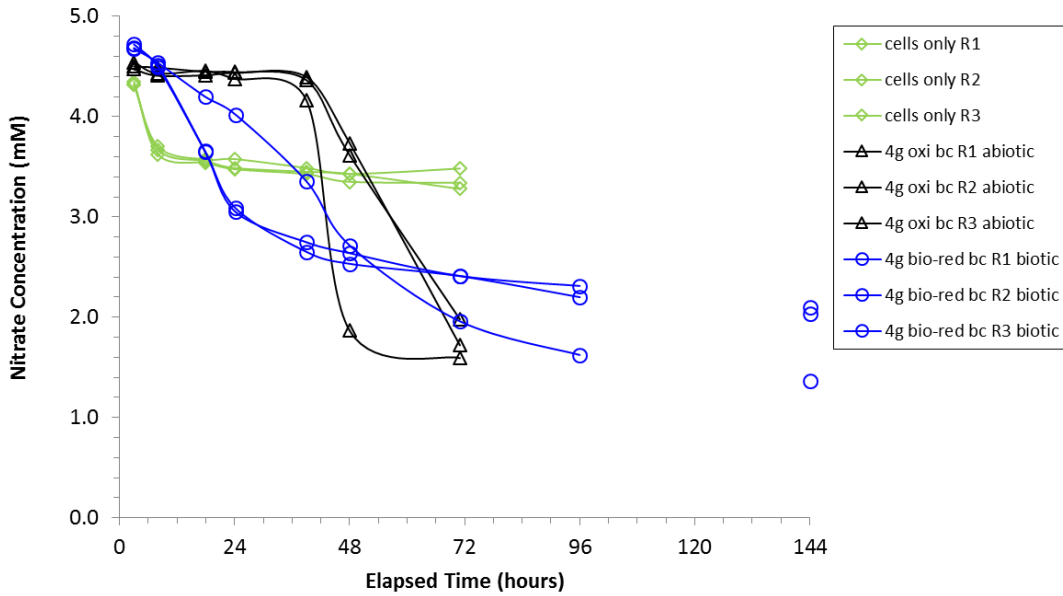


Figure 2. Measured nitrate concentrations as a function of time in serum bottles filled with media plus *G.* cells, media plus 4 g of oxidized (oxi) biochar (bc) (abiotic control), and media plus 4 g of biologically-reduced biochar plus carried over cells from experiment 1 (biotic).

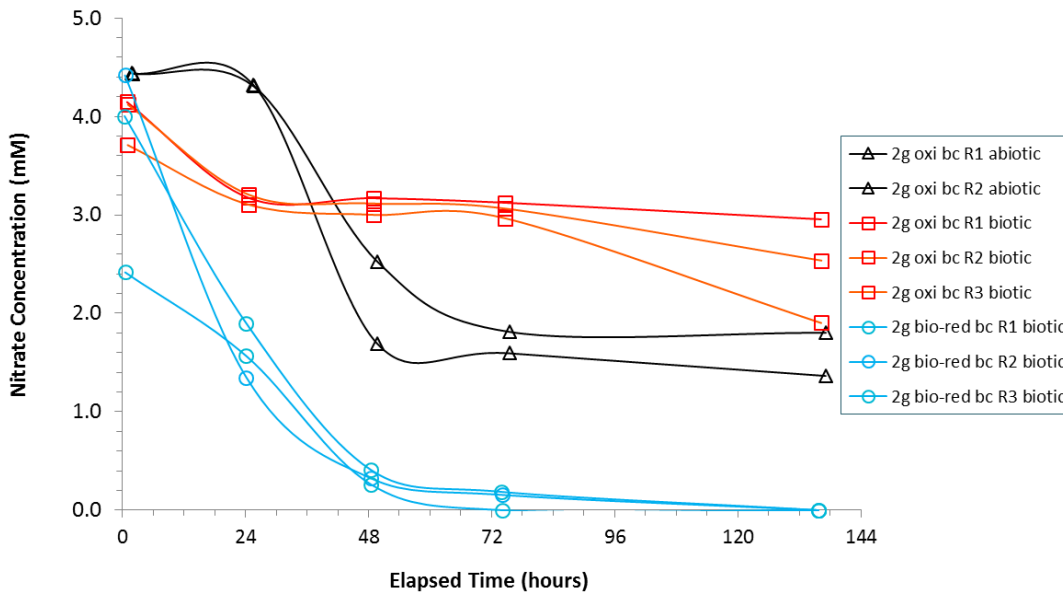


Figure 3. Measured nitrate concentrations as a function of time in serum bottles filled with media plus 2 g of oxidized (oxi) biochar (bc) (abiotic control), media plus 2 g of oxidized biochar and *G.* cells (biotic) and media plus 2 g of biologically-reduced biochar from preliminary experiment 1 plus *G.* cells (biotic).

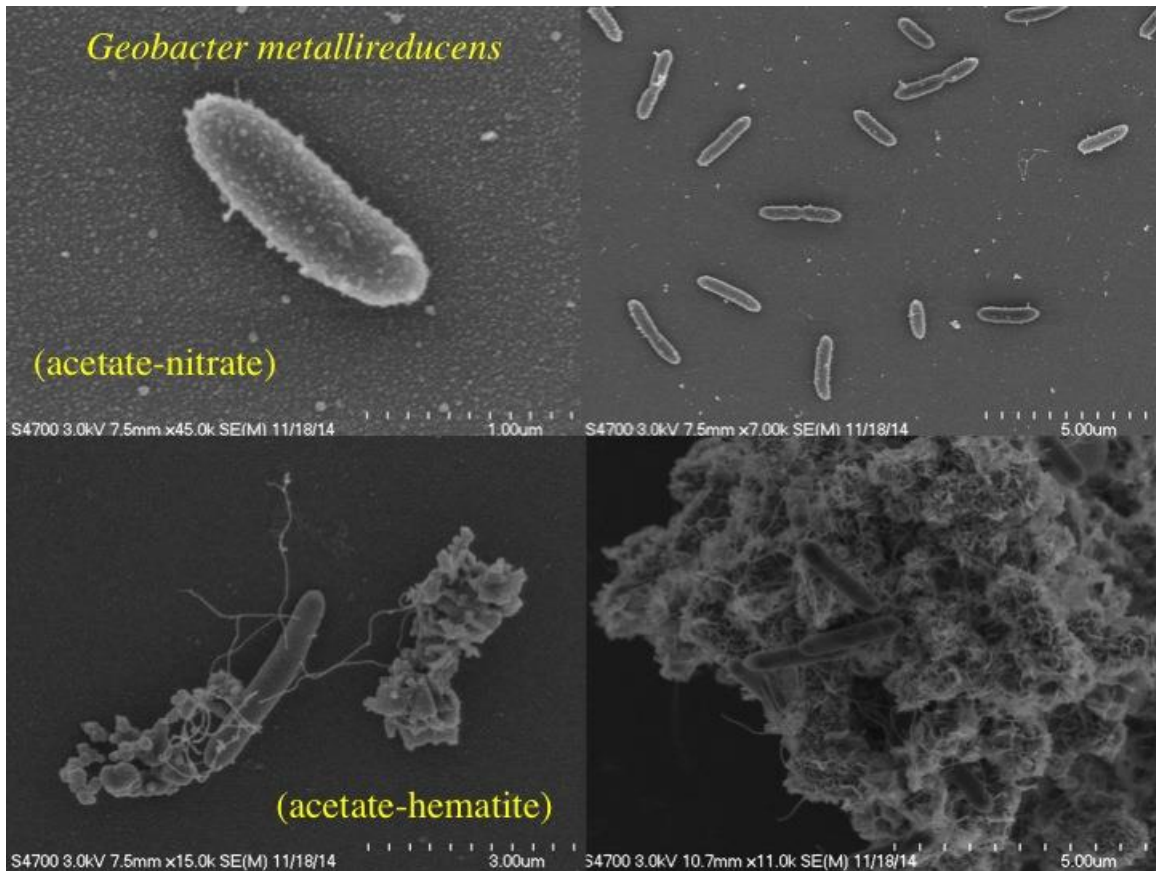


Figure 4. SEM images of *Geobacter metallireducens* from an acetate- and nitrate-fed aqueous culture (top panels) and an acetate- and iron oxide (hematite, a ferric oxide) culture collected from the solution phase (bottom left panel) and the solid phase (bottom right panel), respectively.



## Appendix A

### ATCC medium: 1768 *Geobacter metallireducens* Medium

Ferric citrate (Sigma F-6129).....	13.7 g
Wolfe's Vitamin Solution (see below).....	10.0 ml
Wolfe's Mineral Solution (see below).....	10.0 ml
NaHCO <sub>3</sub> .....	2.5 g
NH <sub>4</sub> Cl .....	0.25 g
NaH <sub>2</sub> PO <sub>4</sub> · H <sub>2</sub> O.....	0.6 g
KCl.....	0.1 g
Sodium acetate.....	6.8 g
Distilled, deionized water.....	1.0 L

Heat about 400 ml of water on a hot stir plate to near boiling. Add ferric citrate, allow it to dissolve, then cool the solution to room temperature in a slurry of ice. Add 400 ml of water; this quickly cools the medium to room temperature. Adjust the pH to 6.0 using 10 N NaOH; when the pH approaches 5.0, add NaOH by drops until the pH level is established. Approximately 6 ml of 10 N NaOH per liter will be needed per liter of medium. Add the remaining ingredients and bring the final volume up to 1.0 L with water. Bubble the medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub>. The final pH should be 6.8-7.0.

**Do not expose this medium to sunlight.**

### **Wolfe's Vitamin Solution:**

Available from ATCC as a sterile ready-to-use liquid (Vitamin Supplement, catalog no. MD-VS).

Biotin.....	2.0 mg
Folic acid.....	2.0 mg
Pyridoxine hydrochloride.....	10.0 mg
Thiamine · HCl.....	5.0 mg
Riboflavin.....	5.0 mg
Nicotinic acid.....	5.0 mg
Calcium D-(+)-pantothenate.....	5.0 mg
Vitamin B12.....	0.1 mg
p-Aminobenzoic acid.....	5.0 mg
Thioctic acid.....	5.0 mg
Distilled water.....	1.0 L

### **Wolfe's Mineral Solution:**

Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS.)

Nitrilotriacetic acid.....	1.5 g
MgSO <sub>4</sub> · 7H <sub>2</sub> O .....	3.0 g
MnSO <sub>4</sub> · H <sub>2</sub> O .....	0.5 g
NaCl.....	1.0 g
FeSO <sub>4</sub> · 7H <sub>2</sub> O .....	0.1 g
CoCl <sub>2</sub> · 6H <sub>2</sub> O .....	0.1 g
CaCl <sub>2</sub> .....	0.1 g
ZnSO <sub>4</sub> · 7H <sub>2</sub> O .....	0.1 g
CuSO <sub>4</sub> · 5H <sub>2</sub> O .....	0.01 g
AlK(SO <sub>4</sub> ) <sub>2</sub> · 12H <sub>2</sub> O.....	0.01 g
H <sub>3</sub> BO <sub>3</sub> .....	0.01 g
Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O.....	0.01 g
Distilled water.....	1.0 L

Add nitrilotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0 L with remaining water and add remaining compounds one at a time.

## Appendix B

### *Geobacter metallireducens*



Figure B1. *G. metallireducens* grown in iron(III)-acetate medium, with iron(III) as the electron acceptor and acetate as the electron donor. When inoculated, the medium turned from amber to dark green as iron(III) was biologically reduced to iron(II).



Figure B2. *G. metallireducens* grown in the Fe(III)-acetate medium were transferred to a modified growth medium with nitrate as the electron acceptor and acetate as the electron donor to establish a nitrate-utilizing *G. metallireducens* culture (nitrate *G.* culture).



Figure B3. *G. metallireducens* grown in nitrate-acetate medium, with nitrate as an electron acceptor and acetate as an electron donor. When inoculated, the medium turns from clear to slightly cloudy and pinkish.

## Appendix C

### Cell Suspension Preparation

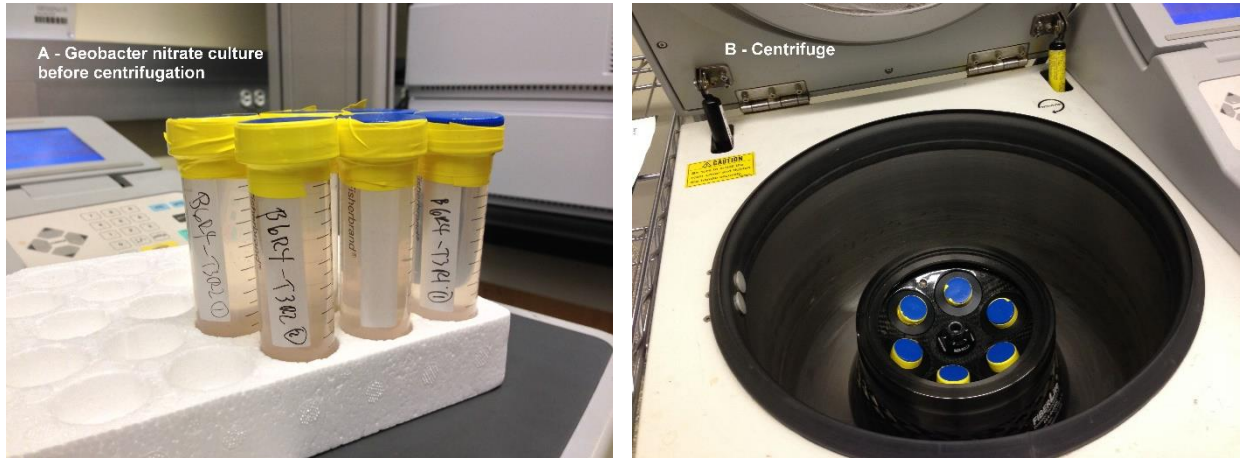


Figure C1. Concentration of *G. metallireducens* cells by centrifugation.

## Appendix D

### Biochar preparation



Figure D1. Sieved biochar, particle size range 0.25-0.50 mm.

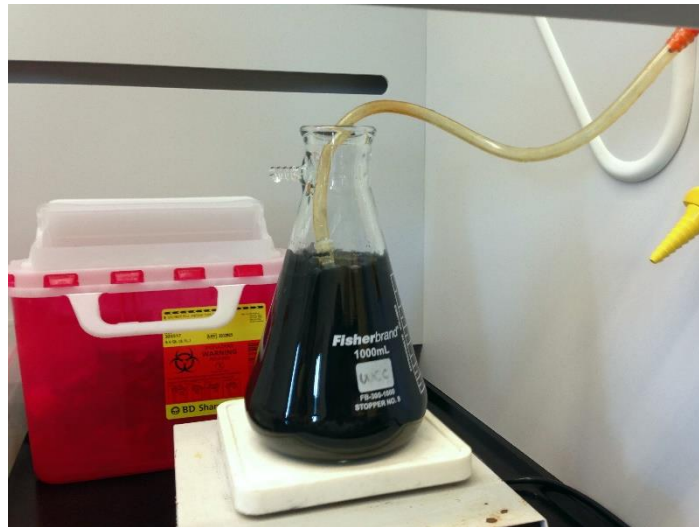


Figure D2. Oxidation of biochar by aeration in deionized water.



**Figure D3. Vacuum filtration of biochar suspension.**



**Figure D4. Vacuum drying of pre-treated biochar.**

Appendix E

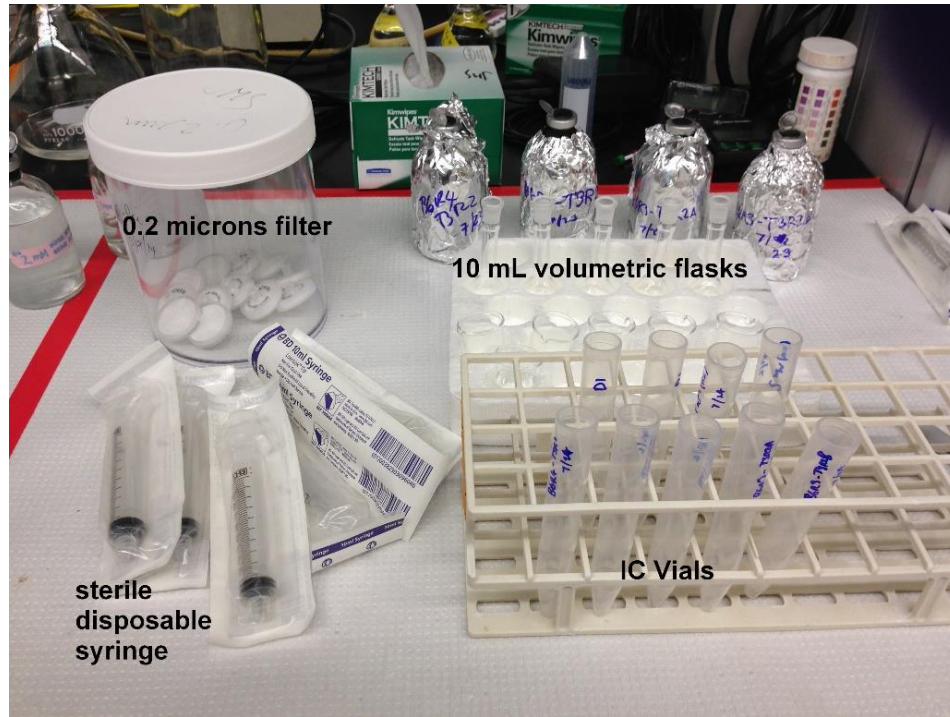


Figure E1. Paraphernalia for sample dilution.

## Appendix F

### IC Standard Calibration Curves

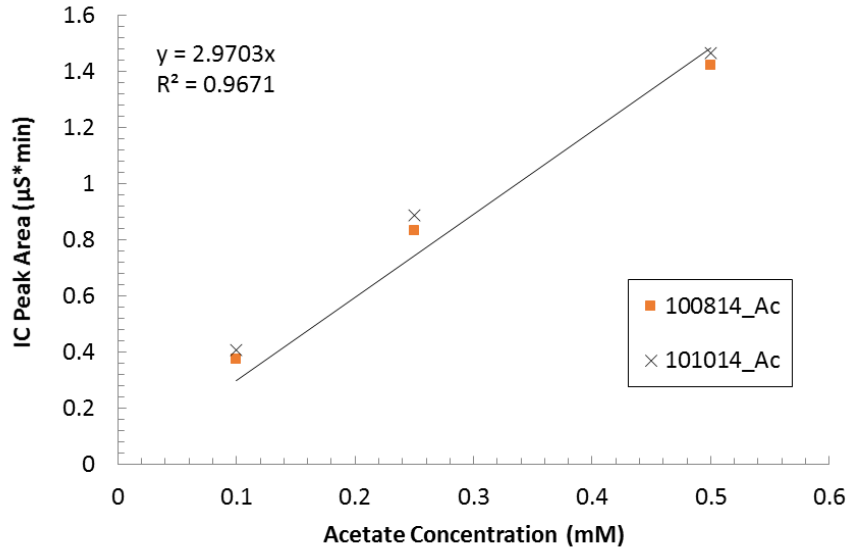


Figure F1. Acetate calibration curve.

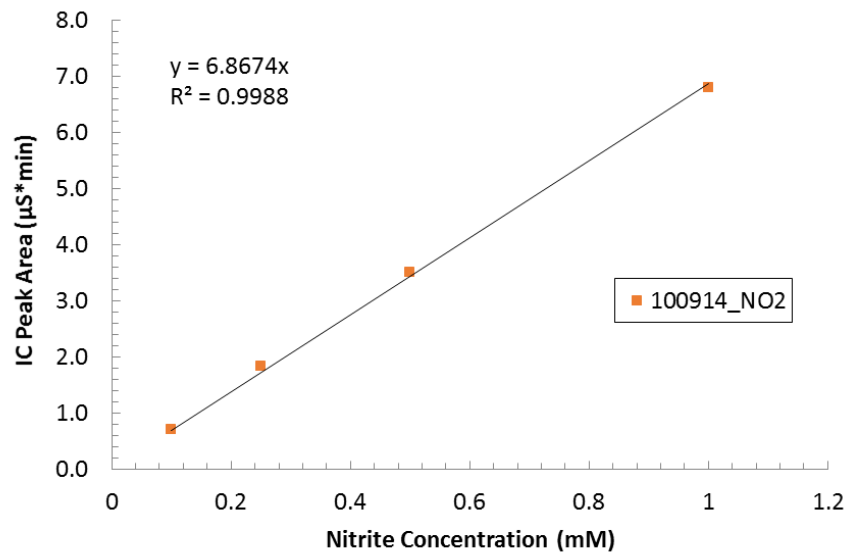


Figure F2. Nitrite calibration curve.



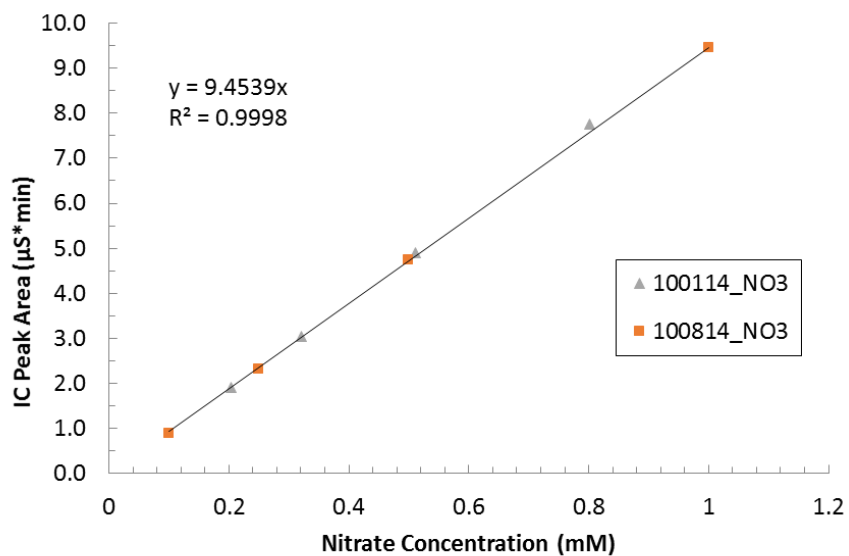


Figure F3. Nitrate calibration curve.

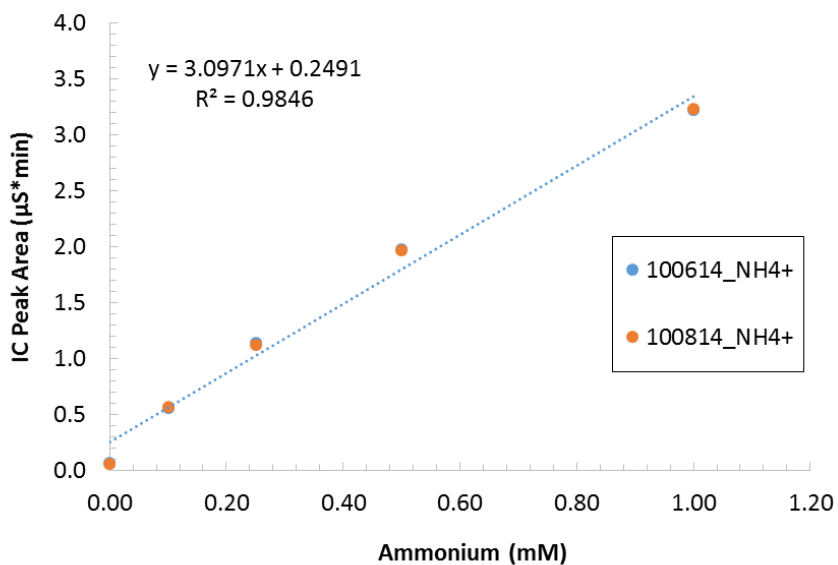


Figure F4.  $\text{NH}_4^+$  calibration curve.

## Appendix G

### Experimental Procedures



**Figure G1. Preparation of serum bottles with 100 mL of blank media (containing no sodium acetate or sodium nitrate).**



Figure G2. Adding concentrated *G. metallireducens* cells to serum bottles.



Figure G3. Sealing the serum bottles with rubber stoppers and aluminum crimps.