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Biochar as a Rechargeable Geobattery to Promote Nitrogen Removal in Stormwater from Roadways

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And
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16. Abstract
Stormwater runoff from roadways is a major source of pollution. State DOTs must comply with Total Maximum Daily Load (TMDL) regulations for nutrients such as nitrate-nitrogen, which is a major cause of water quality impairment. Existing stormwater treatment technologies, such as bioretention cells, do not remove nitrate adequately to meet water quality standards. New technologies are needed that can reduce nitrate more effectively and thus decrease the footprint required for stormwater treatment. Such technologies will not only improve water quality but also result in significant cost savings for state DOTs. We propose that biochar can serve as a rechargeable electron storage medium which, when added to a bioretention cell, can support/promote microbial reductive removal of nitrate in stormwater, and thereby enhance nitrate removal efficiency without increasing treatment footprint. Through batch experiments using a commercial wood-based biochar and the bacterium *Geobacter metallireducens* (GS-15), we showed that air-oxidized biochar served as an electron acceptor to enable acetate oxidation, and that either chemically or microbiologically reduced biochar served as an electron donor for nitrate reduction. The bioavailable electron storage capacity (ESC) of the biochar, estimated based on acetate oxidation and nitrate reduction, was 0.85 and 0.87 mmol e^-/g, respectively. We propose that biochar should be regarded as a rechargeable reservoir of bioavailable electrons in anaerobic environments, and that biochar may be applied to bioretention cells and other engineered systems to promote microbial degradation of nitrate and other pollutants.

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1. 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 to enhance the removal of nutrients from roadway runoff is under investigation at the University of Delaware. Preliminary results show significant removal of ammonium and nearly complete removal of nitrate under laboratory conditions. A series of pilot scale field tests were conducted in 2014 and 2015 and the data substantiated the enhanced removal of nitrogen due to biochar addition. While the mechanism for ammonium sorption by biochar has been explained, a fundamental understanding of how nitrate, the dominant form of nitrogen in stormwater, is removed in bioretention systems is still lacking. Without such an understanding, pilot scale data cannot be rigorously interpreted and designs cannot be developed for full-scale systems.

We hypothesized that biochar can serve as a rechargeable electron storage medium which, when added to a bioretention cell, can support/promote microbial reductive removal of nitrate in stormwater, and thereby enhance nitrate treatment efficiency without increasing treatment footprint. The objective of this project is to test this hypothesis with data from controlled laboratory experiments.

2. APPROACH

We conducted a number of c batch experiments designed specifically to test the hypothesis above. We selected the anaerobic bacterium *Geobacter metallireducens* (GS-15) for this study. This bacterium is ubiquitous in anaerobic subsurface and is known to utilize humic substances, which have similar redox-active functional groups as biochar, as electron donors and acceptors. In addition, *G. metallireducens* can be "trained", or acclimatized, to utilize nitrate as an electron acceptor for growth. We chose a commercial wood-based biochar from The Biochar Company (thebiocharcompany.com). This biochar has been used in field studies, in a pilot-scale bioretention cell in Newark, DE and a full-scale system in Charlottesville, VA. Batch experiments were carried out to evaluate the ability of the biochar, reduced either biologically by GS-15 or chemically with dithionite, to support nitrate degradation by *G. metallireducens*.

3. METHODOLOGY

3.1 Biochar Preparation

Commercially produced biochar (Soil Reef) from hardwood chips through slow pyrolysis at 600˚C was purchased from The Biochar Company, PA. 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. During aeration, 0.2–0.5 mL of 6N H_2SO_4 was added every 0.5–1.0 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. 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). 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

3.2 Microorganism

Geobacter metallireducens (GS-15) was chosen for this study because it can use humic acid as both an electron acceptor and donor but cannot use H_2 as an electron donor, which was used in the glove box and thus might exist in reactors. GS-15, obtained from ATCC (#53774) was grown on 5 mM each of acetate and nitrate in a modified ATCC 1768 medium. This bacterium oxidizes acetate to CO_2 and reduces nitrate dissimilatorily to ammonium through nitrite. After an 18-h incubation at 30 ºC, culture was centrifuged at 1100g for 15 min, washed 4 times with an anoxic medium (N_2/CO_2-purged ATCC 1768 without electron donor, electron acceptor, or NH_4^+) and re-suspended to a density of $7.0(\pm1.6)\times10^9$ cells/mL, as measured by optical density at 600 nm.

3.3.1 Experiment 1: Oxidized Biochar as Electron Acceptor for Acetate Oxidation

Serum bottles (125 mL) were prepared in a glove box ($N_2/CO_2/H_2$, 75:20:5) in quintuplicates, each containing 104 mL of the anoxic medium (above) with known quantities of oxidized biochar (2 or 4 g) and cells (~2×10^8/mL). Cysteine (158 mmol, <5% of the electrons from acetate oxidation) was added to each bottle to scavenge oxygen. Additional bottles were prepared in triplicates as controls: oxidized biochar (no cells), cells only, cells plus cystine (no biochar), and blank (medium only). The pH was 6.9±0.1 throughout each experiment. All reactors were sealed with butyl rubber stoppers and aluminum crimps, foil-wrapped, spiked with 0.4 mmol of sodium acetate (~4.0 mM), and incubated at 30 ºC.

3.3.2 Experiment 2: Microbially Reduced Biochar as Electron Donor for Nitrate Reduction

Upon completion of the acetate utilization experiment, reactors containing 2 g of biochar were placed in a glove box and the (biologically reduced) biochar was retrieved and washed 5 times with 30 mM deaerated bicarbonate buffer and twice with anoxic medium to remove residual acetate and cells. Reactors and controls were set up in triplicates as described above, except either oxidized or biological reduced biochar was used, and ~0.45 mmol of nitrate was spiked instead of acetate.

3.3.3 Experiment 3: Chemically Reduced Biochar as Electron Donor for Nitrate Reduction

To further test our hypothesis, a second nitrate reduction experiment was conducted using chemically reduced biochar. Air-oxidized biochar was reduced in 100 mL of 75 mM sodium dithionite (Fisher, Pittsburgh, PA) solution overnight in a glove box, and washed thoroughly with 30 mM bicarbonate buffer and anoxic medium. The reduced biochar was used to prepare nitrate reduction experiments as described

above, except cysteine was omitted.

3.4 Sample Collection and Analysis

Liquid samples for acetate, nitrite, nitrate and ammonium measurement were collected at various elapsed times during the course of each batch experiment. Sample collection from serum bottles was carried out under a verified protocol to preclude microbial and oxygen contamination. The rubber stopper of each bottle was sterilized with 70% ethanol before sampling. All glass syringes and disposable needles for sampling were flushed with N_2/CO_2 (80:20) multiple times before use. One mL of liquid sample was drawn and diluted 10 fold with deionized water in a 10-mL volumetric flask. After mixing the diluted sample was immediately filtered with 0.22-μm syringe filter (MCE, Millex GS) and transferred into 2 vials: 1.5 mL for NH_4^+ analysis and 8 mL for anion analysis. Samples were analyzed for anions using an ion chromatograph (IC) immediately after sampling. Samples for NH_4^+ measurements were sealed immediately and stored at 4 ˚C, and analyzed at the end of each experiment. NH_4^+ standards were made in parallel using the same preparation methods and storage conditions for quality control, which showed no contamination or loss of NH_4^+ during storage.

Acetate, nitrite and nitrate analyses were performed using a Metrohm 850 Professional IC AnCat unit equipped with a conductivity detector. The mobile phase was 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 total flow rate of 0.7 mL/min. The column oven temperature was constant at 28 ˚C. Ammonium was measured using a Dionex IC (ICS-1100) equipped with an Ion PAC CS16 (5 x 250 mm) and a conductivity detector. The mobile phase was 38 mN sulfuric acid at 1 mL/min. Concentrations of acetate, nitrite, nitrate, and ammonium were obtained based on calibration curves constructed using 0.1–1.0 mM standard solutions of ammonium chloride, sodium acetate, sodium nitrate, and sodium nitrite, individually prepared for each analyte. For quality control, calibration standards were included during IC analysis of each batch of experimental samples.

4. FINDINGS

4.1 Experiment 1: Oxidized Biochar as Electron Acceptor for Acetate Oxidation

As shown in Figure 1, aqueous acetate concentrations were constant in both controls and the blank over 6 days, indicating that physical losses (e.g., sorption to biochar) were minimal and that GS-15 grown on acetate could not utilize acetate without an electron acceptor. In the presence of 2 g of oxidized biochar, acetate was consumed immediately and continuously. The rate of acetate utilization was 0.66±0.10 mM/d for the first 2 d but slowed and approached zero at later times. This suggests air-oxidized biochar could support acetate oxidation by GS-15, presumably by acting as an electron sink, though its capacity to do so appeared to be finite. With 4 g of oxidized biochar, the initial rate of acetate utilization doubled to 1.3±0.1 mM/d, and acetate was completely degraded by day 6. Thus, both the rates and extents of acetate utilization support the hypothesis that air-oxidized biochar could serve as a microbial electron acceptor.

Because cysteine is an electron-donating amino acid, the cysteine/cystine pair could potentially mediate electron transfer in acetate oxidation. Although neither cysteine nor cystine is known to be a substrate for GS-15, it might be possible that the added cysteine was abiotically oxidized by biochar to cystine, which was then used by GS-15 to oxidize acetate. However, no acetate was lost in control containing cells and stoichiometric amount (1.6 mmole) of cystine (Figure 1), indicating cystine was not an electron acceptor for acetate oxidation, and that GS-15 must have transferred electrons from acetate directly to biochar. Assuming acetate oxidation stopped due to the finite electron accepting capacity of 2 g of biochar, and

given that 8 electrons would be exported per acetate molecule oxidized, the ESC of the oxidized biochar accessible to GS-15 was calculated to be 0.77 mmol/g. As discussed below, the actual ESC of the biochar was probably greater than 0.77 mmol/g due to the presence of other electron sources.

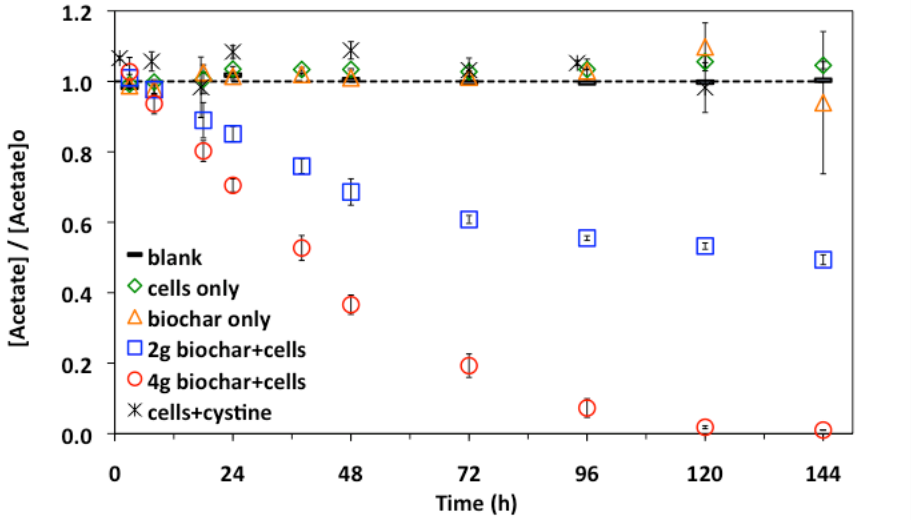

Figure 1. Acetate utilization in batch reactors containing anoxic medium only (blank), cells only (without biochar), cells with 1.6 mmol of cystine (no biochar), 2 g of oxidized biochar without cells (biochar only), and cells plus 2 g or 4 g of oxidized biochar. For biotic biochar reactors and controls, error bars represent one standard deviation from quintuplicate and triplicate reactors, respectively. The initial concentration of acetate was approximately 4 mM for all reactors.

4.2 Experiment 2: Microbially Reduced Biochar as Electron Donor for Nitrate Reduction

Result of a nitrate reduction experiment with biologically reduced biochar is shown in Figure 2(a). Without cells, nitrate was stable in the anoxic medium containing oxidized biochar. Interestingly, in all controls receiving cells, nitrate was removed instantly but only to a limited extent, either with or without oxidized biochar. Ammonium (and traces of nitrite) was detected, indicating that nitrate was indeed reduced. The possible electron sources in these controls were the cysteine and cells added. Indeed, *Geobacter* species are known to store electrons in the periplasmic and outer-surface cytochromes, and rest cells of GS-15 were shown to reduce Pu(VI) and U(VI) without external electron donors. Based on the ammonium yields, we estimated the amount of electrons carried by cysteine and cells combined was 0.173 mmol in each reactor. Accounting for the additional electrons, the ESC of the air-oxidized biochar in the acetate utilization experiments would be *ca*. 0.85 mmol/g.

In contrast to the controls, reactors containing cells and microbially reduced biochar harvested from the acetate utilization experiments showed sustained nitrate removal (Figure 2(b)) and concomitant formation of ammonium. This indicates that the redox reactions of biochar was reversible and that the electrons stored in biochar from acetate oxidation were subsequently retrievable by GS-15 for nitrate reduction.

4.3 Experiment 3: Chemically Reduced Biochar as Electron Donor for Nitrate Reduction

Attempt to include an abiotic control containing microbially reduced biochar without added cells was unsuccessful, because the washing procedure could not eliminate all GS-15 cells associated with biochar and nitrate reduction would commence after an initial lag. In order to verify that reduced biochar cannot reduce nitrate abiotically, and to confirm that biochar reduced either biologically or chemically can be an

electron donor for microbial nitrate reduction, an additional experiment was conducted using dithionite-treated biochar.

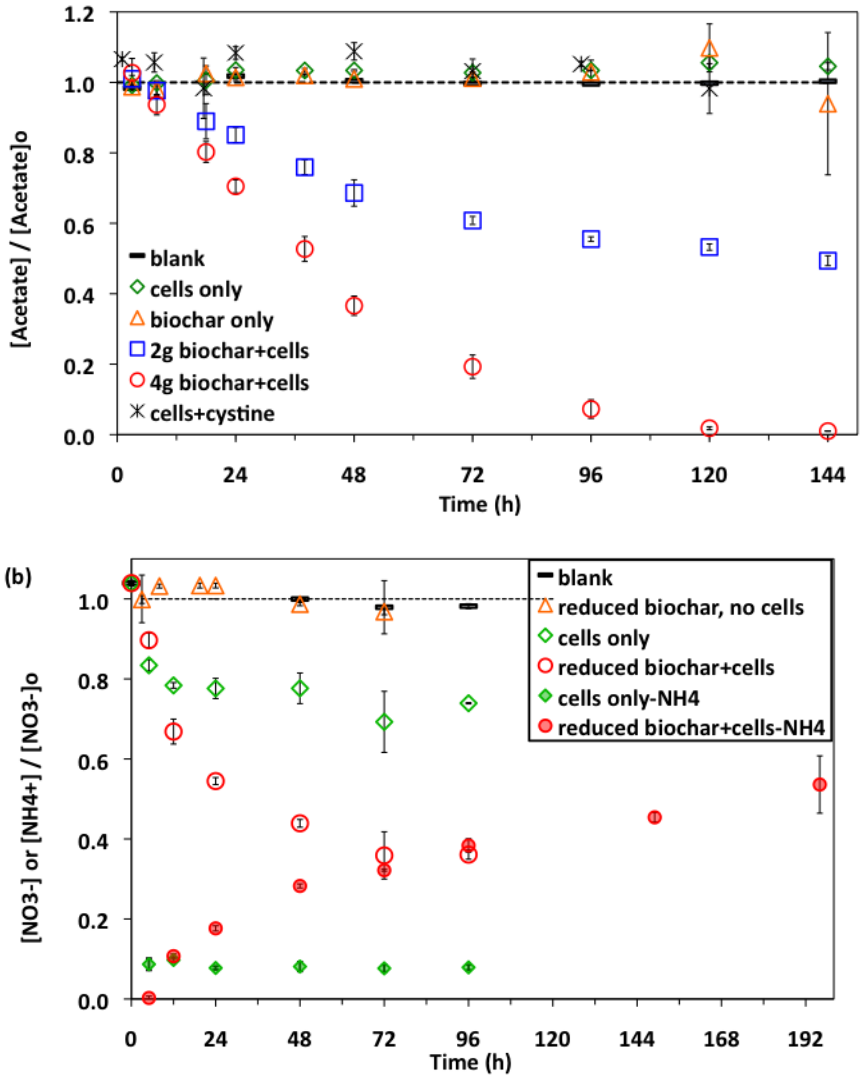

Figure 2. (a) Nitrate reduction in batch reactors containing anoxic medium (blank), cells only, 2 g of oxidized biochar with and without cells, and 2 g of biologically reduced biochar plus cells. (b) Nitrate reduction in reactors containing anoxic medium (blank), cells only, 2 g of dithionite-reduced biochar (no cells), and 2 g of dithionite-reduced biochar plus cells. No cysteine was used in this experiment. NH_4^+ concentrations are also shown for cells-only control and biotic biochar reactors. Initial nitrate concentration was approximately 4.4 mM for all reactors/controls. Error bars represent one standard deviation from triplicate reactors.

As shown in Figure 2(b), nitrate was not removed by dithionite-reduced biochar without cells, and was removed only to a limited extent in the biotic control, as described earlier. The NH_4^+ yields in all controls were 25–30%, suggesting that the nitrate removed from solution was only partially reduced. In reactors with both dithionite-reduced biochar and cells, nitrate was removed faster and more extensively, and the removal stopped at 72 h. Interestingly, NH_4^+ continued to form and reached a plateau at 192 h. Based on

the ammonium yield of 78.0% in Figure 2(b), the bio-accessible ESC of dithionite-reduced biochar was *ca*. 0.87 mmol/g, similar to the value (0.85 mmol/g) estimated earlier based on acetate consumption.

5. CONCLUSIONS

The experimental data clearly illustrate that the wood-based biochar acted as both a reducing agent and an oxidizing agent to enable anaerobic microbial activities including oxidation of organic substrates (acetate) and the reduction of electron acceptors (nitrate). The results suggest 1) biochar behaved as a rechargeable reservoir of microbially available electrons, 2) biologically stored electrons from acetate oxidation were available for the subsequent microbial reduction of nitrate, and 3) biochar, reduced either chemically or biologically, could promote nitrate reduction.

Under similar conditions, such as those in saturated and anaerobic bioretention cells containing soil and biochar, microorganisms capable of reducing nitrate will do so with the support of biochar as an electron donor. This would result in faster and more complete removal of nitrate from stormwater in bioretention systems.

In addition to the findings described above, this study produced a number of products and impacts:

a) The project provided research opportunities for 3 undergraduate students (with or without funding):

Name	**School / Program**	**Time Involved**	**Funding Source**
Hannah Scholes	UD Env. Engineering	summer/fall 2015	MATS-UTC project, DENIN
Marcos Miranda	UD Env. Engineering	summer 2015	MATS-UTC project
Minghan Xian	UD Chem. Engineering	summer/fall 2015	CAIT + independent study / senior thesis

b) This project provided direct (stipend) and indirect support (e.g., supplies) for 2 UD graduate students:
- Yu-Han Yu (Ph.D.)
- Lauren S. Lechner (M.S., DWRC fellowship)

c) One accepted manuscript was partially funded by this project:
- Saquing, J., Yu, Y.-H. and Chiu*, P. C. (2016) "Wood-Derived Black Carbon (Biochar) as a Microbial Electron Donor and Acceptor" *Environmental Science & Technology Letters*, accepted.

d) Results from this study were presented at two major international conferences:
- *Oral:* American Geophysical Union (AGU) 2015 Fall Meeting, San Francisco, CA. "Biochar Addition to Stormwater Treatment Media for Enhanced Removal of Nitrogen."
- *Poster:* Goldschmidt Conference 2015, Prague, Czech Republic. Poster presentation. "Black Carbon (Biochar) as a Rechargeable Electron Donor and Acceptor for Microbial Metabolism."

e) This project has involved the following participants and collaborating organizations:
- Delaware Environmental Institute (DENIN)
- Charles H. Hegberg, reGenesis Consulting Services, LLC
- The Biochar Company (http://thebiocharcompany.com/)

f) Finally, the ideas and preliminary results generated in this project will serve as a basis for proposals we will submit to transportation and other grant agencies in the future.

6. RECOMMENDATIONS

We propose that a biochar of suitable characteristics can be included in the design of a bioretention cell to support microbial denitrification and thereby enhance nitrogen removal during/following a storm event. We further propose that the desired performance of a stormwater treatment system can be engineered in a targeted manner based on the *electron storage capacity* (ESC) of biochar and other design considerations. This study provides experimental evidence that supports the hypothesized nitrogen removal mechanism, as well as information that will guide future design and implementation of field-scale systems. The next (essential and critical) steps toward developing robust and high-performing stormwater treatment systems are to 1) establish a method to reliably determine the ESC of a given biochar and 2) identify and optimize the major parameters that control the ESC of biochar during preparation. This knowledge, combined with further laboratory and field evaluations, will be necessary to gain regulatory approval and bring this new and exciting technology to the market.

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Joey Sanchez at joey.sanchez@rutgers.edu