FINAL REPORT - Part 1

Project Title: Simultaneous Removal of Nitrogen and Phosphorus from Stormwater by Zero-Valent Iron and Biochar in Bioretention Cells

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16. Abstract

Nutrients (N and P) in stormwater are a major cause of water quality impairments in the U.S. Current technologies such as bioretention cells to treat stormwater from roadways do not always remove nutrients sufficiently, and additional land may be needed to achieve the removal required by regulations. To improve performance of bioretention cells, we propose to use zero-valent iron (ZVI) and biochar in bioretention cells to remove nitrate (NO_3^-) and phosphate (PO_4^{3-}) from stormwater *simultaneously*. The primary goals of this project were to 1) understand how nitrate and phosphate are removed by biochar and ZVI, and 2) evaluate the performance of these media in a field-scale bioretention cell. This sub-report addresses the first goal and summarizes the laboratory work conducted at the University of Delaware.

We conducted a series of batch experiments to study microbial nitrate degradation with biochar, and column experiments to assess the removal of phosphate by ZVI. Nitrate degradation was greatly enhanced in the presence of a wood biochar reduced either microbially (by the bacterium GS-15) or chemically (by dithionite). Electron balance calculations show this biochar possessed an electron storage capacity (ESC) of ca. 0.87 mmol/g that was available to microbes for nitrate reduction. Little phosphate removal from anoxic water was achieved by sand alone, whereas a removal of about 30% (8 or 16 ppm P) to nearly 100% (1.6 ppm P) was obtained when 5% ZVI (v/v) was added to sand columns. These results support the use of biochar and ZVI to improve bioretention cell performance with respect to nutrient removal, and justify additional studies to further develop this new stormwater treatment technology for large-scale deployment.

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1. Problem

Stormwater from roadways and agricultural operations is a major contributor to deteriorating water quality in many watersheds in the U.S. and worldwide. Nutrients, mainly nitrogen (N) and phosphorus (P), are a leading cause of impaired water quality. Municipalities and State Departments of Transportation must control their nutrient discharge to comply with the regulations. Current stormwater treatment technologies, such as bioretention cells, do not always remove nutrients sufficiently and may require sizable real estate to achieve the necessary removal. This is particularly true for nitrate, which is among the most prevalent pollutant in natural waters and the most poorly removed nutrient species in bioretention systems. To meet increasingly stringent regulatory requirements with existing technologies will require purchase of more real estate for stormwater treatment at considerable costs. Hence, there are strong environmental and economic incentives to develop better technologies that can achieve higher removal from stormwater and therefore reduce the needed footprint.

To improve performance of bioretention cells with respect to nutrient removal, we propose to add zero-valent iron (ZVI) and biochar, two reactive granular materials derived from waste products, to bioretention cells to remove the major nitrogen and phosphorus species, nitrate (NO_3^-) and phosphate (PO_4^{3-}), from stormwater simultaneously. Specifically, we propose that 1) ZVI will slowly corrode to form iron oxides that can remove phosphate through surface complexation or precipitation, and 2) if placed in the saturated (i.e., anoxic) zone, ZVI and biochar can also enhance nitrate removal by promoting microbial denitrification. The proposed technology represents a new and sustainable approach that uses waste materials to produce environmental and economic benefits. We have obtained preliminary data that confirm the ability of ZVI and biochar to remove the N and P species above. However, a fundamental understanding of the removal mechanism for nitrate is currently lacking. In addition, while shown to be effective in short-term laboratory studies, ZVI and biochar have not been evaluated in combination under field conditions with respect to their ability to remove N and P simultaneously. In order to develop an effective and robust stormwater treatment technology, it is critical that we 1) understand the mechanisms for nutrient removal by ZVI and biochar, and 2) validate the performance of these media at the field scale. These are the primary goals of the proposed study.

2. Approach

We evaluated two waste materials, biochar and ZVI, as amendments to soil media for stormwater treatment. Previous laboratory studies have shown that each amendment alone is capable of removing or transforming nitrogen and/or phosphorus compounds. We proposed that treatment efficiency can be enhanced further by combining biochar and ZVI, which would result in smaller land requirement to achieve a prescribed level of treatment. Laboratory experiments were conducted at the University of Delaware (UD) to evaluate the ability of biochar and ZVI to remove nitrate and phosphate. A field investigation has been conducted by the University of Virginia (UVa) in conjunction with the City of Charlottesville at the Venable Elementary School in Charlottesville, VA, where biochar and ZVI were applied in a retrofitted bioretention facility.

The laboratory study at UD consisted of two major tasks: to evaluate the ability of a wood-based biochar to promote microbial nitrate reduction, and to assess the efficacy of ZVI to remove phosphate. Results of the UD study are summarized in this part (Part 1) of the Final Report. Part 2 of the Report will include results from the VA field study and will be submitted in November, 2016 when the work is completed.

We performed a series of batch experiments designed to illustrate biochar-promoted microbial nitrate reduction. We used the anaerobic bacterium *Geobacter metallireducens* (GS-15) for this study. This bacterium is common in anaerobic subsurface and is known to utilize humic substances, which have similar redox-active functions as biochar. In addition, *G. metallireducens* can be acclimatized to use nitrate as an electron acceptor for growth. We chose a commercial wood-derived 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 the full-scale cell at the Venable site in Charlottesville, VA. The specific goal of the batch experiments was to verify and quantify

the ability of the biochar to support nitrate biodegradation when the biochar was reduced either biologically or chemically.

In addition, we conducted a set of flow-through column experiments to evaluate the efficacy of ZVI to remove phosphate from water. The ZVI selected for these experiments was iron filings from Peerless Metal Powder and Abrasive (Detroit, MI). A series of pulse tests with different phosphate concentrations were performed and the removal efficiency was obtained in each case based on the breakthrough curve. The breakthrough data were then fitted to a reactive transport model to obtain the advection, dispersion, and reaction parameters, which may be useful for assessing phosphate removal in ZVI-augmented bioretention cells.

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 1,000 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 hours. During aeration, 0.2–0.5 mL of 6 N H_2SO_4 was added every 0.5–1.0 hour until the total volume added was 6 mL. The pH of the biochar suspension was monitored after acid addition to ensure the pH stayed around 7.0. After particle settling the suspension was decanted and the water was 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 2,000–2,500 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 suspension.

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 present in the glove box and thus might exist in batch 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, the culture was centrifuged at 1,100g 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 $7.0(\pm1.6) \times 10^9$ cells/mL, which was measured by optical density at 600 nm.

3.3 Batch Experiments with Biologically Reduced Biochar: 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 ($\sim 2\times10^8$/mL). Cysteine (158 mmol, <5% of the electrons from acetate) 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. Upon completion utilization of acetate, reactors containing 2 g of oxiedized 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.

3.4 Batch Experiments with Chemically Reduced Biochar: To further confirm our result (from the experiments in section 3.3), a second nitrate reduction experiment was conducted using chemically reduced biochar. Air-oxidized biochar was reduced in 100 mL solution of 75 mM sodium dithionite (Fisher, Pittsburgh, PA) in the glove box, and then washed thoroughly with 30 mM bicarbonate buffer and an anoxic medium. The dithionite-reduced biochar was then used to prepare nitrate reduction experiments as described above except cysteine was omitted.

3.5 Sample Analyses: Liquid samples for acetate, nitrite, nitrate and ammonium measurement were collected at different 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.

3.6 Medium and Solution Preparation for Column Experiments: Sand (Accusand 40/50, sieved to 250–500 μm) was treated with citrate to remove iron and manganese oxide coatings. This was done by submerging about 800 mL of dry sand in approximately 250 mL of 10 mM sodium citrate solution at ≤ 40 °C for variable times (often overnight). Sodium citrate (10 mM) was used as a complexing agent (Deng and Zhou, 2009). Five-mL samples from the aqueous layer were analyzed using the 1,10-phenanthroline method measured on a UV-vis spectro-photometer at 510 nm for the amount of iron removed from the sand. The sand was then rinsed thoroughly with DI water. This process was repeated four times to ensure that oxides were removed from the sand. This was confirmed by a decreasing amount of iron removed during each wash.

Additionally, the sand was tested to ensure that no phosphorus would be removed by the sand alone. This was done by using 50 mL of the dried, treated sand in 100 mL of deionized water containing 1.69 ± 0.0001235 mg/L PO_4-P. The P concentration in solution was tested after an hour, using the phosphate/molybdate complex and ascorbic acid reduction, measured on UV-vis at 880 nm (Hach PhosVer 3 reagent). All samples were analyzed in triplicate. The ZVI particles used were also sieved to the same size range (250–500 μm) and were used as received without pretreatment.

The solutions used were anoxic and composed of deionized water that was purged with nitrogen gas for 2 hours (per liter), then degassed in a vacuum chamber for 20 minutes (up to –27 in. Hg). After degassing, the solution pH was adjusted to between 8.5 and 9.5 by using 6 N NaOH. This was used as the "blank" influent, as media to prepare other solutions, and to pack the columns. For influent containing phosphorus, the blank solution was

spiked with K_2HPO_4 (final concentration 1.6, 3.2, 8, or 16 mg/L PO_4-P). By using this pH we ensured the predominant species of phosphorus would be HPO_4^- for the entirety of the experiment.

The concentrations of PO_4-P were chosen as dilutions, starting with 16 ppm, 8 ppm was a two-fold dilution, 3.2 was a five-fold dilution, and 1.6 was a ten-fold dilution. Although even the lowest concentration is significantly higher than the concentration commonly observed in the field (Ator and Denver, 2015), it was chosen for two reasons: (1) we did not want the effluent P concentration to be too close to the detection limit of the analytical method in order to ensure confidence in our data and (2) in order to properly analyze our samples, which are 5 mL total, we needed to have enough volume for pH measurement, P analysis, and Fe analysis. A concentration below 1.6 ppm PO_4-P would consume all of the 5-mL sample.

3.7 ZVI Column Experiments for Phosphate Removal: The columns used were acrylic, having a total volume of 114.55 cm^3, and were manufactured in the College of Engineering's Machine Shop. The dimensions are 10.1 cm (L) and 3.8 cm (i.d.). The columns were fitted with mesh screens, to ensure no sand or ZVI particles would be eluted from the column, and nylon barbed tube fittings on either side. The fittings were connected to Tygon tubing (L/S 16, with inside diameter 3.1 mm). The columns were up-flow and the influent entered the bottom tubing from a 1-L reservoir and exited the top of the columns. The solution was pumped through the columns using a peristaltic pump, which was set at 1 mL/min. The flow setting of the pump was calibrated against actual (measured) flow rates.

The columns were wet-packed using the anoxic influent solution (pH-adjusted degassed DI water) to avoid air bubble formation in the column. The control column was packed with sand only, using the citrate-treated sand. The experimental column was packed in three layers. The first 2 cm was citrate-treated sand, the middle 6.1 cm was a mixture of 5% ZVI and 95% sand by volume (or 7.3% ZVI and 92.7% citrate-treated sand by mass), and the final 2 cm was citrate-treated sand. The inlet sand layer helped to even flow distribution before reaching the reactive (i.e., ZVI) center layer of the column.

Once assembled, columns were flushed with blank influent for 3 pore volumes (PVs), and influent was switched to the solution containing phosphate, and effluent sample collection began. A pulse of 1 PV of influent solution (containing P) was conducted before switching the influent back to the P-free blank solution for the remainder of sample collection. For the control column, samples were collected for the first 40 minutes every 10 minutes (~10 mL/sample). From 40 to 110 minutes, samples were collected every 5 minutes (~5 mL/sample). For the remainder of the time, up to 170 minutes, samples were collected every 10 minutes. During the pulse test, pH was monitored periodically, and PO_4-P analysis was performed on every sample. For PO_4-P analysis, samples were diluted accordingly to obtain a measurable concentration, and were analyzed by UV-vis at 880 nm (Hach PhosVer 3 reagent). The phosphate pulse test was repeated four times in total, one for each of the phosphate concentrations (1.6, 3.2, 8, and 16 mg/L PO_4-P). For the experimental (ZVI) column, the process for sampling was similar, with the addition of total iron analysis periodically during pulse test. Total iron was analyzed for using the 1,10-phenanthroline method measured on UV-vis at 510 nm. The pulse tests were conducted from the lowest (1.6 ppm PO_4-P) to the highest (16 ppm PO_4-P) P concentration. After 1 PV, the influent was switched back to the blank solution. Samples were collected every 15 mins (~15 mL) for a total of 8 PVs.

4. Findings

4.1 Nitrate Reduction by Microbially Reduced Biochar: Result of a nitrate reduction experiment with biochar reduced microbially by GS-15 is shown in Figure 1(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, as well as traces of nitrite, was formed indicating that nitrate was indeed reduced. The possible electron sources in these controls were cysteine and the GS-15 cells added. Indeed, *Geobacter* species are known to store electrons in the periplasmic and outer-surface cytochromes, and rest cells of GS-15 have been shown to reduce Pu(VI) and U(VI) without external electron donors (Icopini et al., 2009). Based on the ammonium yields, we estimated the amount of electrons carried by

cysteine and cells combined was 0.173 mmol in each reactor. Subtracting the additional electrons, the electron storage capacity (ESC) of the air-oxidized biochar calculated from the batch experiments was *ca*. 0.85 mmol/g.

In contrast to the controls, reactors containing GS-15 cells and microbially reduced biochar harvested from the acetate reactors showed sustained nitrate removal (Figure 1(a)) and concomitant formation of ammonium (data not shown; see Figure 1(b) and section *4.2* below). This indicates the redox reactions of biochar was reversible and that the electrons stored in biochar from acetate oxidation could be subsequently retrieved by GS-15 for nitrate reduction.

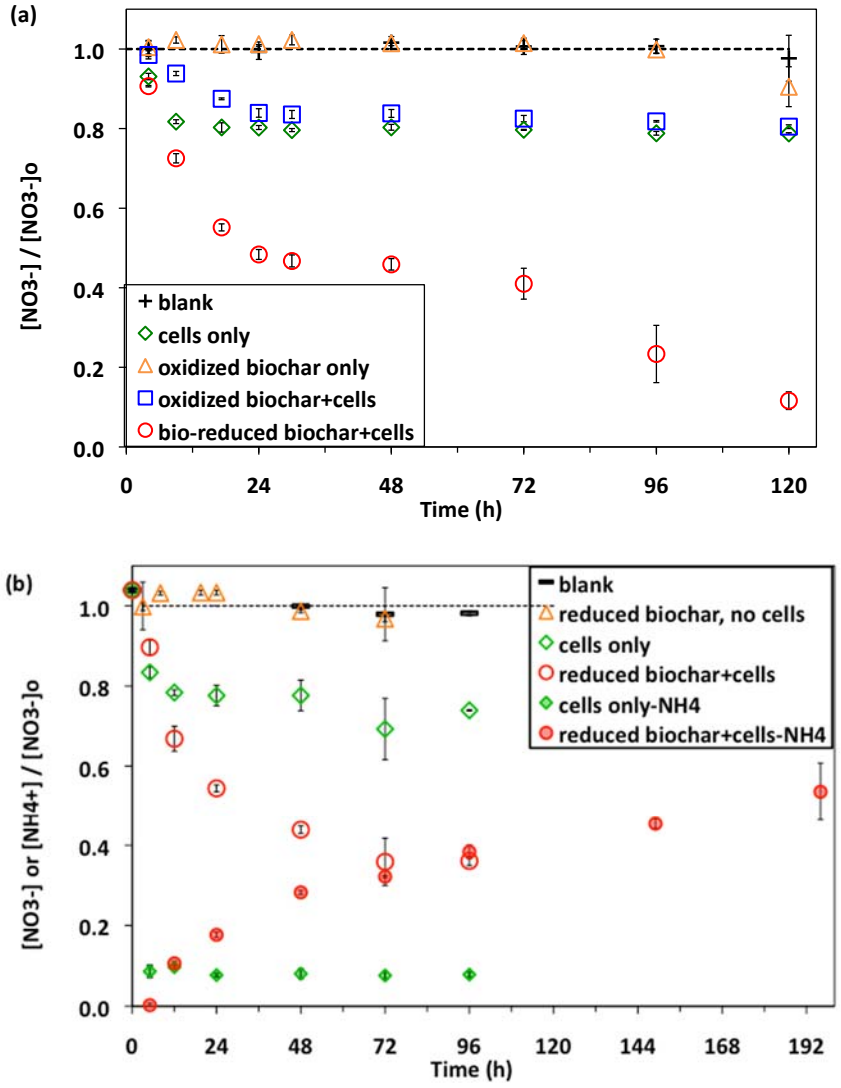

Figure 1. (a) Nitrate reduction in batch reactors containing anoxic medium (blank), cells only, 2 g of oxidized biochar with and without cells, and 2 g of biologically reduced biochar plus cells. (b) Nitrate reduction in reactors containing anoxic medium (blank), cells only, 2 g of dithionite-reduced 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 and controls. Error bars represent one standard deviation from triplicate reactors.

4.2 Nitrate Reduction by Chemically Reduced Biochar: Attempt to include an abiotic control with microbially reduced biochar without added cells was unsuccessful, because the washing procedure used could not eliminate all the GS-15 cells attached to biochar and nitrate reduction would commence after an initial lag. To verify that

reduced biochar cannot reduce nitrate abiotically, and also 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 (i.e., chemically reduced) biochar.

As shown in Figure 1(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 1(b), the microbially accessible ESC of dithionite-reduced biochar was estimated to be 0.87 mmol/g, similar to the value (0.85 mmol/g) calculated above.

4.3 Phosphate Removal by Sand and ZVI: Results of phosphate transport through a sand column are shown in Figure 2 for the four input concentrations of phosphate (1.6, 3.3, 8, and 16 ppm PO_4-P). The entering phosphate ion ($HPO_4^{=}$) was retarded slightly; i.e., had a longer retention time than calculated based on water flow rate, and exhibited a similar degree of dispersion in the sand column regardless of the input P concentration. Using the advection-dispersion-reaction equation (equation 1), the fitted dispersion coefficients (*D*) and retardation factors (R_f) for the migration of phosphate through sand were 2.1 cm^2/min and 1.4, respectively. In addition, the data indicate that the phosphate ion $HPO_4^{=}$ was only minimally removed by sand. Integration of the area under each breakthrough curve gives that the phosphate recovery was 99.6% for 1.6 ppm, 99% for 3.3 ppm, 97% for 8 ppm and 95% for 16 ppm, corresponding to removal efficiencies of 0.4%, 1%, 3%, and 5%, respectively.

$$D\,\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} - \lambda C = \frac{\partial C}{\partial t} \quad \cdots\cdots\cdots\cdots\cdots\cdots [1]$$

Results of phosphate transport through a ZVI-containing column are shown in Figure 3 for the same four input concentrations of phosphate. Similar to the sand-only column results, retardation and dispersion were observed with ZVI and sand. In contrast to the sand column results, however, the amounts of phosphate mass recovered, estimated through integration of the area under each breakthrough curve, were smaller than the corresponding pulse input areas for all four P concentrations. The estimated dispersion coefficients and retardation factors for phosphate transport through ZVI and sand ranged from 1.2 to 1.8 cm^2/min and from 1.5 to 2.0, respectively, not significantly different from those in the sand-only column. In contrast, the phosphate removal efficiencies were *ca*. 100% for 1.6 ppm, 70% for 3.3 ppm, 27% for 8 ppm, and 36% for 16 ppm. Note again that even the lowest concentration (1.6 ppm PO_4-P) was significantly higher than the concentrations observed in typical stormwater (Ator and Denver, 2015). However, using the higher P concentrations in our study would allow us to estimate the amount of phosphate a given mass of ZVI could remove in 1 PV.

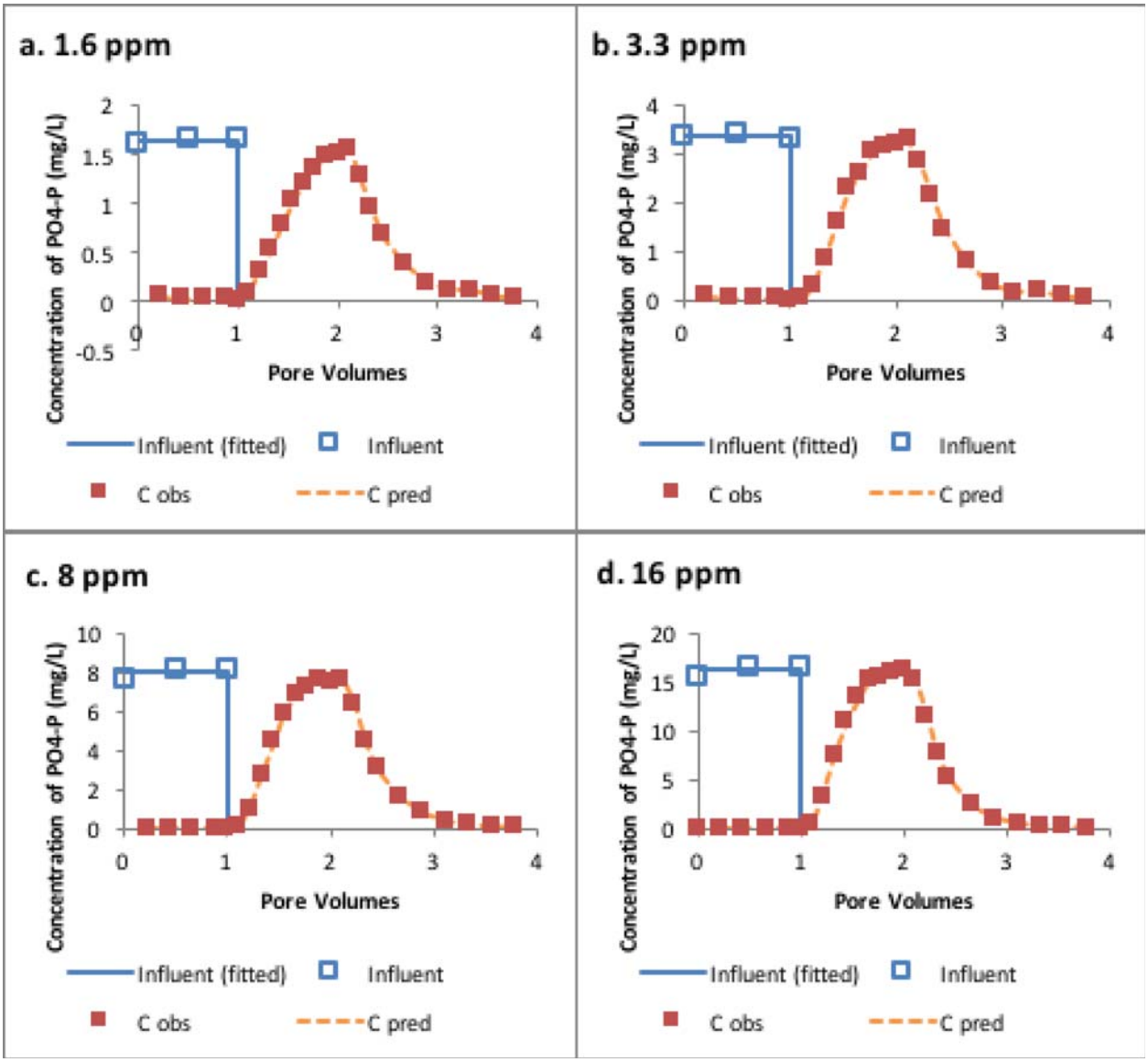

Figure 2. Data from control (sand only) columns for four different input PO_4-P concentrations: (a) 1.6 ppm, (b) 3.3 ppm, (c) 8 ppm, and (d) 16 ppm.

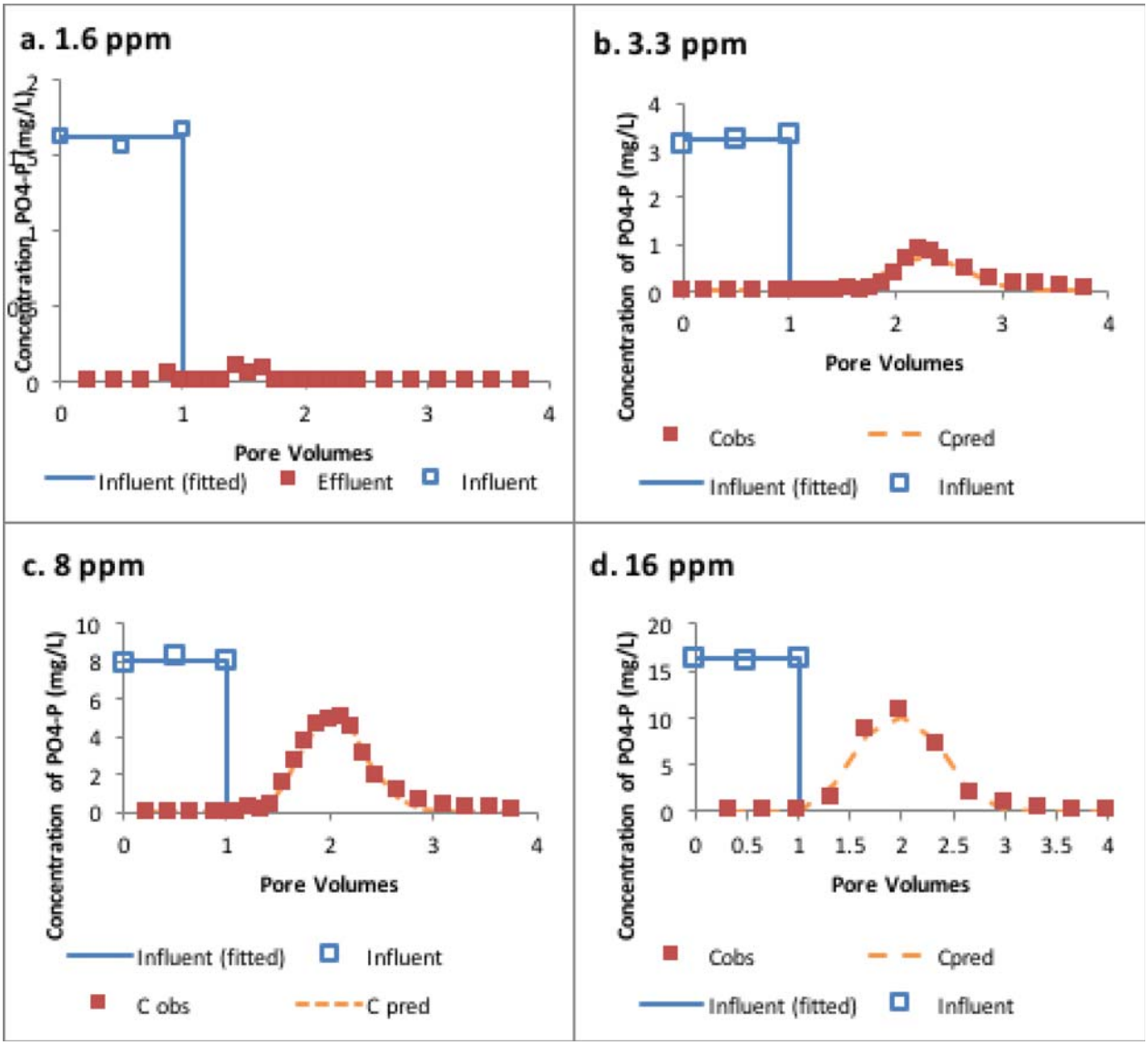

Figure 3. Data from experimental (5% ZVI/95% sand, by volume) column for four different PO_4-P concentrations: (a) 1.6 ppm, (b) 3.3 ppm, (c) 8 ppm, and (d) 16 ppm.

5. Conclusions

The batch experiments clearly show that the wood-based biochar acted as either an electron donor or acceptor to support anoxic microbial activities, including the reduction of nitrate by GS-15. These results demonstrate that (1) biochar behaved like a rechargeable reservoir of microbially accessible electrons, (2) microbially deposited electrons in biochar were bioavailable for subsequent reduction of nitrate, and (3) biochar, reduced either chemically or microbially, could promote microbial nitrate reduction. Under similar conditions, such as those in saturated and anaerobic bioretention cells containing soil and biochar, native microorganisms capable of reducing nitrate are expected do so with biochar as an electron donor. This study hence supports that addition of biochar to a bioretention cell could result in faster and more complete removal of nitrate from incoming stormwater, thus enhancing the performance of the bioretention cell with respect to nitrogen removal. A fuller discussion of the implications and potential engineering applications of the results is provided in Saquing et al. (2016).

Under the experimental conditions, sand retarded the migration of phosphate slightly but was feckless at removing phosphate. In contrast, addition of 5% ZVI (by volume) to sand resulted in marked removal of

phosphate, with an efficiency approaching 100% at 1.6 ppm PO_4-P, which is already higher than the average total phosphorus concentration of *ca*. 0.4 ppm observed under field conditions (Ator and Denver, 2015). Our study thus supports the addition of ZVI as a reactive medium in bioretention cells for enhanced removal of phosphate from stormwater.

6. Recommendations

We propose that a biochar of suitable characteristics (e.g., ESC) can be included in the design of a bioretention cell to promote microbial denitrification and thereby enhance nitrogen removal during/following a storm event. We further propose that performance of a stormwater treatment system can be engineered based on the electron transfer and storage properties of biochar along with other design parameters. This study provides experimental evidence that supports the hypothesized nitrate removal mechanism, as well as information that could guide the design and implementation of field-scale systems. Specifically, the location (i.e., depth) and amount of biochar to be incorporated into a bioretention cell can be estimated based on its microbially available ESC and the target stormwater volume and nitrate concentration. The study also demonstrates the effectiveness of ZVI to remove phosphate from water, supporting ZVI addition to bioretention cells to promote phosphorus removal. However, we should note that the study was short-term in nature and was performed under pristine laboratory conditions; hence the data may not be extrapolated to field conditions or large-scale systems. Further laboratory and field investigations should be conducted to validate the biochar and ZVI results, in order to gain regulatory approval and bring this new and exciting technology to the market.

7. Data Analysis

The calculations of ESC based on nitrate reduction to NH_4^+ with 2 g of dithionite-reduced biochar (Figure 1(b)) are presented below.

a) ESC of the biochar, based on nitrate losses (assuming reduced fully to NH_4^+, gaining 8 e^- per nitrate ion)
= (nitrate loss in reduced biochar+cells reactors – nitrate loss in cell-only control) × solution volume × (number of e^- per nitrate) ÷ biochar mass
= (3.138 – 1.389) mM × 0.104 L × 8 e^-/nitrate ÷ 2 g biochar = 0.728 mmol e^-/g biochar

b) ESC of the biochar, based on NH_4^+ production in Figure 1(b) (8 e^- transferred per NH_4^+ ion)
= (NH_4^+ formed in reduced biochar+cells reactors – NH_4^+ formed in cell-only control) × solution volume × (number of e^- per NH_4^+) ÷ biochar mass
= (2.443 – 0.360) mM × 0.104 L × 8 e^-/NH_4^+ ÷ 2 g biochar = 0.867 mmol e^-/g biochar

The nitrate and ammonium concentrations are averages from triplicate reactors. The higher ESC based on NH_4^+ production was due mainly to the lower NH_4^+ yield (0.360/1.389 = 25.9%) in the cells-only control than in the reduced biochar+cells reactors (2.443/3.138 = 78.0%). Hence the nitrate lost from solution was reduced only to a limited extent in the cells-only control, as noted earlier. We thus suggest the value based on NH_4^+ production, 0.867 mmol e^-/g, would be a more accurate estimate of the ESC of the biochar. This ESC value is also in better agreement with that (0.85 mmol e^-/g) obtained based on acetate utilization with air-oxidized biochar.

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