



## Short communication

## Impact of temperature and hydraulic retention time on pathogen and nutrient removal in woodchip bioreactors

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## ABSTRACT

Woodchip denitrification bioreactors are an important edge-of-field practice for treating agricultural drainage; however, their ability to filter microbial pollutants has primarily been explored in the context of wastewater treatment. Upflow column reactors were constructed and tested for *E. coli*, *Salmonella*,  $\text{NO}_3\text{-N}$ , and dissolved reactive phosphorus (DRP) at hydraulic retention times (HRTs) of 12 and 24 h and at controlled temperatures of 10 and 21.5 °C. Influent solution was spiked to  $30 \text{ mg L}^{-1} \text{ NO}_3\text{-N}$ ,  $2\text{--}8 \times 10^5$  *E. coli* and *Salmonella*, and  $0.1 \text{ mg L}^{-1}$  DRP. Microbial removal was consistently observed with removal ranging from 75 to 78% reduction at 10 °C and 90–96% at 21.5 °C. The concentration reduction ranged from  $2.75$  to  $9.03 \times 10^4$  for both organisms. HRT had less impact on microbial removal than temperature and thus further investigation of removal under lower HRTs is warranted. Nitrate concentrations averaged 96% reduction (with load removal of  $14.6 \text{ g N m}^{-3} \text{ d}^{-1}$ ) from 21.5 °C columns at 24 HRT and 29% reduction (with load removal of  $8.8 \text{ g N m}^{-3} \text{ d}^{-1}$ ) from 10 °C columns at 12 HRT. DRP removal was likely temporary due to microbial uptake. While potential for removal of *E. coli* and *Salmonella* by woodchip bioreactors is demonstrated, system design will need to be considered. High concentrations of these microbial contaminants are likely to occur during peak flows, when bypass flow may be occurring. The results of this study show that woodchip bioreactors operated for nitrate removal have a secondary benefit through the removal of enteric bacteria.

## 1. Introduction

Carbon-based denitrification bioreactors are becoming an important edge-of-field technology for nitrate removal from tile drainage waters in areas of intensive agricultural production, such as the Upper Midwestern United States. In this environment, the primary carbon source implemented and tested has been woodchips. Studies of field scale woodchip bioreactor systems have reported from 12 to 76% removal (Christianson et al., 2012), with nitrate removal rates being impacted by hydraulic retention time (HRT), temperature (Christianson et al., 2012), and carbon source (David et al., 2016). While woodchip bioreactors are popular with farmers, the scale of implementation needed to meet nitrate reduction goals is far from being achieved. For example, the Iowa Nutrient Reduction Strategy estimated that installation of woodchip bioreactors on all tile drained land would reduce nitrate loading by 18% (INRS, 2017). Even the more practical implementation of woodchip bioreactors on 60–70% of drained land (scenarios NCS1 and NCS8) require implementation of 112,000 to 131,000 bioreactors treating just over 30 ha of drained land each. To

date approximately 60 are operating in Iowa. With renewed emphasis on drainage water quality combined with farmer interest in implementation of woodchip bioreactors, guidance is needed to optimize the design of these systems to maximize nutrient reduction.

Even in heavily drained areas of the Upper Midwestern United States, pathogen contamination of surface waters is a significant water quality concern. The presence of pathogens is typically determined by the presence of FIB such as *E. coli* in freshwater and enterococci in marine waters (U.S. EPA, 2012). According to the U.S. EPA 2014 303d list of impaired waters, pathogens are the leading cause of water quality impairments in the U.S. for assessed rivers and streams. Pathogens are the leading cause of water quality impairments in rivers and streams in several states dominated by tile drainage, including Iowa (2014 data), Illinois (2010 data), and Indiana (2006 data) ([https://iaspub.epa.gov/waters10/attains\\_nation\\_cy.control](https://iaspub.epa.gov/waters10/attains_nation_cy.control)). Extensive work has demonstrated the presence of and quantified concentrations of FIB and pathogens downstream of manure-amended lands (Soupir et al., 2006; Haack et al., 2016). The presence of microbial contaminants in surface waters presents a risk to public health through contamination of irrigation or

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drinking water or through recreational exposure.

Much of the focus of drainage water quality research has been on nitrate export, but recent works highlight the potential movement of other contaminants through drainage systems, especially in manure-amended areas. Hruby et al. (2016) reported fecal indicator bacteria (FIB) and *Salmonella* in drainage from poultry manured amended plots, with detection frequencies ranging from 35 to 90%. Peak concentrations of *E. coli* ( $6.6 \times 10^3$  CFU 100 mL<sup>-1</sup>), *Salmonella* ( $2.8 \times 10^3$  CFU 100 mL<sup>-1</sup>), and enterococci ( $6.6 \times 10^5$  CFU 100 mL<sup>-1</sup>) were found in drainage from no-till managed plots. Similarly, Pappas et al. (2008) investigated *E. coli* and enterococci concentrations in drainage from swine manure amended plots; peak *E. coli* concentrations were 82 CFU 100 mL<sup>-1</sup> and peak enterococci concentrations were  $1.2 \times 10^3$  CFU 100 mL<sup>-1</sup>; both concentrations were observed in plots receiving late winter manure applications. FIB and pathogen movement to drainage is impacted by timing between manure application and precipitation (Samarajeewa et al., 2012). The design of woodchip bioreactors to remove multiple pollutants, could also have a positive impact on local water quality, specifically through contaminant reduction of phosphorous and manure-derived enteric bacteria. Further this would advance the application of woodchip bioreactors as a potential management practice for treatment of storm water in urban areas and effluent from private septic systems. While great need exists for improved management practices to reduce pathogen movement to surface waters, limited work has been conducted on the woodchip bioreactor as a practice to reduce pathogen or FIB concentrations (Rambags et al., 2016).

Reduction of microbial contaminants in a woodchip bioreactor is possible via several mechanisms. First of all, filtration of drainage water has potential to retain bacteria on the surfaces of the woodchips, as has been previously demonstrated in sand-based filters (Torkelson et al., 2012; Ahammed and Davra, 2011). Retention in the woodchip bioreactor could enhance removal through natural decay or predation (Haig et al., 2015). Further it is likely that microorganisms interact with woodchip surfaces through cellular properties such as surface structures (flagella or fimbriae), cell surface charge and hydrophobicity, or extracellular polymeric substances (Liao et al., 2015). Field observations support the use of woodchip bioreactors for microbial contaminant removal; however, all reports were conducted on woodchip bioreactors placed downstream of primary or secondary domestic wastewater discharges. Rambags et al. (2016) reported 2.9 log *E. coli* and 3.9 log bacteriophage reduction when a woodchip bioreactor was used to treat “secondary-treated septic effluent”. The tested system differed from the systems of the Upper Midwestern U.S. in that the average HRT of the system was 8 days, while woodchip bioreactors intercepting drainage are designed for a minimum HRT of 3 h during peak flow conditions (NRCS, 2015). Another study by Tanner et al. (2012), also focused on domestic wastewater treatment, reported mean *E. coli* reduction of ~1.2 log units (approximate HRT of 3.3 d) while Robertson et al. (2005) reported similar removal rates with HRTs ranging from 1.7 to 5.4 days.

Another important potential function of woodchip bioreactors is the removal of phosphorous. Drainage is a significant source of P to surface waters in agricultural watersheds. In an extensive review, King et al. (2015) reported P concentrations in drainage ranging from < 0.01 to > 8.0 mg/L, generally above concentrations needed to stimulate eutrophication. Edge-of-field interception and treatment of drainage for P removal has great potential but to date, few published studies have examined P removal in woodchip bioreactors. The limited works available have augmented the pure woodchip reactor with various amendments including biochar (Bock et al., 2015), gravel or zeolite (Ibrahim et al., 2015), drinking water treatment residuals (Zoski et al., 2013), or recycled steel byproduct (Hua et al., 2016). These augmentations have been mixed in with the woodchip reactor or designed as a separate component of the reactor.

To address the limited knowledge regarding the performance of

woodchip bioreactors as a conservation practice to remove bacterial contaminants in drainage from manure amended agricultural lands, we conducted multiple columns studies. We focused our study on *E. coli*, the U.S. EPA recommended FIB for freshwater sources and *Salmonella*. Triplicate column studies were conducted at two HRTs, 12 and 24 h, and at 2 temperatures, 10 °C and 21.5 °C (room temperature) to separately assess the impact of HRT and temperature on removal of bacteria from drainage. Reductions in nutrients typical of agricultural drainage (nitrate and dissolved reactive phosphorus (DRP)) were also assessed. Results of the study are useful to assess woodchip bioreactors as a potential conservation practice to address multiple contaminants and are especially useful in watershed planning when manure-derived contaminants such as FIB and phosphorus are a concern.

## 2. Materials and methods

Two sets of triplicate upflow bioreactor columns were used to conduct a paired experiment to evaluate nutrient removal and the fate of potential pathogens in packed bed bioreactors. Each column was packed with weathered woodchips from the same supplier as those described in Hoover et al. (2015). One set of columns was maintained under controlled temperature at 10 °C, and the second set held at room temperature, 21.5 °C. Hydraulic retention time (HRT) was held at 12 h and 24 h for each set of columns. Experiments were conducted over a four month period.

### 2.1. Column design

The columns used for the 21.5 °C experiments were constructed of clear acrylic, each measuring approximately 50.8 cm in height with an internal diameter of 13.5 cm. The second set of columns were used for the 10 °C study and were constructed of schedule 40 PVC with dimensions of 41.2 cm height and 15.2 cm internal diameter. Both sets of columns have similar internal volumes of 7.3–7.5 L. The design and construction of the columns is described previously by Hoover et al. (2015). Briefly, ports to connect tubing to the influent and effluent ends of the columns were attached to endplates sealed with rubber gaskets. Perforated (0.3 cm, randomly spaced holes) acrylic plates were fit at both ends to distribute flow. A multichannel peristaltic pump (Model No. ISM834 Ismatec REGLO, Cole-Parmer, Wertheim, Germany) was used to control flow rate to the individual columns in both sets of experiments. Prior to the initiation of experiments, columns were flushed to remove excess carbon from the system. Composite flow volumes were collected and weighed every 24 or 48 h to determine the volume of water collected. This confirmed flow rate throughout the study, and adjustments were made as necessary to maintain pre-determined flow rates.

The sets of columns had similar outflow flow rates, measured as outflow volume divided by elapsed time, of 2.6 mL min<sup>-1</sup> at 24-h HRT in both 21.5 °C RT and 10.0 °C studies, and 5.4 mL min<sup>-1</sup> and 5.6 mL min<sup>-1</sup> at 12-h HRT in 21.5 °C and 10.0 °C columns, respectively. The average gravitational pore volume of the columns was 4.1 L. The achieved HRTs for the 21.5 °C study were 21.8-h and 12.6-h based on calculated flow rates and measured gravitational porosity. The HRTs for the 10.0 °C study were somewhat higher, achieving 25.2-h and 12.4-h.

### 2.2. Influent drainage solution

Synthetic tile drainage solution was prepared using deionized water. The synthetic tile water contained micronutrients to support bacterial growth, and was modified from a recipe previously reported by Nadelhoffer (1990), and used by Hoover et al. (2015). Experiments were conducted at a target NO<sub>3</sub>-N concentration of 30 mg L<sup>-1</sup> and PO<sub>4</sub>-P concentration of 0.1 mg L<sup>-1</sup> entering the woodchip bioreactors.

Bacteria inoculated influent was continuously applied to the columns for a minimum of five-day periods, followed by a flushing period

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