3-2018

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

Michelle L. Soupir
Iowa State University, msoupir@iastate.edu

Natasha L. Hoover
Iowa State University, nlhoover@iastate.edu

Thomas B. Moorman
United States Department of Agriculture

Ji Yeow Law
Iowa State University, jiyeow@iastate.edu

Bradley L. Bearson
United States Department of Agriculture

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

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, NO3-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 mg L−1 NO3-N, 2–8 × 105 E. coli and Salmonella, and 0.1 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 × 104 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 g N m−3 d−1) from 21.5 °C columns at 24 HRT and 29% reduction (with load removal of 8.8 g N m−3 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.

Keywords
Woodchip bioreactor, Management practice, Salmonella, Escherichia coli, Water quality

Comments
This article is published as Soupir, M. L., N. L. Hoover, T. B. Moorman, J. Y. Law, and B. L. Bearson. "Impact of temperature and hydraulic retention time on pathogen and nutrient removal in woodchip bioreactors." *Ecological Engineering* 112 (2018): 153-157. DOI: 10.1016/j.ecoleng.2017.12.005. Posted with permission.

Ecological Engineering 112 (2018) 153–157

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Short communication

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

M.L. Soupir[a,*], N.L. Hoover[a], T.B. Moorman[b], J.Y. Law[a], B.L. Bearson[b]

[a] Department of Agricultural and Biosystems Engineering, Iowa State University, 3358 Elings Hall, Ames, IA, 50011, USA
[b] USDA, Agricultural Research Service, National Laboratory for Agriculture and the Environment, 1015 N University Boulevard, Ames, IA, 50011, USA

ARTICLE INFO

Keywords:
Woodchip bioreactor
Management practice
Salmonella
Escherichia coli
Water quality

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*, NO_3-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 mg L^{-1} NO_3-N, 2–8 × 10^5 *E. coli* and *Salmonella*, and 0.1 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 × 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 g N m^{-3} d^{-1}) from 21.5 °C columns at 24 HRT and 29% reduction (with load removal of 8.8 g N m^{-3} 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). 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

* Corresponding author.
E-mail address: msoupir@iastate.edu (M.L. Soupir).

https://doi.org/10.1016/j.ecoleng.2017.12.005
Received 19 September 2017; Received in revised form 5 December 2017; Accepted 6 December 2017
Available online 10 January 2018
0925-8574/

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* (6.6×10^3 CFU 100 mL^{-1}), *Salmonella* (2.8×10^3 CFU 100 mL^{-1}), and enterococci (6.6×10^5 CFU 100 mL^{-1}) 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^{-1} and peak enterococci concentrations were 1.2×10^3 CFU 100 mL^{-1}; 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 fimbrae), 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 compartment 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^{-1} at 24-h HRT in both 21.5 °C RT and 10.0 °C studies, and 5.4 mL min^{-1} and 5.6 mL min^{-1} 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_3-N concentration of 30 mg L^{-1} and PO_4-P concentration of 0.1 mg L^{-1} 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

without bacterial addition until effluent bacteria concentrations returned to levels less than 100 cfu 100 mL^{-1}. This process was repeated at least twice under each experimental condition. During the flushing periods, nutrient concentrations in the influent solution were maintained as described above.

After an initial flush period, the synthetic tile drainage was augmented with *E. coli* (wild strain environmental isolate) and *Salmonella enterica* serovar Typhimurium strain BBS 1144. *S.* Typhimurium strain BBS 1144 (*invGEABCIJ spaOPQRS sicA sipBC*):*neo* was constructed using recombineering as previously described (Bearson et al., 2014). Briefly, a nalidixic acid resistant *S.* Typhimurium UK1 strain containing pKD46 (Datsenko and Wanner, 2000) was transformed with the linear knockout fragment oBBI 456/457-*neo*. This fragment was synthesized by PCR amplification of oBBI 92/93-*neo* using primers oBBI 456 (**gcg gaaattatcaaatattattcaattggcagacaaatgaag**catagagcagtgacgtagtcgc) and oBBI 457 (**cagcacgtttttaatactgtggctttcagtggtcagtttatcgat**agctgaatgagtgacgtgc). The sequence of the oBBI 456 and oBBI 457 primers in bold are homologous to upstream *invG* and downstream *sipC* in the *S.* Typhimurium genome, respectively. The underlined primer sequence anneals to the oBBI 92/93-*neo* fragment for PCR amplification. BBS 1114 is resistant to nalidixic acid and kanamycin and has an internal deletion (~14 kb) in *Salmonella* pathogenicity island-1 (SPI-1) that removed many of the genes encoding Type 3 Secretion System-1 (T3SS-1). T3SS-1 is a nanosyringe-like organelle that facilitates the entry of *Salmonella* into the host cell (Moest and Méresse, 2013). Without T3SS-1, *Salmonella* should be unable to invade the host cell and is therefore attenuated for infection via the oral route. The use of this avirulent strain provided significant advantages in biosafety. Additionally, naladixic acid was used to eliminate the growth of *E. coli* on the XLD medium.

Salmonella and *E. coli* were cultured separately. Isolated colonies from streak plates (*E.coli* grown on modified mTEC and *Salmonella* on XLD augmented with 30 mg L^{-1}–60 mg L^{-1} nalidixic acid) were transferred to brain heart infusion (BHI) broth and cultured at 37 °C. Spectrophotometer measurements of cultures were made to approximate log-phase, and 0.5 mL of each culture was immediately added to 14 mL of phosphate buffered solution to be used as an inoculant to the synthetic drainage water. Column influent solution was prepared in 40–60 L batches, with 0.5–1.0 mL of the microbial inoculant added to each batch. The microbial inoculant was stored at room temperature and used for up to 72 h. The average *Salmonella* concentrations measured in the influent were 20,149 (± 14,467) cfu 100 mL^{-1} and 63,464 (± 38,698) cfu 100 mL^{-1} at 21.5 °C and 10 °C, respectively. *E. coli* concentrations were 44,605 (± 26,186) cfu 100 mL^{-1} and 75,139 (± 38,359) cfu 100 mL^{-1} at 21.5 °C and 10 °C, respectively. Standard deviation is shown in parentheses.

2.3. Sample collection and analysis

Effluent samples were collected daily during inoculation periods, and *Salmonella* and *E. coli* were enumerated using the membrane filtration technique within 24 h of sample collection (U.S. EPA method 1603). In addition to measuring the influent bacteria concentrations, we needed to adjust results for natural decay since it occurred in both the influent container and the woodchip columns. Therefore, samples were collected from the influent container at the same time as collection of effluent samples. This resulted in concentration values that could be directly compared for bacteria removal by the bioreactor. Dilutions were performed when necessary to achieve optimal bacteria plate counts between 20 and 80 colonies. *E. coli* was cultured on Difco modified mTEC and *Salmonella* was cultured on Difco XLD agar infused with 30–60 ppm nalidixic acid. The addition of the antibiotic to the medium eliminated *E. coli* growth on the agar which has previously been found to interfere with *Salmonella* growth (Hruby et al., 2016). Nalidixic acid was initially added at 30 ppm, but this was increased to 60 ppm to fully inhibit undesired growth.

Effluent nutrient samples were collected as composite samples from the individual columns. Each column drained into separate effluent containers during a collection period of 24 or 48 h, dependent on HRT. Influent samples were collected at the same time as the effluent samples. Samples were analyzed using an AQ2 discrete autoanalyzer (Seal Analytical Inc., Mequon, WI). Nitrate + nitrite was analyzed following AQ2 method EPA-114-A, Rev. 7 (equivalent to US EPA method 353.2 Rev. 2); and DRP was analyzed using AQ2 method EPA-118-A Rev. 5 (equivalent to US EPA Method 365.1, Rev. 2.0). The detection limit for DRP is 0.002 mg P L^{-1}.

2.4. Phosphorus sorption to woodchips

A supplimental experiment was conducted to evaluate P-sorption by woodchips sterilized at 105 °C and nonsterile woodchips. All woodchips used for the experiment were air dried or oven sterilized, then ground and sifted through a 2 mm sieve followed by a 0.85 mm sieve to achieve a consistent woodchip size. One gram of the ground woodchips was measured into twelve 50 mL centrifuge tubes, and 25 mL of solution containing 1.12 mg/L P was added.

2.5. Data analysis

Reduction is expressed as the mean difference in concentration (mg L^{-1}) between column influent and effluent at each time step, and as that difference expressed as a percentage of influent concentration. Column influent was collected on the same day as effluent to account for natural decay of bacteria. Statistical comparisons of analyte reduction were made between column treatments including HRT and temperature using JMP Statistical Software. First, data were tested for normal distribution using Q–Q plots. For normally distributed data, Levene's test was used to test for variance equality followed by *t*-test. For non-normally distributed data, the Wilcoxon Rank-Sum Non-Parametric Test was conducted. Non-detects were assumed to be 0.5 the detection limit. Dates with influent NO_3-N concentrations above 35 mg L^{-1} or below 25 mg L^{-1} and influent DRP concentrations below 0.05 mg L^{-1} and above 0.15 mg L^{-1} were excluded from analysis.

3. Results and discussion

3.1. Woodchip bioreactors have potential to reduce E. coli and Salmonella

The woodchip bioreactor columns were effective at removing organisms under all experimental conditions. Percent reductions were greater than 75% for both *Salmonella* and *E. coli* at 10 °C, and greater than 87% for both at 21.5 °C (Table 1). When comparing differences in removal between HRTs of 12 and 24 h, *Salmonella* and *E. coli* reductions were statistically greater only under 21.5 °C conditions. These results support previous studies reporting retention of FIB and viruses in woodchip bioreactors (Rambags et al., 2016) but at HRT values more typical of woodchip bioreactors designed to treat agricultural drainage.

Natural bacterial die-off (or decay) was accounted for in the experimental design by collecting influent samples at the same time as effluent. Therefore, any decay that occurred in the influent reservoir was assumed to also occur in the columns since column effluent was sampled daily (corresponding to 1 or 2 pore volumes, depending on the HRT). Greater reduction at room temperature is likely due to higher die-off rates in the 21.5 °C columns or increased predation (Haig et al., 2015).

The influent concentrations of *E. coli* and *Salmonella* used in these experiments likely exceeds the concentrations observed under field conditions. Elevated FIB concentrations in both surface and drainage systems have been observed at peak flows resulting from storm events. Tomer et al. (2010) reported *E. coli* concentrations that peaked above 4.8×10^4 cells 100 mL^{-1} at a 156 ha subbasin tile outlet in the Tipton Creek watershed. The drainage basin is dominated by poorly drained

Table 1
Salmonella and *E. coli* reduction by woodchip bioreactors operated with 12 and 24 h HRTs, temperatures of 10 °C or 21.5 °C.

Experimental Conditions			Reduction[a]			
Temperature	HRT	n	*Salmonella*		*E. coli*	
°C	h		CFU 100 mL^{-1}	%	CFU 100 mL^{-1}	%
10	12	36	$3.75 \times 10^4(1.75 \times 10^4)$	76% aA	$5.50 \times 10^4(3.80 \times 10^4)$	78% aA
	24	18	$9.03 \times 10^4(3.36 \times 10^4)$	77% aA	$6.87 \times 10^4(3.16 \times 10^4)$	75% aA
21.5	12	30	$6.79 \times 10^4(2.30 \times 10^4)$	90% aB	$4.84 \times 10^4(2.79 \times 10^4)$	91% aB
	24	33	2.75×10^4 (1.06×10^4)	94% bB	$3.80 \times 10^4(2.03 \times 10^4)$	96% bB

[a] Reduction is expressed as the mean difference in concentration (mg 100 L$-^1$) between column influent and effluent, or that difference expressed as a percentage of influent concentration. Lower case letters indicate significance between HRT at a set temperature. Standard deviation shown in parentheses. Upper case letters indicate significance between temperatures at a set HRT.

soils with surface intakes reported in potholes and roadside ditches. Thus, the elevated peak *E. coli* concentrations during the event is a combination of drainage and runoff routed through surface intakes. At the field scale, Hruby et al. (2016) reported peak concentrations of bacteria under poultry manure amended field plots on the order of 10^5 cfu 100 mL^{-1} enterococci and 10^2 cfu 100 mL^{-1} *E. coli* during peak flow in drainage from no-till and chisel plow managed plots, respectively. Bacterial export followed the rising limb of the drainage hydrograph or peaked prior to the hydrograph peak, indicating macropore export. Even a 90% reduction of these concentrations would leave residual concentrations that would be problematic in a recreational setting; current recreational water quality criteria in the U.S. are a geometric mean value of 126 cfu 100 mL^{-1} (U.S. EPA, 2012).

Based on field and watershed monitoring studies, peak concentrations of bacteria may also occur when the capacity of woodchip bioreactors is exceeded and excess drainage is routed through bypass flow. Woodchip bioreactors designed to treat tile drainage include a bypass component to prevent drainage backup into crop fields and to limit the development of preferential flow paths in the bioreactor. When bypass flow occurs, the treatment capability of the woodchip bioreactor is limited and the fraction of bacteria removed during these conditions would be reduced. However, the differences between percent removal between 12 and 24 h HRTs for any temperature or organism varied by less than 5%. This seemingly small impact of flow on bacterial removal is promising, and future work should examine both *Salmonella* and *E. coli* removal at lower HRT values.

Future work should also explore the mechanism of removal to better inform the longevity of the observed findings. Weathered woodchips were used in this study, indicating that the results are not dependent upon fresh woodchips. Further, if the mechanism of removal is retention followed by natural decay, decreased performance with time would not be expected.

The reductions of bacteria in water flow herein compare favorably to other edge of field strategies to remove pathogens and indicator organisms from agricultural waters. For example, vegetative barriers have been used in agriculture for a number of years to reduce losses of sediments and nutrients. Coyne et al. (1998) found that grass filter strips were more effective for sediment removal than for removal of enterococci (68–74%) from poultry manure. Collins et al. (2004) obtained greater removal of *E. coli* and *Campylobacter* at low runoff flows (< 95% removal) than high flows (0–15% removal). Mechanism for removal of bacteria by filter strips includes sedimentation and entrapment of particulate associated bacteria and infiltration of bacteria in runoff water. Similar removal mechanisms would apply to wetlands designed to remove nitrate from agricultural drainage; however, inputs from wildlife have the potential to make wetlands a source of fecal indicators to downstream waters. Further, organisms increase survival by attaching to particles (Auer and Niehaus, 1993) and can survive in bottom sediments only to be resuspended during higher flows (Jamieson et al., 2005; Rehmann and Soupir, 2009). Other conservation practices such as saturated buffers and controlled drainage have yet to be assessed for their potential to reduce export of FIB or enteric pathogens to surface waters.

3.2. Nitrate was impacted by HRT and temperature

Nitrate removal was evaluated in this study primarily to confirm woodchip bioreactor function and results are presented in Table 2. As expected, increasing HRT and temperature both significantly increased percent removal. Twenty-nine percent removal occurring at 10 °C and HRT of 12 h compared to 48% removal when the HRT was increased to 24 h. Similarly, NO_3-N reduction increased from 67 to 96% when the 21.5 °C HRT was increased from 12 to 24 h. Increased residence time allows for greater percent removal; however,comparison of load reductions at 12 and 24-h HRTs indicates better overall reduction with the lower HRT. At 21.5 °C, an average load removal of 22.5 g N m^{-3} d^{-1} was observed at the 12-h HRT, while an average load reduction of 14.6 g N m^{-3} d^{-1} was achieved at the 24 h HRT. At 10 °C, removal rates were 8.8 g N m^{-3} d^{-1} and 7.7 g N m^{-3}d^{-1} at 12 h and 24 h HRTs, respectively. These results are comparable to those reported by Hua et al. (2016), who measured NO_3-N removal at 24-h HRT at influent concentrations of 20 and 50 mg L^{-1}, with resulting load reductions of 10.1 g N^{-3} d^{-1} and 18.9 g N^{-3} d^{-1}, respectively; as well as other studies (Hoover et al., 2015).

3.3. DRP removal occurs

For phosphorus removal studies, the target influent concentration was set at 0.1 mg L^{-1} and the average influent concentration was 0.09 mg P L^{-1} and 0.10 mg P L^{-1} at 12-h and 24-h HRTs, respectively. At the beginning of the experiments (10 °C and 24 HRT), P was observed in effluent (0.1 mg L^{-1} increase) at greater concentrations than the influent. The flush was possibly due to microbial accumulation

Table 2
Nutrient removal by woodchip bioreactors operated with 12 and 24 h HRT and temperatures of 10 °C or 21.5 °C.

Experimental Conditions			Reduction[a]			
Temperature	HRT	n	NO_3-N		DRP	
°C	h		mg L^{-1}	%	mg L^{-1}	%
10	12	15	8.8 (6.0) aA	29%	0.06(0.03)aA	72%
	24	15	15.2(2.9) bA	48%	−0.11(0.15)bA	−217%
21.5	12	30	20.9(3.7) aB	67%	0.02(0.0)2aA	64%
	24	45	28.7(1.5) bB	96%	0.06(0.05)bB	85%

[a] Reduction is expressed as the mean difference in concentration (mg L$-^1$) between column influent and effluent, or that difference expressed as a percentage of influent concentration. Standard deviation is shown in parentheses. Lower case letters indicate significance between HRT at a set temperature. Upper case letters indicate significance between temperatures at a set HRT.

while the bioreactors were stored prior to restart. After 15 days and decreasing the HRT to 12, DRP was removed (0.06 mg L^{-1}), similar to observations from the 21.5 °C study. The reduction in DRP was statistically different between HRT conditions at 21.5 °C, with an average of 0.04 mg L^{-1} greater removal at the 24 HRT (Table 2).

The reported fate of phosphorus in woodchip bioreactors has been variable, with multiple studies indicating an increase in phosphorus at the bioreactor outlet, and others suggesting moderate P removals. Gottschall et al. (2016) reported TP and DRP removals in a field study using pilot scale bioreactors of 21% and 19%, respectively. A batch study by Sharrer et al. (2016) showed a release of DRP, with woodchips contributing 0.74 and 1.09 mg/L increase in effluent concentrations. A pilot-scale reactor flush from the same study had a larger concentration increase of 5.03 mg/L.

The variation in P removal reported by others likely indicates that sorption to the wood is not occurring. Ibrahim et al. (2015) reported maximum adsorption values (q_{max}) of 9 mg/g for DRP adsorption woodchips based on the Langmuir isotherm fit; much lower than 14.2 and 18.3 mg/g reported for pea gravel and zeolite, respectively. The observed removal in this study could be due to temporary microbial uptake, which was tested by a woodchip sorption experiment. The 24–48 h sorption results showed an increase in solution P equilibrating with the sterilized woodchips, with an average concentration of 2.05 (± 0.34) mg/L P (data not shown). However, phosphorus was removed from solution by the nonsterile woodchips, with an average final solution P concentration of 0.11 (± 0.11) mg/L from the 1.12 mg/L initial P concentration. The results suggest that part of the change in P could be attributed to reduction in microbial biomass by autoclaving and microbial growth in the non-sterile system.

4. Conclusions

We demonstrated potential for *Salmonella* and *E. coli* removal by woodchip bioreactors designed to treat high flows from agricultural drainage. Temperature affected bacteria removal, with greater removal occurring at 21.5 °C when compared to 10 °C conditions. Further, differences in *Salmonella* and *E. coli* removal at 12 and 24 HRT were only significantly different under the 21.5 °C conditions, and thus investigation into microbial removal at higher flows is recommended, especially considering the lack of conservation practices capable of removing microorganisms from agricultural drainage.

Acknowledgements

This project was funded by the Iowa Soybean Association. The authors would like to thank Leigh Ann Long for water quality analysis and undergraduate research assistants Rene Schmidt and Benjamin Morrison for assistance with sample collection and analysis.

References

Ahammed, M.M., Davra, K., 2011. Performance evaluation of biosand filter modified with iron oxide-coated sand for household treatment of drinking water. Desalination 276, 287–293.
Auer, M.T., Niehaus, S.L., 1993. Modeling fecal-coliform bacteria .1. Field and laboratory determination of loss kinetics. Water Res. 27 (4), 693–701.
Bearson, B.L., Bearson, S.M.D., Kich, J.D., Lee, I.S., 2014. An *rfaH* mutant of *Salmonella enterica* serovar Typhimurium is attenuated in swine and reduces intestinal colonization, fecal shedding and disease severity due to virulent *Salmonella* Typhimurium. Front. Vet. Sci. 1, 9.
Bock, E., Smith, N., Rogers, M., Coleman, B., Reiter, M., Benham, B., Easton, Z.M., 2015. Enhanced nitrate and phosphate removal in a denitrifying bioreactor with biochar. J. Environ. Qual. 44 (2), 605–613.
Christianson, L.E., Bhandari, A., Helmers, M., Kult, K., Sutphin, T., Wolf, R., 2012. Performance evaluation of four field-scale agricultural drainage denitrification bioreactors in Iowa. Trans. ASABE 55 (6), 2163–2174.
Collins, R., Donnison, A., Ross, C., McLeod, M., 2004. Attenuation of effluent-derived faecal microbes in grass buffer strips. N. Z. J. Agric. Res. 47, 565–574.
Coyne, M.S., Gilfillen, R.A., Villalba, A., Zhang, Z., Rhodes, R., Dunn, L., Blevins, R.L., 1998. Fecal bacteria trapping by grass filter strips during simulated rain. J. Soil Water Conserv. 53, 140–145.
Datsenko, K.A., Wanner, B.L., 2000. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc. Natl. Acad. Sci. U. S. A. 97 (12), 6640–6645.
David, M.B., Gentry, L.E., Cooke, R.A., Herbstritt, S.M., 2016. Temperature and substrate control woodchip bioreactor performance in reducing tile nitrate loads in east-central Illinois. JEQ 45, 822–829.
Gottschall, N., Edwards, M., Craiovan, E., Frey, S.K., Sunohara, M., Ball, B., Zoski, E., Topp, E., Khan, I., Clark, I.D., Lapen, D.R., 2016. Amending woodchip bioreactors with water treatment plant residuals to treat nitrogen, phosphorus, and veterinary antibiotic compounds in tile drainage. Ecol. Eng. 95, 852–864.
Haack, S.K., Duris, J.W., Kolpin, D.W., Focazio, M.J., Meyer, M.T., Johnson, H.E., Oster, R.J., Foreman, W.T., 2016. Contamination with bacterial zoonotic pathogen genes in US streams influenced by varying types of animal agriculture. Sci. Total Environ. 563, 340–350.
Haig, S.J., Schirmer, M., D'Amore, R., Gibbs, J., Davies, R.L., Collins, G., Quince, C., 2015. Stable-isotope probing and metagenomics reveal predation by protozoa drives E-coli removal in slow sand filters. ISME J. 9, 797–808.
Hoover, N., Bhandari, A., Soupir, M., Moorman, T., 2015. Woodchip denitrification bioreactors: impact of temperature and hydraulic retention time on nitrate removal. J. Environ. Qual. (Spec. Sect.) 43 (3), 803–812. http://dx.doi.org/10.2134/jeq2015.03.0161.
Hruby, C.E., Soupir, M.L., Shelley, M., Moorman, T.B., Kanwar, R.S., 2016. Effects of tillage and poultry manure application rates on Salmonella and fecal indicator bacteria concentrations in tiles draining des moines lobe soils. J. Environ. Manage. 171, 60–69.
Hua, G.H., Salo, M.W., Schmit, C.G., Hay, C.H., 2016. Nitrate and phosphate removal from agricultural subsurface drainage using, laboratory woodchip bioreactors and recycled steel byproduct filters. Water Res. 102, 180–189.
INRS. Iowa Nutrient Reduction Strategy, 2017. A Science and Technology-based Framework to Assess and Reduce Nutrients to Iowa Waters and the Gulf of Mexico. Available at: http://www.nutrientstrategy.iastate.edu/sites/default/files/documents/2017 INRS Complete_Revised2017_12_11.pdf (Accessed 04 January 2018).
Ibrahim, T.G., Goutelle, A., Healy, M.G., Brennan, R., Tuohy, P., Humphreys, J., Lanigan, G., Brechignac, J., Fenton, O., 2015. Mixed agricultural pollutant mitigation using woodchip/pea gravel and woodchip/zeolite permeable reactive interceptors. Water Air Soil Pollut. 226 (3), 11.
Jamieson, R., Joy, D.M., Lee, H., Kostaschuk, R., Gordon, R., 2005. Transport and deposition of sediment-associated Escherichia coli in natural streams. Water Res. 39 (12), 2665–2675.
King, K.W., Williams, M.R., Macrae, M.L., Fausey, N.R., Frankenberger, J., Smith, D.R., Kleinman, P.J.A., Brown, L.C., 2015. Phosphorus transport in agricultural subsurface drainage: a review. J. Environ. Qual. 44 (2), 467–485.
Liao, C., Liang, X., Soupir, M.L., Jarboe, L.R., 2015. Cellular, particle and environmental properties influencing attachment in waters: a review. J. Appl. Microbiol. 119 (2), 315–330.
Moest, T.P., Méresse, S., 2013. Salmonella T3SSs: successful mission of the secret(ion) agents. Curr. Opin. Microbiol. 38–44.
NRCS, 2015. Conservation Practice Standard Denitrifying Bioreactor. Code 605.
Nadelhoffer, K.J., 1990. Microlysimeter for measuring nitrogen mineralization and microbial respiration in aerobic soil incubations. Soil Sci. Soc. Am. J. 54, 411–415.
Pappas, E.A., Kanwar, R.S., Baker, J.L., Lorimor, J.C., Mickelson, S., 2008. Fecal indicator bacteria in subsurface drain water following swine manure application. Trans. ASABE 51, 1567–1573.
Rambags, F., Tanner, C.C., Stott, R., Schipper, L.A., 2016. Fecal bacteria, bacteriophage, and nutrient reductions in a full-scale denitrifying woodchip bioreactor. J. Environ. Qual. 45 (3), 847–854.
Rehmann, C.R., Soupir, M.L., 2009. Importance of interactions between the water column and the sediment for microbial concentrations in streams. Water Res. 43, 4579–4589. http://dx.doi.org/10.1016/j.watres.2009.06.049.
Robertson, W.D., Ford, G.I., Loombardo, P.S., 2005. Wood-based filter for nitrate removal in septic systems. Trans. ASAE 48, 121–128. http://dx.doi.org/10.13031/2013.17954.
Samarajeewa, A.D., Glasauer, S.M., Lauzon, J.D., O'Halloran, I.P., Parkin, G.W., Dunfield, G.K.E., 2012. Bacterial contamination of tile drainage water and shallow groundwater under different application methods of liquid swine manure. Can. J. Microbiol. 58 (5), 668–677.
Sharrer, K.L., Christianson, L.E., Lepine, C., Summerfelt, S.T., 2016. Modeling and mitigation of denitrification 'woodchip' bioreactor phosphorus releases during treatment of aquaculture wastewater. Ecol. Eng. 93, 135–143.
Soupir, M.L., Mostaghimi, S., Yagow, E.R., Hagedorn, C., Vaughan, D.H., 2006. Transport of fecal bacteria from poultry litter and cattle manures applied to pastureland. Water Air Soil Pollut. 169, 125–136. http://dx.doi.org/10.1007/s11270-006-1808-x.
Tanner, C.C., Sukias, J.P.S., Headley, T.R., Yates, C.R., Stott, R., 2012. Constructed wetlands and denitrifying bioreactors for on-site and decentralized wastewater treatment: comparison of five alternative configurations. Ecol. Eng. 42, 112–123. http://dx.doi.org/10.1016/j.ecoleng.2012.01.022.
Tomer, M., Wilson, C., Moorman, T., Cole, K., Heer, D., Isenhart, T., 2010. Source-pathway separation of multiple contaminants during a rainfall-runoff event in an artificially drained agricultural watershed. J. Environ. Qual. 39 (3), 882–895.
Torkelson, A.A., da Silva, A.K., Love, D.C., Kim, J.Y., Alper, J.P., Coox, B., Dahm, J., Kozodoy, P., Maboudian, R., Nelson, K.L., 2012. Investigation of quaternary ammonium silane-coated sand filter for the removal of bacteria and viruses from drinking water. J. Appl. Microbiol. 113, 1196–1207.
U.S. EPA, 2012. 2012 Recreational Water Quality Criteria. EPA 820-F-12-061. Available at: http://water.epa.gov/scitech/swguidance/standards/criteria/health/recreation.
Zoski, E.D., Lapen, D.R., Gottschall, N., Murrell, R.S., Schuba, B., 2013. Nitrogen, phosphorus, and bacteria removal in laboratory-scale woodchip bioreactors amended with drinking water treatment residuals. Trans. ASABE 56 (4), 1339–1347.