Biochar Amended Bioretention Systems for Nutrient and Fecal Indicator Bacteria Removal from Urban and Agricultural Runoffs

Md Yeasir Arif Rahman
University of South Florida

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Biochar Amended Bioretention Systems for Nutrient and Fecal Indicator Bacteria Removal from Urban and Agricultural Runoffs

by

Md Yeasir Arif Rahman

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Environmental Engineering
Department of Civil and Environmental Engineering
College of Engineering
University of South Florida

Co-Major Professor: Sarina Ergas, Ph.D.
Co-Major Professor: Mahmood Nachabe, Ph.D.
Mauricio Arias, Ph.D.
Aydin Sunol, Ph.D.
Bina Nayak, Ph.D.

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Dedication

Thank you, God, for your mercy and grace and for all the blessings that you have bestowed upon me and my family. It is our faith in you that lift us up and gives us the strength to face life’s obstacles and appreciate its rewards.

With my deepest gratitude, I dedicate this dissertation to my beloved family: my parents, and my wife. To my wonderful parents, Khan and Yasmin, thank you for the love and support that you have given me throughout the years. It was with your unconditional love and continuous encouragement throughout my life, especially during my education years that I was able succeed and become the person I am today. Having to study abroad, away from my family for seven years must be one of the most difficult experiences that I have faced. At last I have completed my long academic journey, and it is finally time for me to complete. I also want to thank my incredible and loving wife Laiel Safayeti, who has been my rock through thick and thin, for taking care of me and for holding down the fort while I was busy. You have stood by my side, away from your family and home and it would be impossible to have done this without you. There are no words to describe how grateful I am for you and for everything you have done. I am glad that I am done with my education so that I get to spend more time with you.

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Table of Contents

List of Tables ......................................................................................................................... iv

List of Figures ........................................................................................................................ v

Abstract ....................................................................................................................................... viii

Chapter 1: Introduction ........................................................................................................... 1
  1.1 Motivation ......................................................................................................................... 1
  1.2 Bioretention-A Low Impact Development (LID) Technology ....................................... 1
  1.3 Potential Impact of Bioretention on Nitrogen Removal .................................................. 2
  1.4 Potential Impact of Bioretention on E. coli Removal ......................................................... 3
  1.5 Biochar ............................................................................................................................. 4
  1.6 The Impact of Biochar on Contaminant Removal ............................................................. 4
    1.6.1 Biochar as a Media Amendment for Nutrient Removal ........................................... 5
    1.6.2 Biochar as a Media Amendment for E. coli Removal .............................................. 5
  1.7 Overall Goal and Research Questions .............................................................................. 6

Chapter 2: Biochar Amendment of Stormwater Bioretention Systems for Nitrogen and
Escherichia coli Removal: Effect of Hydraulic Loading Rates and Antecedent Dry Periods ........ 8
  2.1 Abstract ................................................................................................................................... 8
  2.2 Introduction .......................................................................................................................... 8
  2.3 Material and Methods ......................................................................................................... 12
    2.3.1 Porous Media ................................................................................................................ 12
    2.3.2 Batch Adsorption Experiments .................................................................................... 13
    2.3.3 Synthetic Stormwater .................................................................................................... 13
    2.3.4 Column Setup ............................................................................................................... 14
    2.3.5 Water Quality Analysis ................................................................................................. 16
    2.3.6 Data Analysis ............................................................................................................... 16
  2.4 Results and Discussion ....................................................................................................... 17
    2.4.1 Physicochemical Media Properties ............................................................................... 17
    2.4.2 Batch Study ................................................................................................................... 17
    2.4.3 Acclimation Phase ......................................................................................................... 18
    2.4.4 Hydrological Aspect of Column Study ......................................................................... 19
    2.4.5 Phase 2 Average Water Quality Performances ............................................................. 21
    2.4.6 Effect of Volume Applied on Column .......................................................................... 23
    2.4.7 Effect of Hydraulic Loading Rate .................................................................................. 26
    2.4.8 Effect of Antecedent Dry Period ................................................................................... 28
2.5 Conclusions

Chapter 3: Water Quality and Hydraulic Performance of Biochar Amended Biofilters for Management of Agricultural Runoff
3.1 Abstract
3.2 Introduction
3.3 Materials and Methods
  3.3.1 Materials
    3.3.1.1 Porous Media
    3.3.1.2 Dairy Runoff Preparation
  3.3.2 Experimental Program
    3.3.2.1 Phase I: Biochar Selection
    3.3.2.2 Phase II: Effect of Amendment Rate and ADP on Water Quality
    3.3.2.3 Phase III: Effect of ADP in Consecutive Experiments
    3.3.2.4 Phase IV: Effect of Amendment Rate on Hydraulic Performance
  3.3.3 Analytical Methods
  3.3.4 Mathematical Model and Data Analysis
3.4 Results and Discussions
  3.4.1 Phase I: Biochar Selection
  3.4.2 Phase II: Effect of Amendment Rate on Water Quality
    3.4.2.1 Overall Nutrient Removal Performance
    3.4.2.2 Effect of ADPs on Water Quality in Consecutive Experiments
    3.4.2.3 N-species Breakthrough
    3.4.2.4 Phase-III: Biochar Amendment Rate Comparison – Hydraulics
3.5 Conclusions

Chapter 4: Removal of Fecal Indicator Bacteria from Dairy Runoff Using Biochar Amended Bioretention
4.1 Abstract
4.2 Introduction
4.3 Materials and Methods
  4.3.1 Porous Media
  4.3.2 Chemical Composition of Dairy Runoff
  4.3.3 Experimental Program
    4.3.3.1 Phase I: Biochar Selection
    4.3.3.2 Phase II: Effect of Amendment Rates on E. coli Removal
    4.3.3.3 Phase III: Pilot Modified Bioretention Systems
  4.3.4 Analytical Methods
  4.3.5 Data Analysis
4.4 Results and Discussion
  4.4.1 Biochar Selection
  4.4.2 Effect of Biochar Amendment Rate on FIB Removal
List of Tables

Table 2.1: Effluent N-species concentrations in three columns during the acclimation stage .................................................................19

Table 2.2: Effect of ADPs on mass removal efficiency for N-species, DOC and E. coli for S, SBC1 and SBC2 .................................................................29

Table 2.3: Target synthetic stormwater (SSW) chemical composition and operating conditions during each experimental phase .........................................................34

Table 2.4: Physicochemical characterization of two biochars used in this study .................................................................34

Table 2.5: Overall mass contaminant removal performance for the three columns .................................................................35

Table 3.1: Operating conditions during each experimental phase .................................................................42

Table 3.2: Overall N-species and organic carbon removal for SBC-20 and SBC-50 columns. .................................................................49

Table 3.3: Calibrated Parameters for the HYDRUS model to simulate the breakthrough curve .................................................................55

Table 4.1: Experimental design of different phases on E. coli removal .................................................................64

Table 4.2: Overall E. coli and Enterococci removal performance for biochar amended sand .................................................................69

Table 4.3: E. coli removal performance for pilot studies for four bioretention systems considering different pore water infiltration with 7-days ADPs and 0.10 cm$^3$/cm$^2$/min HLR .................................................................72

Table B.1: Physicochemical characterization of media .................................................................104

Table B.2: Effect of three different flow rates/HRTs of three different columns for contaminants removal based on mass removal .................................................................104
List of Figures

Figure 1.1: Schematics of a typical stormwater biofilter with and without an internal water storage zone .................................................................2

Figure 2.1: Batch experiment of NH4+ and NO3- adsorption for Sand (S), Biochar 1 (BC 1) and Biochar 2 (BC 2)..................................................................................................................................18

Figure 2.2: Moisture content at three different depths (i. surface, ii. mid depth: 15 cm from the top surface and iii. bottom depth: 30 cm from the surface) of the three columns (i. Sand, ii. SBC1 and iii. SBC2) for 7- and 30-days ADPs........20

Figure 2.3: a) Percent water retention with error bars for the three columns considering eighteen events. b) Average water retention of three porous media in the three columns.................................................................................................................................21

Figure-2.4: Pollutant concentration profiles (a: TAN; b: NOx; c: DON, d: TN, ....e: E. coli and f: DOC) of S, SBC1 and SBC2 bioretention systems for 3.5 hours and 7 ADP stormwater experiment with a high HLR ...........................................................................................................25

Figure 2.5: Effect of high, medium and low HLRs on sand (S), sand with Biochar 1 (SBC1) and sand with Biochar 2 (SBC2) biofilters for removal of a) TAN, b) NOx, c) DON, d) TN, e) DOC and f) E. coli different columns for contaminants removal.................................................................................................................................27

Figure 2.6: Pollutant concentration profiles (a: TAN, b: NOx, c: DON) of S (Left) and SBC2 (Right) bioretention systems for 3-,7- and 30-day ADPs stormwater experiments with 3.5-hour storm events at medium HLR.................................................................................................................................31

Figure 2.7: Pollutant concentration profiles (d: TN, e: DOC, f: DON) of S (Left) and SBC2 (Right) bioretention systems for 3-,7- and 30-day ADPs stormwater experiments with 3.5-hour storm events at medium HLR.................................................................................................................................32

Figure 3.1: Comparison of N-species (TAN, NOx and DON) concentrations in influent dairy runoff and effluent from three columns (S: Sand, SBC1: Sand with biochar 1 and SBC2: Sand with biochar 2). Error bars show standard deviations for four experiments .................................................................................................................................48

Figure 3.2: TAN and TN removal for SBC-20 and SBC-50 for four consecutive experiments with 7-day ADP and 0.1 cm/min HLR............................................................51
Figure 3.3: Pollutant concentration profiles (a: TAN; b: NO3-; c: DON, d: TN, and e: TOC) for SBC-20, and SBC-50 bioretention systems with 12-day ADP and high HLR. The red lines indicate the number of pore volumes.................................53

Figure 3.4: Volumetric moisture content (cm3/cm3) with depth for varying ADPs: a) SBC-20 and b) SBC50..............................................................54

Figure 3.5: Tracer breakthrough curves and model simulations for: a) SBC-20 and b) SBC-50 .................................................................56

Figure 3.6: Vertical profile of a) moisture content of the SBC-20 and SBC-50 columns at different time intervals ........................................56

Figure 4.1: Cross-sectional diagrams of (a) sand modified bioretention cell with plant (SP), (b) sand modified bioretention cell (S), (c) biochar amended sand modified bioretention cell with plant (BP) and (d) biochar amended sand modified bioretention cell (B) (units are in cm)........................................66

Figure 4.2: Effluent E. coli concentrations normalized to influent (C0) for columns packed with (a) Sand (S), (b) mixture of sand and high surface area biochar (SBC1), and (c) mixture of sand and high cation exchange capacity biochar (SBC2) for a 3.5 hour experiment with 7 day ADP and HLR of 0.10 cm3/cm²/min. Influent E. coli concentration was 6.0 x10⁶ ± 1.55x10⁶ CFU/100 ml .................................................................69

Figure 4.3: E. coli breakthrough profile for SBC-20 and SBC-50 columns for a) 7-days and b) 28-days ADPs ......................................................71

Figure 4.4: Overall performance of E. coli removal for pilot studies for four modified bioretention systems ..................................................73

Figure 5.1: Schematic of two bioretention systems: (a) conventional and (b) modified..............76

Figure 5.2: Cross-sectional diagrams of (a) sand modified bioretention cell with plant (SP), (b) sand modified bioretention cell (S), (c) biochar amended sand modified bioretention cell with plant (BP) and (d) biochar amended sand modified bioretention cell (B) (units are in cm)........................................79

Figure 5.3: Overall N-species removal efficiency (a: TAN; b: NO3-; c: DON; d: TN and e: TOC) for four modified bioretention systems (BP: biochar with plant bioretention; B: biochar amended bioretention; SP: sand amended bioretention with plant and S: sand bioretention system)..........................82

Figure 5.4: Pollutant breakthrough curve of (a) TAN and (b) TN for four modified bioretention considering 222 ml/min flow rate for 4.5 hours dairy runoff experiment. .................................................................83
Figure 5.5: Photographs of two modified bioretention systems (a) sand with plant, biochar with plant after twelve runoff experiments ................................................................. 84

Figure A1: Experimental setup of the lab scale experiment for three different media for urban runoff management .................................................................................................. 102

Figure A2: Experimental setup of the Pilot scale experiments for dairy runoff Management .................................................................................................................. 102

Figure A3: Future recommended works for layered systems on biochar amended bioretention systems .................................................................................................................. 103

Figure B1: Influent and effluent relationship for three different HRTs for three columns ....... 105

Figure C1: E. coli log removal for four pilot systems including BP, B, SP and S .................. 106

Figure C2: Flow rates of four pilot systems including BP, B, SP and S during the experiment ................................................................................................................................ 106
Abstract

Excessive nitrogen and fecal indicator bacteria (FIB) in stormwater runoff from urban areas or dairy farm operations impair water bodies resulting algal blooms, gastrointestinal illness, and economic losses. Recent advancements in stormwater management, including low impact development (LID) techniques, have provided engineers with a variety of tools to use in place of traditional catch basins and retention ponds. One promising LID technology for runoff management is bioretention, which consists of a shallow depression containing engineered porous media and vegetation. Prior studies have shown that modified bioretention systems that include an internal water storage zone (IWSZ) to promote denitrification can improve total nitrogen (TN) and FIB removal. However, limited adsorption, nitrification, ammonification and denitrification lead to wide range of removals have been reported for total ammonia nitrogen (TAN), dissolved organic nitrogen (DON), dissolved organic carbon (DOC) and total nitrogen (TN) in sand based bioretention systems. Moreover, limited surface area (SA) and porous structure of sand hinder FIB attachment, resulting low removal efficiencies. Therefore, amending engineering media with an appropriate adsorbent can enhance bioretention performance.

Biochar is a promising low cost adsorbent that can be added to bioretention media to improve TN and FIB removal in urban and dairy farm runoff. It is a carbon rich by-product of waste materials pyrolyzed at high temperature under oxygen limited conditions. The type of feedstock, production process and pyrolysis temperatures are key factors that influence biochar properties. In general, biochar has a high cation exchange capacity (CEC), moisture content (MC),
SA, porosity (n), pore size distribution (PSD), hydrophobicity, and ash content. The high nutrient retention and water holding capacity (WHC) of biochar aids plant growth, which can help inactivate pathogens and promote nitrification and denitrification by releasing root exudates. The overall goal of this research was to expand the current knowledge of biochar amended sand bioretention systems to manage TN and FIB removal from urban stormwater and agricultural runoff. This dissertation research was conducted in three phases, which included both laboratory-scale and pilot-scale studies with mathematical modeling.

Selection of media was initially carried out by characterizing both biochar and sand based on pH, hydraulic conductivity (K), bulk density (BD), electrical conductivity (EC), grain size distribution (GSD), n, MC, CEC, SA and PSD. Based on the physicochemical properties, masonry sand was used as the main medium for all three phases of research.

In Phase I, the influence of biochar properties on the fate of N-species and Escherichia coli (E. coli) removal in bioretention systems was investigated through batch and column studies using sand media, with and without biochar addition, for treating urban runoff. Two different commercial wood-chip based biochars were tested that were produced at different temperatures. In abiotic batch experiments, significantly higher TAN adsorption was observed for biochar (3.5 mg/g) than sand (0.05 mg/g) due to the higher CEC of biochar. Data also showed that, biochar had very low NOx (NO₃⁻ + NO₂⁻) adsorption capacity. TAN, DOC, and E. coli removals were significantly higher in biochar-amended columns due to biochar’s high CEC, pH, microporous structure, carbon content and SA. TAN adsorption resulted in increased nitrification during the antecedent dry periods (ADPs) when aerobic conditions developed. MC data revealed that saturated conditions prevailed toward the bottom of biochar-amended columns for several days after the storm event due to the high WHC of biochar, which favored denitrification and TN
removal. \textit{E. coli} removal was a strong function of SA and hydrophobicity; greater than 6 log \textit{E. coli} removal was observed in the column amended with high SA biochar. Biochar amended columns also showed more stable TAN, DOC and \textit{E. coli} effluent concentrations under varying hydraulic loading rates (HLRs) and ADPs. Therefore, biochar with higher SA was selected for the next phases of experiments.

In Phase II, the effect of biochar amendment rate on nitrogen species, DOC and FIB removals and hydraulic performance was evaluated in biofilter columns treating dairy farm runoff. One of the key differences between urban and dairy runoff are the compositions of runoff. Dairy runoff has a higher ionic strength, higher concentrations of DON, DOC, suspended and dissolved particles than urban runoff. Two biofilter columns with different biochar fractions (20\% and 50\% by volume) were operated at varying HLRs and ADPs. TAN, DON and DOC removals were significantly higher for the higher biochar fraction amended column. The higher biochar amendment rate increased the surface charge availability for TAN adsorption (71\%), even with more complex influent compared to the column with the lower biochar amendment rate (34\%). The high CEC of biochar increased TAN retention during the application period, allowing for nitrification during the ADPs when aerobic conditions developed in the media pores. However, low effluent NOx concentrations were observed from both systems. Biochar high SA also resulted in greater retention of DON and DOC by adsorption. The high WHC of biochar and presence of adsorbed DOC enhanced denitrification. Therefore, TN removal was significantly higher with the higher biochar amendment rate (65\%) compared with the lower biochar amended column (39\%). Significantly higher \textit{E. coli} removals were observed compared with Enterococci in both columns, indicating a greater attachment affinity to the biochar surface for \textit{E. coli}. However, there were no significant differences in \textit{E. coli} or Enterococci removals between the two columns with different
biochar fractions. Moreover, longer ADPs were found to enhance *E. coli* removal in the higher biochar fraction column.

A variable saturation flow model of biochar amended biofiltration was developed using HYDRUS-1D software. The model was calibrated using data from conservative tracer and moisture content studies. Model results showed that the high microporous structure of the biochar increases the time needed to reach full saturation, lowers the saturated conductivity and increases the hydraulic retention time in the medium.

In Phase III, pilot-scale studies were conducted with four modified bioretention systems that included an IWSZ. Experiments were designed to test TN and FIB removal performance with and without biochar and with and without plants (Muhlenbergia). Higher DOC adsorption in the IWSZ in systems with biochar favored denitrification, resulting in higher TN removal (>96%) in both biochar (B) and biochar with plant (BP) bioretention systems. Due to high moisture and nutrient retention, better plant growth was observed in BP compared with sand with plant (SP). The presence of plants also influenced N-species removal. The inclusion of plants, biochar and an IWSZ in pilot-scale systems resulted in the best *E. coli* removal (> 5 log *E. coli* removal). Plants can improve *E. coli* removal through predation and competition by rhizosphere microbes or inactivation by antimicrobial compounds from root exudates. Higher *E. coli* removals in pilot units with an IWSZ may have been due to the longer retention times and/or the anoxic conditions present in the IWSZ.

Future research should be carried out considering other pollutants i.e. phosphorous, metals, pesticides and viruses, which also cause water quality impairment. Pilot and field-scale research should also be carried to investigate maintenance requirements for biochar amended bioretention.
Chapter 1: Introduction

1.1 Motivation

Human activities, such as urbanization and agriculture, can permanently modify the nature, form and behavior of receiving water bodies, leading to multiple negative hydrological, ecological, and public health impacts. Non-point sources of pollution, mainly nutrients (N and P) and fecal indicator bacteria (FIB) from humans and animals, are worldwide causes of surface water contamination (Wurtsbaugh et al., 2019; USEPA, 2012). Fecal contamination limits shellfish harvesting, results in beach closings and effects economic activity in coastal areas (Lee et al., 2006; McLellan et al., 2003; Parker et al., 2010). Anthropogenic activities, such as excessive fertilizer usage in urban and agricultural regions or improper human and livestock waste management practices, are worldwide causes of eutrophication (Erickson et al., 2013). Nutrient contamination results in overgrowth of algae, seagrass mortality, development of hypoxic zones in water bodies and ground water contamination (Lian et al., 2019; Islam et al., 2018). The National Academy of Engineering (NAE) and the United Nations Environment Programme (UNEP) listed nitrogen cycle management as one of the engineering’s “Grand Challenge” because of the harmful environmental effects of nitrogen discharges (UNEP, 2007). Therefore, proper treatment of contaminated stormwater runoff is essential before discharge of the effluent to surface water bodies.

1.2 Bioretention-A Low Impact Development (LID) Technology

Low impact development (LID) is a design approach that can help to maintain predevelopment hydrology and improve surface water quality. Bioretention systems, also known
as rain gardens or biofilters, are some of the most effective LID practices. Bioretention systems are shallow depressions adjacent to developed impervious surfaces consisting of different porous media layers, vegetation, and an optional overflow pipe (Figure 1.1). To enhance water quality treatment performance, bioretention systems can be modified by addition of an internal water storage zone (IWSZ), containing an electron donor such as wood chips or tire chips (Figure 1) (Lopez et al., 2020; Lopez et al., 2017). The main goal of modified bioretention systems is to enhance denitrification of nutrient-rich stormwater by providing the appropriate retention time, anoxic conditions, and electron donor in the IWSZ (Ergas et al., 2010).

Figure 1.1: Schematics of a typical stormwater biofilter with and without an internal water storage zone.

1.3 Potential Impact of Bioretention on Nitrogen Removal

In conventional bioretention systems, nitrification \((\text{NH}_4^+ \rightarrow \text{NO}_3^-)\) is promoted in the aerobic filter medium. However, in conventional bioretention systems total nitrogen (TN) removal
is typically low because the systems lack conditions needed for ammonification, nitrification and
denitrification (Li et al., 2014; Hsieh and Davis, 2005). Following a storm, drainage releases the
soil water, allowing air to fill the pores, thus providing oxygen needed for nitrification of adsorbed
NH$_4^+$. However, sand has limited CEC therefore, NH$_4^+$ adsorption is low. Line and Hunt (2009)
reported variable and often limited NH$_4^+$ removal performance (-39% to 87%) due to the low
cation exchange capacity (CEC) of media and observed net negative NOx (NO$_2^-$+NO$_3^-$) removal
(384 to −57%). Moreover, dissolved organic nitrogen (DON) adsorption and ammonification is
limited in the sand media (Sharkey and Hunt, 2005; Zinger et al., 2007).

Although inclusion of IWSZ works well for treatment of contaminants in urban runoff, the
high concentrations of TAN, DON and dissolved organic carbon (DOC) present in dairy runoff
present a challenge for these systems. During storm events, these pollutants are transported through
the bioretention media with the runoff and are not retained long enough for complete
biodegradation. Therefore, amending the filter medium with high CEC and SA materials has the
potential to improve NH$_4^+$, DON and DOC adsorption during infiltration for both urban and dairy
runoff.

1.4 Potential Impact of Bioretention on E. coli Removal

In an assessment of the International Stormwater Best Management Practices (BMP)
database, Clary et al. (2008) concluded that conventional bioretention or sand filtration is the most
feasible BMP to remove bacterial contamination from stormwater runoff. However, limited
hydraulic permeability, improper design criteria, inappropriate media or media amendment and
lack of regular maintenance reduced the performance of the systems. Also, although laboratory-
and field-based studies of conventional sand media biofilters show that they have some potential
to remove FIB (Garcia-Albacete et al., 2014; Hathaway et al., 2011; Chandrasena et al., 2014;
Zhang et al., 2010) research on the effect of IWSZ addition on FIB removal is limited. Metal oxide, metal hydroxides, antimicrobial coatings, zeolite, activated carbon and other geo-media amendments have shown potential for better FIB removal in laboratory-scale bioretention systems in short term studies. Additional research is needed on fundamental design criteria for bioretention, and the selection of media or media amendment for removal of FIB.

1.5 Biochar

Biochar is the by-product of an organic feedstock produced at high temperature under oxygen limiting conditions. Biochar feedstocks include materials of biological origin, such as manures, animal-litters, and lignocellulosic biomass, including crop residues and wood biomass. The microporous structure of biochar shows a high degree of chemical and microbial stability, which enhances the availability of macro-nutrients such as nitrogen (N). Biochar amendment alters alkalization of soil pH and increases electrical conductivity (EC), CEC and surface area (SA). Their incorporation into soils influences soil structure, texture, porosity, particle size distribution and density (Atkinson et al., 2010). This microscopic physical structure of biochar largely depends on its: i) feedstocks; ii) production technology; and iii) heating time (Lehmann et al., 2011).

1.6 The Impact of Biochar on Contaminant Removal

Biochar has been recognized as an amendment material to reduce the bioavailability of contaminants in the environment and exhibits a great potential for pollution remediation and removal of water contaminants (Sohi, 2012; Ahmad et al., 2014; Rajapaksha et al., 2016). Prior studies have demonstrated the potential of biochar amendment for TN and phosphorous (Laird et al. 2010), heavy metals (Uchimiya et al., 2011), pesticides (Cao et al., 2009) and E. coli (Afrooz et al., 2017; Mohanty et al., 2014; Mohanty et al., 2014a; Mohanty et al., 2013; Abit et al., 2012) removal.
1.6.1 Biochar as a Media Amendment for Nutrient Removal

Amendment of bioretention system media with biochar for nutrient removal in is quite new. Due to its high CEC, biochar helps to retain NH$_4^+$ in stormwater. Ding et al. (2010) showed slow vertical movement of NH$_4^+$ in bamboo biochar amended sand columns due to high CEC. Yao et al. (2012) showed that biochar pyrolysis temperature $>$600°C can limit leaching of NH$_4^+$ and NO$_3^-$. The author found that a soil column with 2% biochar amendment reduced leaching of NH$_4^+$ and NO$_3^-$ by 34.7% and 34%, respectively, compared with an un-amended soil column. Tian et al. (2016) investigated two different biochars (poultry litter and wood) performance for stormwater treatment and found that biochar produced at high pyrolysis temperature increased NH$_4^+$ adsorption and helped to limit the amount of organic and inorganic N released. Tian et al. (2016) also found that SA was negatively correlated with NH$_4^+$ adsorption, while there was a linear correlation between CEC and NH$_4^+$ sorption. Studies of biochar amended sand biofilters with an IWSZ for nutrient removal are limited. Afrooz et al. (2017) investigated a biochar-sand biofilter with an IWSZ for nutrient removal and found that addition of biochar helped remove nutrients from net leaching to net removal.

1.6.2 Biochar as a Media Amendment for *E. coli* Removal

Due to its low cost (Ahmad et al., 2014) and high adsorption capacity, biochar amended sand biofilters have been studied for removing FIB at laboratory scale. Recent studies showed that wood derived biochar produced at higher pyrolysis temperature achieved higher *E. coli* removal compared with low temperature manure derived biochar (Abit et al., 2012). In laboratory scale experiments, Mohanty et al. (2014) concluded that biochar with higher surface charge and hydrophobicity along with lower volatile mater can accelerate *E. coli* removal in biochar amended sand biofilters. Mohanty and Boehm, 2014 showed that exclusion of fine particle biochar (<125
µm) resulted in lower *E. coli* removal. Although prior studies have shown promise for FIB removal, one important difference between urban and dairy runoff is the chemical composition of runoff matrix. Dairy runoff contains high DOC, suspended and dissolved particles and higher ionic strength compared with urban runoff. In prior laboratory studies, organic matter was shown to decrease removal capacity of *E. coli* in biochar amended biofilters (Mohanty and Boehm, 2014; Mohanty and Boehm, 2015). Therefore, the effectiveness of biochar for FIB removal from agricultural runoff with high concentrations of DOC and suspended particulates is not known. Moreover, Afrooz and Boehm (2017) reported insignificant influence of the IWSZ on FIB removal performance while another study showed enhanced FIB removal in a saturated biochar biofilter compared with an unsaturated biofilter. Therefore, research at both laboratory and field scale with varying amendment rates using complex influent runoff compositions need to be address.

### 1.7 Overall Goal and Research Questions

The overall goal of this research was to expand the current knowledge of biochar amended sand bioretention systems to manage FIB and N removal from urban stormwater and dairy runoff. The following questions guided this research:

1. How do physicochemical properties of biochar and rate of biochar amendment affect biological nitrogen transformations in biochar amended bioretention systems?
2. How do physicochemical properties of biochar and rate of biochar amendment affect FIB removal in biochar amended bioretention systems?
3. How does the composition of the influent (e.g., urban stormwater compared with dairy runoff) affect N-species and FIB removal in biochar amended bioretention systems?
4. How does the rate of biochar amendment affect the hydraulic performance of bioretention systems?
5. How does the presence of an IWSZ and plants affect the long term performance of N-species and *E. coli* removal in modified bioretention systems amended with biochar?

This dissertation is divided into six chapters. Chapter 2 presents the results of side-by-side column studies, with and without biochar for N-species and FIB removal from urban runoff. The effect of biochar physicochemical properties on treatment performance was also investigated. Chapter 3 presents the results of side-by-side column studies for two different biochar amendment rates on N-species removal from dairy runoff. The effect of different fractions of biochar amendment on hydraulic performances was also investigated. Chapter 4 presents the results of side-by-side column studies with two different biochar amendment rates on FIB removal from dairy runoff. The inclusion of IWSZ and plants on *E. coli* removal from dairy runoff was also addressed. Chapter 5 presents the effect of IWSZ and plants on N-species removal from dairy runoff. Chapter 6 presents the overall conclusion and recommendation for future work.
Chapter 2: Biochar Amendment of Stormwater Bioretention Systems for Nitrogen and *Escherichia coli* Removal: Effect of Hydraulic Loading Rates and Antecedent Dry Periods

2.1 Abstract

Bioretention systems improve stormwater infiltration and water quality; however, sand-based bioretention media has limited total nitrogen (TN) and fecal indicator bacteria (FIB) removal. In this study, the fate of N-species and *E. coli* in bioretention systems was investigated through batch and column studies using sand media with and without biochar addition. Variables investigated included biochar characteristics, hydraulic loading rate (HLR) and antecedent dry period (ADP). Total ammonia nitrogen (TAN), dissolved organic carbon (DOC), and *E. coli* removals were significantly higher in biochar-amended columns due to biochar’s high cation exchange capacity and specific surface area. TAN adsorption resulted in increased nitrification during the ADP when aerobic conditions developed. Moisture content data revealed that saturated conditions prevailed toward the bottom of biochar-amended columns for several days, favoring denitrification and TN removal. Biochar amended columns also showed more stable TAN, DOC and *E. coli* effluent concentrations under varying HLR and ADP.

2.2 Introduction

Pathogenic microorganisms and nutrients from non-point sources are major worldwide causes of surface water contamination. Nutrient runoff results in eutrophication (Wurtsbaugh et al., 2019), algae growth, and increased hypoxic zones. Microbial contamination in runoff water,

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such as fecal matter from animals and humans, causes gastro-intestinal illness, limits shellfish harvesting and may result in beach closures (Parker et al., 2010). Therefore, stormwater Best Management Practices (BMPs) are needed to mitigate non-point source pollution from urban sources. Low Impact Development (LID) technologies, such as biofilters, green roofs and permeable pavement, aim to restore predevelopment hydrology and reduce nutrient and pathogen loads to water bodies.

Biofilters, or bioretention systems, have emerged as effective LID BMPs. These systems consist of vegetated shallow depressions adjacent to impervious surfaces, with layers of pervious material, including mulch, sand, and gravel. Conventional bioretention systems efficiently remove suspended solids, phosphorus, hydrocarbons, and heavy metals (Bratieres et al., 2008; Jay et al., 2019; Mahmoud et al., 2019). However, a number of studies (Davis 2008; Hsieh and Davis, 2005; Line and Hunt, 2009) have reported variable and often limited performance in removing total nitrogen (TN) due to the low cation exchange capacity (CEC) of sand. In a field-based bioretention study, Line and Hunt, (2009) reported that NH$_4^+$ removal varied from -39 to 87% and observed a net negative removal of NOx ($\text{NO}_2^-$ + $\text{NO}_3^-$) (384 to -57%) due to nitrification. Therefore, enhancing the CEC by amending the filter medium has the potential to improve NH$_4^+$ adsorption during infiltration. Following a storm, drainage releases the soil water, allowing air to fill the pores, thus providing oxygen needed for nitrification of adsorbed NH$_4^+$. Enhanced nitrification can be coupled with inclusion of a submerged zone with a source of electron donor, such as wood chips, to promote denitrification of $\text{NO}_3^-$ to gaseous nitrogen (N$_2$) (Ergas et al., 2010; Lynn et al., 2015).

Bioretention systems design and operational conditions influence N-transformations in the porous media. An important design parameter is the Hydraulic Retention Time (HRT), which is the average time the contaminated stormwater spends in contact with the biofilter porous medium.
Bratieres et al. (2008) reported that lower Hydraulic Loading Rates (HLRs; and therefore, longer HRT) improved TN, NH$_4^+$, NO$_3^-$ and Org-N removal in biofilter systems. However, Jay et al., (2019) observed no relationship between TN removal and HLR in column studies with different bioretention media. Moreover, high HLR can result in ponding and stormwater runoff bypassing the biofilters. Variations in Antecedent Dry Period (ADP), which is the time interval between two successive rain events, when soil water redistributes, drains or evaporates, also affects biofilter performance, particularly, net leaching of NO$_3^-$ after rewetting. As mentioned previously, following the passage of a storm, pore water is replaced by oxygenated air, which promotes nitrification of adsorbed NH$_4^+$ during the ADP. Moreover, evapotranspiration reduces the effluent volume during long ADPs. Hence, the relationship between ADP and N-transformations should be further explored to optimize bioretention design.

In an analysis of the International Stormwater BMP database, Clary et al., (2008) concluded that bioretention or sand filtration systems are the most feasible BMPs for fecal indicator bacteria (FIB) removal from stormwater. However, wide variability in FIB removal has been reported (Hathaway and Hunt, 2011; Mahmoud et al., 2019; Zhang et al., 2010) due to differences in porosity, hydrodynamic dispersion, and surface characteristics of media and bacteria. Due to their ease of measurement and abundance in contaminated waters, Escherichia coli (E. coli), are often used to indicate the presence of fecal wastes in runoff. Major mechanisms for E. coli removal in porous media are attachment, straining, predation and die-off. Attachment is influenced by media surface characteristics, whereas straining is controlled by pore and particle sizes (Zhang et al., 2010).

Biochar is the by-product of a pyrolysis at high temperature of an organic feedstock, such as wood chips, under oxygen limited conditions. Feedstock properties and pyrolysis temperature
affect biochar properties, such as particle size distribution, density, CEC and surface area (Mukherjee et al., 2011; Suliman et al., 2016). Due to its low cost and high adsorption capacity, biochar amended sand biofilters have been studied for removing nitrogen (Laird et al., 2010; Tian et al., 2016) and E. coli (Bolster and Abit, 2012; Nabiul Afrooz and Boehm, 2017) at different scales.

Several prior studies have investigated the ability of biochar to retain NH$_4^+$ from stormwater (Ding et al., 2010; Yao et al., 2012). In 600°C pyrolyzed bamboo biochar amended sand columns, Ding et al. (2010) attributed the slowed vertical movement of NH$_4^+$ to increased CEC. Yao et al. (2012) found that addition of high pyrolysis temperature (>600°C) wood biochar reduced leaching of NH$_4^+$ and NO$_3^-$, while low pyrolysis temperature biochars (300-450ºC) were not as effective. Tian et al. (2016) investigated the performance of two different biochars (poultry litter and wood feedstocks) in stormwater column studies; biochar produced at a high pyrolysis temperature released limited amounts of organic and inorganic N. The authors showed that a 10% (w/w) biochar amended sand column with a steady infiltration rate over 8 hours achieved 90% NH$_4^+$ adsorption but only 0.24-0.61% of NOx removal. However, the authors did not investigate the effect of biochar addition on NH4+ adsorption and nitrification rate with varying HLRs and ADPs.

Several prior studies have investigated E. coli removal in biochar amended biofilters. Abit et al., (2012) showed that sand amended with 2% (w/w) wood derived biochar improved E. coli removal compared with poultry litter derived biochar at two different flow rates. During the low flow rate study (0.06 cm/min), in which the media was only 50% saturated, higher E. coli removal was observed compared to the high flow rate study (0.13 cm/min) when the media was fully saturated. However, these experiments were conducted for a short duration (30 min) with less than
1 pore volume of influent and without biofilm acclimation, which would be present under field conditions. Mohanty and Boehm, (2014) showed that exclusion of fine particle biochar (<125 μm) resulted in lower *E. coli* removal than a column with fine biochar (0.42 compared to 1.39 log *E. coli* removal). The authors also found that biochar with low surface area, which had higher hydrophobicity and low volatile matter content, was more effective at *E. coli* removal. Abit et al., (2012) showed that 700ºC pyrolyzed poultry litter biochar had a higher surface area (9 m²/g) than 350ºC pyrolyzed biochar (1.1 m²/g), which helped in retaining more *E. coli* in column studies.

Prior studies of biochar amended bioretention systems have investigated either N-species or pathogen removal, while stormwater managers need to consider both simultaneously. These studies have also been carried out for short durations and did not examine the effect of repeated wetting and drying cycles. The aim of this research was to gain a deeper understanding of the fate of nitrogen and *E. coli* under more realistic operation conditions through infiltration tests in bench-scale sand columns with and without biochar. The objectives of this study were to investigate the effects of: 1) biochar properties, 2) HLRs, and 3) ADPs on removal of *E. coli* and N-species in urban runoff. This is the first study to investigate the effect of HLR and ADP on the performance of biochar amended bioretention columns for both nutrient and FIB removal.

### 2.3 Material and Methods

#### 2.3.1 Porous Media

Three different sands and two commercial biochars were characterized for pH, hydraulic conductivity (K), bulk density (BD), porosity (n), moisture content (MC), electrical conductivity (EC), grain size distribution (GSD), CEC, surface area (SA) and pore size distribution (PSD), as described below. Three sands (i. concrete, ii. masonry and iii. local sand) were obtained from Seffner Rock & Gravel in Tampa, Florida, USA. Two-wood derived biochars were acquired from
Biochar Supreme (Environmental Ultra, Everson, WA; (BC1)) and Biochar Now (Loveland, CO; (BC2)). Biochar Supreme (BC1) was pyrolyzed at 900-1,000°C and Biochar Now (BC2) was pyrolyzed at 550°C. Prior to use, biochars were sieved and hand crushed to a particle size between 0.15 mm -1.00 mm to produce homogenous mixture with sand.

GSD and MC were measured using ASTM D6913/D6913M–17 and ASTM D2974, respectively. Hydraulic conductivity (K) was measured by the Falling Head Test Method (ASTM D5084 – 03). The Ammonium Acetate Method (Chapman, 1965) (Method 9080) was used to measure CEC. SA and pore size distributions were measured using a Quantachrome Instrument (P/N 05061-1.5 Rev a Quantachrome Instruments, AUTOSORB-1, AS1Win Version 1.5X, 2008). SA was measured by N₂ gas adsorption-desorption using the Brunauer-Emmett-Teller (BET) method. Mesopore and micropore size distributions were analyzed by Barrett, Joyner and Halenda and Horvath-Kawazoe (HK) methods, respectively. A total of 20 adsorption and 20 desorption points were selected for the isotherms and degas temperature was 150°C for 10 hr.

2.3.2 Batch Adsorption Experiments

Batch experiments were performed in duplicate to evaluate adsorption of NH₄⁺ and NOx by sand, BC1 and BC2 under abiotic conditions. Briefly, media materials were soaked overnight in deionized (DI) water, rinsed and then oven dried at 103.5°C. Subsequently, 1.5 g of each material was added to an Erlenmeyer flask that contained 100 ml of sterile NH₄⁺/NO₃⁻ solution (13 mg NH₄⁺-N /l and 8.5 mg NO₃⁻-N /l). Flasks were place on a shaker table at 150 rpm and 23°C for 24 hours prior to NH₄⁺ and NO₃⁻ analysis.

2.3.3 Synthetic Stormwater

The column study was performed in two phases: i) acclimation and ii) urban runoff, with different Synthetic Stormwater (SSW) compositions (Table 2.3). The acclimation phase was used
to establish nitrifying and denitrifying biofilms before introducing SSW. Target concentrations of N-species (1 mg/l each of \( \text{NH}_4^+ \)-N, \( \text{NO}_3^- \)-N, DON) and \( E. coli \) (10\(^7\) CFU/100ml) for the urban runoff phase was consistent with the literature (Le Fevre et al., 2015; Lynn et al., 2015). SSW was prepared by adding stock solutions of \( E. coli \), potassium nitrate (KNO\(_3\)), ammonium chloride (NH\(_4\)Cl) and ground oak leaf extract to filtered local groundwater. Oak leaves collected from University of South Florida were used as source of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) (Lopez-Ponnada et al., 2020). Briefly, oak leaves were dried and ground to a fine powder using a coffee grinder. Subsequently, 9 gm of ground oak leaves were added to a 1x1 inch mesh bag, sealed and then submerged in 800 ml of DI water overnight. This yielded an oak leaf extract stock solution with approximately 11 mg/l of DON.

\( E. coli \) K12 (ATCC 10798) was used for the \( E. coli \) stock solution, which was prepared according to Mohanty et al. (2013). \( E. coli \) samples were collected over time from duplicate cultures and both absorbance at 600 nm and Colony Forming Units (CFU) were counted for each sample to develop a calibration curve for estimating initial \( E. coli \) concentrations. The stationary phase cultured \( E. coli \) were centrifuged, the supernatant was discarded, and the pellet was re-suspended in phosphate buffer saline (PBS) before adding to SSW.

2.3.4 Column Setup

Three different columns were setup with: i) sand (S), ii) sand with biochar 1 (SBC1) and iii) sand with biochar 2 (SBC2). The volumetric ratio of sand and biochar was 70:30, which corresponded to 3.10% and 4.75% of biochar by mass, for BC1 and BC2, respectively. Koflo 2000 ml Calibration Columns (Fisher Scientific, IL) with 7.2 cm internal diameter and 50 cm height were used for the bench-scale columns. The depth of the media in each column was 30 cm. From the bottom of the column there was: i) steel mesh, ii) 7.62 cm gravel layer (#3/4 downgrade white
river gravel, Seffner Rock & Gravel, Tampa, FL), iii) another steel mesh wrapped with geotextile to prevent fines from clogging the gravel, iv) 30 cm of sand or sand amended with biochar and v) 20 cm free board for ponding (Appendix A: Figure A1). SSW was applied to the surface using a peristaltic pump (Masterflex L/S, Cole-parmer) connected to a feed container.

On the first day of the acclimation phase, 275 ml of supernatant from the Falkenburg Advanced Wastewater Treatment Plant, (FAWTP) Tampa, FL was added to each column as an inoculum. FAWTP carries out nitrification/denitrification in an oxidation ditch process. During days 1 to 4 of acclimation, column effluent was continuously recirculated to the top of the column to enhance biofilm growth (Ergas et al., 2010). N-species concentrations (NH₄⁺ and NOₓ) were measured every 24 hrs. During days 5 to 8, columns were fed 2500 ml of SSW#1 daily, without recirculation at a HLR of 0.73 cm³/cm²/min. Effluent TN concentrations were measured daily to gain insight into N-species transformations. When similar effluent concentrations of NH₄⁺, NOₓ and TN were achieved for at least two days, acclimation of the units was considered completed. The urban runoff phase investigated the effects of biochar addition on E. coli and N transformations at varying HLR and ADP (Table 1). Experiments were conducted at three different HLRs: i) low (0.098 cm³/cm²/min), ii) medium (0.18 cm³/cm²/min) and iii) high (0.25 cm³/cm²/min). To calculate the influent volumes, it was assumed that a bioretention cell would occupy 5% of the drainage area (Brown et al., 2011). For the low HLR, a 0.25-inch rainfall event over 3.5-hrs results in a HLR of 0.098 cm³/cm²/min, which scaled down to a flow rate of 240 ml/hr. For the medium and high HLRs, 0.5-inch and 1-inch rainfall events were considered, respectively, corresponding to 420 ml/hr and 600 ml/hr flow rates over 3.5 hours. Depending on the HLR, 2-5 pore volumes (PVs) of water were applied. Varying ADP experiments were
conducted at medium HLR (0.18 cm$^3$/cm$^2$/min) with 1, 3, 7 and 30 dry days between successive SSW events.

2.3.5 Water Quality Analysis

Influent and effluent samples were collected at 15-minute intervals during each SSW event. Effluent volume was measured gravimetrically to calculate mass load pollutant reductions. *E. coli* was enumerated using the membrane filter method with modified membrane-Thermotolerant Escherichia coli Agar (m-TEC; EPA Method 1603). Each sample was measured in duplicate at three dilutions in PBS. Total ammonia nitrogen (TAN: NH$_3$ + NH$_4^+$) and NOx (NO$_3^-$ + NO$_2^-$) were measured by the gas diffusion conductivity method with a Timberline Ammonia Analyzer (Timberline Instruments, Boulder, CO). TN and DOC were measured with a Shimadzu TOC-V CSH TOC/TN Analyzer (Shimadzu Scientific Instruments, Columbia, MD). DON was calculated by difference between TN and total inorganic nitrogen (TIN). Method detection limits for TAN, NOx, TN and DOC were 0.05mg/l, 0.05mg/l, 0.03 mg/l, and 0.11 mg/l, respectively.

2.3.6 Data Analysis

Concentrations of *E. coli* in feed solutions were measured at the start and end of each experiment to confirm that growth/death did not occur during the experimental period. Log removal values were calculated using the following:

$$E. coli \text{ Log Removal} = -\log_{10} \frac{C}{C_0} \quad \text{.......................................................... (i)}$$

where, $C_0$ and $C$ are the influent and effluent concentrations, respectively.

Pollutant mass removal (Rx) was calculated using the following:

$$R_X = \frac{\sum N C_0 V_0 - CV}{C_0 V_0 N} \quad \text{.......................................................... (ii)}$$

where, X refers to the pollutant (e.g., TAN); N is the total number of effluent samples; $V_0$ and V are the influent and effluent volume (L), respectively.
One-way analysis of variance (ANOVA) was performed with Tukey’s post-hoc test to identify statistically significant differences in measured parameters. Statistical analyses were considered significant level at $p < 0.05$.

2.4 Results and Discussion

2.4.1 Physicochemical Media Properties

Physicochemical properties of the biochars are shown in Table 2.4. Elemental analysis revealed that BC1 had lower molar ratios of H/C, O/C and O+N/C than BC2, indicating that BC2 could be more interactive with polar compounds than BC1. Similar results were observed in studies where higher pyrolysis temperatures increased aromaticity and lowered polarity of biochar (Suliman et al., 2016). BC1 had a higher pH than BC2, most likely because of the higher heating rate during pyrolysis and increased mineral ash content. Table 2.4 also shows that BC2 had a higher CEC than BC1. The higher pyrolysis temperature of BC1 could result in lower CEC due to the loss of volatile matter with negatively charged functional groups during pyrolysis (Mukherjee et al., 2011).

BC1 had almost four times more SA than BC2. Although both biochars were produced from wood chips, significant differences in SA can be attributed to increased carbonization at higher pyrolysis temperature for BC1. Prior research found similar results, where oak biochar SA increased by over 100 times from 2 to 225 m$^2$/g by increasing the pyrolysis temperature from 450$^\circ$ to 650$^\circ$ C (Mukherjee et al., 2011). Bulk densities of both biochars were within the range of published data.

2.4.2 Batch Study

Abiotic batch experiment results for three media materials (S, BC1 and BC2) for NH$_4^+$ and NO$_3^-$ adsorption are shown in Figure 1. Significantly higher NH$_4^+$ adsorption was observed for
biochar (3.5 mg/g) than sand (0.05 mg/g) due to higher CEC. However, differences between BC1 and BC2 were not significant (Figure 2.1). In contrast, both biochar and sand had very low NO₃⁻ adsorption capacity. BC1 had slightly higher NO₃⁻ adsorption (0.3 mg/g) compared with the other materials (~0.23 mg/g). Prior studies also showed limited NO₃⁻ adsorption by biochar, depending on biochar feedstock and pyrolysis temperature (Yao et al., 2012). Gai et al., (2014) found that washing the biochar with acid and DI water reduced the ash content and created more sites for NO₃⁻ adsorption due to protonation of the surface. NO₃⁻ leaching from unwashed biochar was also observed in a preliminary study in our laboratory (data not shown).

Figure 2.1: Batch experiment of NH₄⁺ and NO₃⁻ adsorption for Sand (S), Biochar 1 (BC 1) and Biochar 2 (BC 2)

2.4.3 Acclimation Phase

Effluent concentrations of TAN, NOx and TN for the three columns (S: Sand, SBC1: Sand with Biochar 1, and SBC2: Sand with Biochar 2) during the acclimation phase are shown in Table 2.1. After four days of recirculation, effluent TAN concentrations for S, SBC1 and SBC2 decreased (p<0.05) by 48%, 65% and 67%, respectively. The lowest effluent TAN concentration was observed with SBC2, most likely due to its higher CEC compared with SBC1. After four days of recirculation, NOx concentrations for S, SBC1 and SBC2 columns increased significantly.
(p<0.05) by 261%, 253% and 84%, respectively. The results indicate that nitrification was occurring in all columns and that simultaneous nitrification-denitrification was occurring in SBC2.

Table 2.1: Effluent N-species concentrations in three columns during the acclimation stage

<table>
<thead>
<tr>
<th>Day</th>
<th>With Recirculation (Day 1 to 4)</th>
<th></th>
<th>Day</th>
<th>Without Recirculation (Day 5 to 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TAN (mg/L-N)</td>
<td>NOx (mg/L-N)</td>
<td></td>
<td>TAN (mg/L-N)</td>
</tr>
<tr>
<td>S</td>
<td>SBC1</td>
<td>SBC2</td>
<td>SBC1</td>
<td>SBC2</td>
</tr>
<tr>
<td>1</td>
<td>6.6</td>
<td>7.21</td>
<td>6.69</td>
<td>1.09</td>
</tr>
<tr>
<td>2</td>
<td>6.0</td>
<td>5.98</td>
<td>4.31</td>
<td>1.54</td>
</tr>
<tr>
<td>3</td>
<td>5.9</td>
<td>4.85</td>
<td>3.64</td>
<td>2.08</td>
</tr>
<tr>
<td>4</td>
<td>3.7</td>
<td>2.54</td>
<td>2.20</td>
<td>3.93</td>
</tr>
</tbody>
</table>

Effluent TN concentrations were measured for next four days (days 5 through 8) with daily operation with SSW#1 without recirculation. During this period only TN was measured due to analytical constraints. Compared with the sand column, both biochar amended columns achieved greater TN removal (Table 2.1). Lower effluent TN concentrations in biochar amended columns were most likely due to development of saturated conditions, which favored denitrification.

2.4.4 Hydrological Aspect of Column Study

Bioretention geo-media are typically selected based on hydraulic conductivity and porosity to minimize overflow and peak flow during storm events (Davis 2008; Hsieh and Davis, 2005; Mahmoud et al., 2019). Although sand is common bioretention media, fine sand limits hydraulic conductivity. In addition, although sand has good porosity, it has poor water retention. Therefore, biochar could be an appropriate amendment because its pore structure increases storage (Table 2.4) and promotes nitrification and denitrification. Moisture content (MC) for the three columns (S, SBC1 and SBC2) for 7- and 30-day ADPs are shown in Figure 2. Media samples were collected from the surface, middle (15 cm from the surface) and bottom (30 cm from the surface) of each column. As expected, as ADPs increased, MC of all columns decreased due to drainage and evaporation. Evaporation increases air and oxygen availability in pore space, thus promoting
nitrification of adsorbed NH$_4^+$. MC data also revealed that saturated conditions prevailed toward the bottom of the biochar amended columns compared to the sand column, which persisted for several days (ADP7), thus promoting denitrification (Figure 2.2). Prior studies showed that an increase in biochar pyrolysis temperature from 300 to 500°C enhanced available MC of soil (Peake et al., 2014). Most likely, higher fraction of micropores in SBC1 retained more infiltrated water compared to meso- or macro-pores due to capillary, thus providing a higher MC even for an ADP of 30-days.

Figure 2.2: Moisture content at three different depths (i. surface, ii. mid depth: 15 cm from the top surface and iii. bottom depth: 30 cm from the surface) of the three columns (i. Sand, ii. SBC1 and iii. SBC2) for 7- and 30-days ADPs.

Figure 2.3 shows the average percent water retention for eighteen simulated storm events for the three columns. An overall comparison shows that biochar increased water retention compared with sand. The average water retention was 0.036, 0.059 and 0.053 g of infiltrated stormwater per gram of S, SBC1 and SBC2 media, respectively. Prior studies also showed improved water retention with biochar application to agricultural soils (Abel et al., 2013; Novak et al., 2012). According to Abel et al. (2013), addition of biochar to sand altered the bulk density and increased water retention. A comparison of SBC1 and SBC2 (Figure 3b) showed that BC1
had a higher water holding capacity (0.79 g of infiltrated water/g of biochar) compared with BC2 (0.37 g of infiltrated water/g of biochar), resulting in saturated media conditions. The higher pyrolysis temperature of BC1 increased its water holding capacity compared with BC2.

Prior studies showed inconsistent results regarding the effect of biochar amendment on hydraulic conductivity. Some studies have observed an increase (Ibrahim et al., 2013) while others reported a decrease (Uzoma et al., 2011) in hydraulic conductivity with biochar amendment. In this study, biochar amendment slightly reduced the hydraulic conductivity compared with sand (Appendix B: Figure B.1). This can be attributed to the moderate biochar application rate (<5%) and biochar particle sizes (0.25-0.15mm: 50% of total added biochar) used in this study. Similar findings were reported by Uzoma et al (2011) where addition of 0.5% and 0.9% (w/w) wood-derived biochar reduced the hydraulic conductivity of sand.

2.4.5 Phase 2 Average Water Quality Performances

Overall removal performances for TAN, NOx, DON, TN, DOC and E. coli for 18 stormwater experiments are shown in Table 2.5. Both biochar amended columns had significantly higher TAN removals compared with sand (p<0.05), indicating that biochar amendment enhanced
NH₄⁺ adsorption. Although BC2 had a higher CEC, TAN mass removals for biochar amended columns were not significantly different from each other (p<0.05), which is consistent with the batch experiments (Figure 1). The results indicate that 30% (v/v) (5% w/w) biochar amendment is adequate for TAN removal from urban stormwater. Prior studies (Ding et al., 2010; Tian et al., 2016) used higher fractions of biochar (10% w/w) and reported a range of TAN removals, from 15% to >90% (Tian et al., 2016). However, these were short-term batch experiments (Gai et al., 2014; Tian et al., 2016) or column studies (Tian et al., 2016; Yao et al., 2012) without acclimation. No significant differences were observed for overall NOx, DON or TN mass removals between columns. However, a slightly higher effluent NOx mass was observed for the biochar amended columns compared with the sand column (Table 4). This was likely due to the lower hydraulic conductivity (K) of the biochar amended columns, which reduces the flow velocity and increases HRT. The longer HRT provides greater opportunity for nitrifying bacteria to convert TAN to NOx. TAN adsorption by biochar also provides opportunities for nitrification during the ADP, which will be discussed later. Biochar addition also increased effluent pH (Biochar: 9.5-10.2, sand: 7.9), which favors nitrification. Similar results were observed by Case et al. (2012), where increasing pH values from 7.5 to 9 resulted in higher nitrification rates compared to un-amended sand.

DOC removals were significantly higher (p<0.05) in biochar amended columns than sand. Significantly higher DOC removal was observed in SBC1 than SBC2, most likely due to greater SA and pore volume of BC1. Prior studies showed leaching of DOC from biochar pyrolyzed at low/medium temperature (300-600°C), which resulted in nano- or micro-pore blockage (Ulrich et al., 2015). DON removal mainly occurs due to surface adsorption and ammonification; however, no significant differences were observed in DON removal between the three columns.
E. coli removals were significantly different in all three columns (p<0.05). Throughout the course of experiments, effluent E. coli concentrations from SBC1 were below detection limits (<30 CFU/100 ml), therefore log removals could not be calculated. SBC2 had an order of magnitude higher E. coli removal than S (4.23±0.61 vs. 3.43±1.05 log removal). The higher SA of SBC1 was the most likely reason for the increased E. coli attachment compared with SBC2 or sand. Similar results were reported by Mohanty and Boehm, (2014), where SA significantly influenced E. coli removal. Prior studies reported that surface adsorption of natural organic matter reduced E. coli removal (Mohanty et al., 2014, 2013); however, in this study high E. coli removal was observed in biochar amended columns despite the addition of DOC (oak leaf extract).

2.4.6 Effect of Volume Applied on Column

Effluent concentration profiles over time for the three columns for a 3.5-hour storm event with high HLR and 7-day ADP are shown in Figure 2.4. The upper horizontal scales show the approximate SSW pore volumes applied. Data were divided into two periods: 1) ≤1 PV (~46 min into the experiment) and 2) >1 PV until the end of the experiment (46 min to 210 min).

Higher TAN removal was observed in biochar amended columns than the sand column (Figure 2.4 (a)). During the first PV, SBC2 had lowest average effluent TAN concentration (0.022 mg/l) compared with SBC1 (0.071 mg/l) and S (0.041 mg/l). During this time period, it is likely that fresh infiltrating water displaced resident soil solution that was retained in the column during the ADP. An ADP of 7 days provided the time and oxygen required for nitrification in the unsaturated medium; therefore, effluent TAN concentrations were low even for the sand column. Once one pore water was displaced (>1-4.5 PV), both biochar amended columns showed significantly lower effluent TAN concentrations compared to the sand column due to its adsorption
onto biochar. Effluent TAN concentrations for SBC2 were close to zero for the rest of the experiment.

Effluent NOx concentrations largely depended on the activity of bacteria in the porous media, the porous structure of the media and ADPs between storms. In the first 46 minutes, the highest effluent NOx concentrations were observed in SBC2. Data from this time period revealed 134% export of NOx for SBC2, whereas SBC1 and S columns had 77% and 60% NOx export, respectively. Lower MC (25.7%) and water retention (0.37 g of water/gram of BC2) in SBC2 suggests that greater fractions of pores were filled with air, which replaced the drained water during ADP. This promoted the development of aerobic conditions during the 7-day ADP and enhanced nitrification in SBC2 compared with SBC1 (MC: 27.9% and water retention of BC1: 0.79 g of water/gram of BC1). Once the pore water was displaced by newly infiltrated stormwater (PV>1) NOx concentrations decreased; however, SBC1 continued to export NOx, most likely because adsorbed TAN continued to be nitrified due the greater number of meso- and micropores and favorable pH (9.9±0.22) compared to S (7.86±0.12) and SBC2 (8.68±0.17). During period 1, greater TN removal was observed with SBC1 compared with SBC2 and S columns. As discussed previously, higher water retention and MC of BC1 compared with BC2 promoted saturation and waterlogging, which favored denitrification during the ADPs. After one PV was applied, SBC2 had lower effluent TN concentrations than SBC1 due to formation of a thin saturated zone that was visible at the bottom of the column. Poor TN removal was observed in the S column because of lower NH4+ adsorption, lower MC and lower available DOC.

SBC1 and SBC2 showed greater *E. coli* removal than the S column throughout the experiment. Up to one PV, all three columns had effluent *E. coli* levels BDL. This suggests that seven days of ADP were adequate for *E. coli* die-off in the pore water. However, during period 2,
the S biofilter showed poor removal of *E. coli* compared to biochar amended columns, most likely because of the lower SA for *E. coli* attachment. Similar trends were observed for effluent DOC concentrations. Therefore, both *E. coli* and DOC treatment largely depends on the surface properties of the biochar.

![Figure 2.4](image-url)

**Figure 2.4** (a-e): Pollutant concentration profiles (a: TAN; b: NOx; c: DON, d: TN, e: *E. coli* and f: DOC) of S, SBC1 and SBC2 bioretention systems for 3.5 hours and 7 ADP stormwater experiment with a high HLR.
2.4.7 Effect of Hydraulic Loading Rate

The effect of varying HLRs on N-species, *E. coli* and DOC mass removal are shown in Figure 2.5. Experiments were carried with a 3-day ADP to eliminate the effect of this variable. Statistical significance was analyzed by i) comparison of each treatment system for three HLRs and ii) comparison among the three systems for each HLR (Appendix B: Table B.2).

TAN removal significantly decreased (p<0.05) from 98.5% to 67% for the sand column with increasing HLR. Increasing HLR increases the flow velocity and shortens the HRT in the column, reducing the opportunity for nitrification of TAN to NOx. In contrast, both biochar amended columns showed similarly high TAN removals without significant change in performance with increasing HLR (Figure 2.5). Therefore, biochar amended bioretention could be an appropriate choice for TAN removal, even at high HLR, because of its high ammonium adsorption capacity.

Significantly higher NOx export was observed at high HLR compared with low or medium HLR for all columns. In contrast, Lopez-Ponnada et al., (2020) observed that HLR had no significant effect on NOx removal in field studies with a conventional bioretention unit with sand media. Comparison among HLRs for SBC1 showed higher NOx removal (28%) at medium HLR and higher export (-47%) at high HLRs, respectively, compared to SBC2 (NOx removal: 11% and NOx export: -42.6%). Mineralization of DON can possibly lead to higher NOx export.

DON removal significantly decreased for both SBC1 (61% to 27%) and SBC2 (56% to 33%) columns with increasing HLR. The longer HRT during the low HLR experiments probably led to greater adsorption and mineralization of DON for SBC1 and SBC2, resulting in greater DON removal compared to the high HLR. In contrast, DON export was observed from the S column
during the high HLR experiment, possibly due to sloughing of microbial biomass. Competition between DON and DOC adsorption could also result in lower DON removal at high HLR.

**1st and 2nd superscripted letters represent significantly different (p < 0.05) considering consequence of each treatment system for different HRTs and effect of each HLR on different treatments, respectively, using Tukey tests.**

Figure 2.5: Effect of high, medium and low HLRs on sand (S), sand with Biochar 1 (SBC1) and sand with Biochar 2 (SBC2) biofilters for removal of a) TAN, b) NOx, c) DON, d) TN, e) DOC and f) *E. coli* different columns for contaminants removal
DOC removal significantly decreased from 74% to 67% for the sand column at high HLRs. Interestingly, the best DOC removal was observed for biochar amended columns at medium HLR, possibly due to better mass transfer of DOC to the biochar surface at higher liquid velocity.

Comparison of *E. coli* removal for each system at varying HLRs showed that only the S column had significantly (p=0.034) lower *E. coli* removal with increasing HLRs. *E. coli* removals were not significantly affected by HLR in biochar amended columns (p=0.075). Effluent *E. coli* concentrations from SBC1 were < 30 CFU/100 ml (BDL) regardless of HLR. Effluent *E. coli* concentrations increased at medium and high HLR for SBC2, indicating that greater contact time favored adsorption of *E. coli* to the media surface for the less hydrophobic material.

2.4.8 Effect of Antecedent Dry Period

A summary of the effect of ADPs on N-species, DOC and *E. coli* mass removal efficiency is provided in Table 2.2. Figures 2.6 and 2.7 show breakthrough curves for S and SBC2 for 3, 7 and 30-days ADPs during 3.5-hour storm experiments. The performance of SBC1 and SBC2 were similar, therefore, only SBC2 is compared with S. All experiments were performed at an HLR of 0.18 cm³/cm²/min to eliminate the effect of this variable.

TAN removal did not significantly differ for S, SBC1 and SBC2 columns with varying ADPs, as shown in Table 2.2. However, NOx removals varied significantly with ADP for biochar amended columns. This is likely due to the combined effect of media saturation and nitrifying and denitrifying microbial activity during the ADPs. In general, shorter ADP led to NOx removal and longer ADPs resulted in NOx export. Media moisture content remained high during a 3-day compared to the 7- or 30-day ADPs (Figure 2.2), which led to greater NOx removal (Table 2.2). This was likely due to depletion of oxygen from water-logged zones at the bottom of the column, which favors denitrification. At longer ADPs (7 and 30 days) the column had time for water to
drain and pores to fill with air, leading to additional nitrification and NOx export. In contrast, a prior study of biochar amended bioretention by Nabiul Afrooz and Boehm (2017) observed NOx removal (35%-40%) rather than export for 3- and 7-day ADPs. Prior studies have shown that biochar addition stimulates nitrification in agricultural fields (Prommer et al., 2014).

Table 2.2: Effect of ADPs on mass removal efficiency for N-species, DOC and *E. coli* for S, SBC1 and SBC2.

<table>
<thead>
<tr>
<th>ADPs</th>
<th>Media</th>
<th>TAN (%)</th>
<th>NOx (%)</th>
<th>DON (%)</th>
<th>TN (%)</th>
<th><em>E. coli</em> (%)</th>
<th>DOC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-day</td>
<td>S</td>
<td>87.3± 6.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>17.4± 24.9&lt;sup&gt;ba&lt;/sup&gt;</td>
<td>43.7± 11.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>41.4± 14.7&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>99.7± 0.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>77.8± 5.4&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBC1</td>
<td>98.7± 2.1&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>15.3± 7.9&lt;sup&gt;ba&lt;/sup&gt;</td>
<td>61.0± 3.6&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>50.1± 2.9&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>100± 0&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>92.3± 3.5&lt;sup&gt;aa&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>SBC2</td>
<td>98.9± 1.8&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>11.0± 11.2&lt;sup&gt;ba&lt;/sup&gt;</td>
<td>55.6± 8.6&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>46.4± 7.4&lt;sup&gt;ba&lt;/sup&gt;</td>
<td>99.9± 0&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>90.2± 1.2&lt;sup&gt;aa&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>7-day</td>
<td>S</td>
<td>72.5± 30.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>-49.1± 75.3&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>-66.6± 161.5&lt;sup&gt;ba&lt;/sup&gt;</td>
<td>30.3± 16.4&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>99.6± 0.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>77.8± 6.2&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBC1</td>
<td>98.5± 2.9&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>-104.4± 37.0&lt;sup&gt;bb&lt;/sup&gt;</td>
<td>-45.8± 55.5&lt;sup&gt;ba&lt;/sup&gt;</td>
<td>32.7± 7.9&lt;sup&gt;ba&lt;/sup&gt;</td>
<td>100± 0&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>96.6± 0.4&lt;sup&gt;bb&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>SBC2</td>
<td>98.9± 2.1&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>-38.6± 4.0&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>-24.8± 48.9&lt;sup&gt;ba&lt;/sup&gt;</td>
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<td>90.4± 1.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>30-day</td>
<td>S</td>
<td>77.0± 30.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>-28.9± 43.3&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>45.9± 15.3&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>41.2± 19.1&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>99.9± 0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>75.1± 7.9&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBC1</td>
<td>99.8± 0.4&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>-47.8± 23.8&lt;sup&gt;ba&lt;/sup&gt;</td>
<td>41.9± 9.3&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>44.3± 6.4&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>100± 0&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>93.7± 1.3&lt;sup&gt;aa&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>SBC2</td>
<td>99.9± 0.2&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>-48.5± 24.1&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>48.8± 9.5&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>48.3± 6.8&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>99.9± 0&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>89.5± 1.6&lt;sup&gt;aa&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

**1<sup>st</sup> and 2<sup>nd</sup> superscripted letters represent significantly different (p < 0.05) considering consequence of each treatment system for different ADPs and effect of each ADP on different treatments, respectively, using Tukey tests.**

No significant differences were observed for DOC removal with varying ADPs. This may be due to bio regeneration of biochar adsorption capacity or diffusion of DOC into internal pores during the ADP. Prior studies found either no or limited DOC removal in biochar amended bioretention systems due to pore blockage (Nabiul Afrooz and Boehm, 2017). *E. coli* removal also did not significantly vary with ADP for any of the systems.

N-species and *E. coli* breakthrough profiles for S and SBC2 columns for 3-, 7- and 30-day ADPs are shown in Figure 2.6 and Figure 2.7. Removal efficiency varied with the number of PVs
of water applied, as discussed in Section 3.6. A comparison between ADP7 and ADP30 for S and SBC2 shows that significantly higher NOx export was observed during the first PV of water applied (approximately the first 75 minutes) regardless of the media type due to nitrification of adsorbed TAN during the drainage phase; once the first PV was displaced with fresh water, the effluent solution was enriched with NOx. Greater NOx export from the S column was most likely due to aerobic conditions during the ADPs. Subramaniam et al. (2016) also noted that the effect of ADPs on NO$_3^-$ removal was more pronounced during the first PV and that denitrification mainly occurred during the drainage phase rather than the infiltration phase only if a water-logged zone was present during the ADP. There was no clear pattern of DON removal with three different ADPs. However, export of DON during the first PV was observed, which might be due to desorption of previously adsorbed DON or sloughing of biomass that died during the longer ADP (ADP30).

A comparison between S and SBC2 columns showed higher E. coli log removal for biochar amended column for 3, 7 and 30-day of ADPs. Higher E.coli log removal was observed for ADP7 for SBC2 column compared with ADP3 and ADP30. No significant differences were observed for S column for three different ADPs. The higher die-off rate or competition among microbes led to greater E. coli log removal for ADP7 in biochar amended sand column. Moreover, 1st PV water showed greater log removal in biochar amended column (SBC2) compared with sand based bioretention system due to unfavorable conditions and greater competition among the microbes led higher die-off.
Figure 2.6: Pollutant concentration profiles (a: TAN; b: NOx; c: DON) of S (Left) and SBC2 (Right) bioretention systems for 3-, 7- and 30-day ADPs stormwater experiments with 3.5-hour storm events at medium HLR.
Figure 2.7: Pollutant concentration profiles (d: TN, e: DOC and f: *E. coli*) of S (Left) and SBC2 (Right) bioretention systems for 3-, 7- and 30-day ADPs stormwater experiments with 3.5-hour storm events at medium HLR
2.5 Conclusions

Nitrogen and *E. coli* removal mechanisms were investigated in sand bioretention columns, with and without biochar amendment. Biochar addition increased TAN adsorption during the wetting period, providing substrate for nitrification during the drainage period. The higher moisture content and presence of adsorbed DOC in biochar amended columns favored denitrification, resulting in higher TN removals. *E. coli* removal was a strong function of SA; greater than 6 log *E. coli* removal was observed in the column amended with high SA biochar. Biochar addition also resulted in more stable effluent N-species and *E. coli* concentrations despite variations in HLR and ADP.

This research addressed the effect of biochar amendment for urban runoff management. Future works should be carried out to investigate the effect of different biochar amendment rates on N-species and FIB removal from different non-point sources pollution mitigation. Also, biochar has higher moisture content, therefore, adding different amendment rates can also behaves differently for hydrologic aspects of the systems. Moreover, different runoff has different influent concentrations. Therefore, future research should be conducted to observe the effect of more complex runoff influents on biochar amendment systems.
Table 2.3: Target synthetic stormwater (SSW) chemical composition and operating conditions during each experimental phase

<table>
<thead>
<tr>
<th>Phase</th>
<th>Chemical Composition</th>
<th>Operating conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>NH₄⁺ (mg/l)</strong></td>
<td><strong>NO₃⁻ (mg/l)</strong></td>
</tr>
<tr>
<td>SSW#1: Acclimation</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>SSW#2: Urban stormwater</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2.4: Physicochemical characterization of two biochars used in this study.

<table>
<thead>
<tr>
<th>Biochar</th>
<th>Organic carbon (%)</th>
<th>Ash (%)</th>
<th>H/C</th>
<th>O/C</th>
<th>(O+N)/C</th>
<th>pH</th>
<th>CEC cmol/kg</th>
<th>SA m²/g</th>
<th>Mesopore cc/g</th>
<th>Micropore cc/g</th>
<th>Pore Volume cc/g</th>
<th>BD g/cc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochar 1</td>
<td>80.1</td>
<td>5.8</td>
<td>0.56</td>
<td>0.09</td>
<td>0.09</td>
<td>10.12</td>
<td>10.57</td>
<td>537</td>
<td>0.15</td>
<td>0.19</td>
<td>0.36</td>
<td>0.10</td>
</tr>
<tr>
<td>Biochar 2</td>
<td>81.7</td>
<td>1.2</td>
<td>0.7</td>
<td>0.106</td>
<td>0.11</td>
<td>8.5</td>
<td>13.63</td>
<td>136</td>
<td>0.062</td>
<td>0.061</td>
<td>0.13</td>
<td>0.19</td>
</tr>
</tbody>
</table>

*CEC: Cation Exchange Capacity; SA: Surface Area; BD: Bulk Density
Table 2.5: Overall mass contaminant removal performance for the three columns

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TAN</td>
<td>1.00 ± 0.4</td>
<td>78.3 ±13.1a</td>
<td>76.3</td>
<td>6.7</td>
<td>99.2 ± 1.3b</td>
<td>100</td>
<td>0.2</td>
<td>99.5 ± 0.7b</td>
<td>99.9</td>
<td>0.1</td>
</tr>
<tr>
<td>NOx</td>
<td>0.98 ± 0.5</td>
<td>-14.5±(-41.2)d</td>
<td>-0.6</td>
<td>17.6</td>
<td>-36.7 ±(-62.8)d</td>
<td>-13.3</td>
<td>20.2</td>
<td>-27.9 ±(-47.2)d</td>
<td>-12.2</td>
<td>19.4</td>
</tr>
<tr>
<td>DON</td>
<td>1.16 ± 0.2</td>
<td>41.0 ±20.5d</td>
<td>38.6</td>
<td>12.5</td>
<td>46.8 ± 26.5^d</td>
<td>43.9</td>
<td>11.8</td>
<td>50.2 ± 24.9^d</td>
<td>52.4</td>
<td>11.2</td>
</tr>
<tr>
<td>TN</td>
<td>3.14 ± 0.4</td>
<td>39.4 ±10.1d</td>
<td>39.3</td>
<td>36.9</td>
<td>44.6 ± 14.1^d</td>
<td>40.5</td>
<td>31.6</td>
<td>47.6 ± 11.6^d</td>
<td>46.3</td>
<td>30.4</td>
</tr>
<tr>
<td>DOC</td>
<td>19.93 ± 8.1</td>
<td>74.7 ±10.8a</td>
<td>75.1</td>
<td>91.5</td>
<td>92.5 ± 5.1b</td>
<td>93.4</td>
<td>22.4</td>
<td>86.3 ± 7.8c</td>
<td>88.9</td>
<td>39.1</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>2.24x10^7 ± 1.80x10^7</td>
<td>3.4 ±1.1a</td>
<td>3.0</td>
<td>N/A</td>
<td>BDL</td>
<td>-</td>
<td>N/A</td>
<td>4.2± 0.6c</td>
<td>4.3</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Values across a row followed by a different superscripted letter are significantly different (p < 0.05) among the three columns using Tukey tests.
BDL=Below Detection Limit.

*E. coli* influent concentration unit: CFU/100 ml; Mass Removal and median in log10 scale.
*S*: Sand column; SBC1: Sand amended Biochar 1 column; SBC2: Sand amended Biochar 2 column
Chapter 3: Water Quality and Hydraulic Performance of Biochar Amended Biofilters for Management of Agricultural Runoff

3.1 Abstract

This research evaluated the effect of biochar amendment rate on nitrogen species and organic carbon removals and hydraulic performance in biofilter columns treating dairy farm runoff. Initial studies compared the performance of sand columns amended with two types of biochar with different specific surface area (SA) and cation exchange capacity (CEC) with an un-amended sand column. The results showed that biochar enhanced N-species removal due to its unique physicochemical properties. In subsequent tests, two biofilter columns with different biochar fractions (20% and 50% by volume) were operated at varying hydraulic loading rates and antecedent dry conditions. Total nitrogen, ammonia, organic nitrogen and organic carbon removals were significantly higher in the column with the higher biochar fraction. The high CEC of biochar increased ammonium retention during the application period, allowing for nitrification during the antecedent dry periods (ADPs) when aerobic conditions developed in the media pores. High biochar SA also resulted in greater retention of DON and DOC by adsorption. A variable saturation flow model of biochar amended biofiltration was developed using HYDRUS-1D software. The model was calibrated using data from conservative tracer and moisture content studies. Model results showed that the high microporous structure of the biochar increases the time needed to reach full saturation, lowers the saturated conductivity and increases the hydraulic retention time.
in the medium. This calibrated model can be used to design field scale biofilter systems for managing agricultural runoff.

3.2 Introduction

Excess nutrients in agricultural runoff are a major worldwide cause of eutrophication, resulting in algal blooms, fish kills, loss of biodiversity and nitrate contamination of drinking water sources (Lian et al., 2019; Islam et al., 2018). Compared with other non-point nutrient sources as discussed in chapter 2, such as urban, forestry and highway runoff, livestock waste runoff contributes high levels of nitrogen and phosphorus to receiving waters (Kato et al., 2009; Boesch et al., 2001). On dairy farms, nutrient rich runoff is generated from uncovered manure stockpiles, deposits of urine and fecal wastes and milking and cleaning operations (Lansing & Martin, 2006; Wright and Graves, 1998). Characteristics of dairy runoff depends on several factors, including farm size, weather conditions and manure management practices (Kato et al., 2009; Longhurst et al., 2000).

In the US, livestock manure is typically managed by stabilization in anaerobic lagoons followed by irrigation reuse or direct effluent discharge. Bulk manure treatment strategies include composting, aerobic lagoons, chemical precipitation or anaerobic digestion (Kato et al., 2009; Horn et al., 1991), whereas passive strategies, including vegetated buffer strips and constructed wetlands, are becoming common for manure runoff treatment (Liang et al., 2020; Loannidou et al., 2020; Koelsch et al., 2006; Hay et al., 2006). Although passive approaches remove organic matter and suspended solids, nutrient removal is usually low due to high organic loading rates and short hydraulic retention times (HRTs), which is the period of time for influent solution to pass through the treatment medium (Mantovi et al., 2003). In addition, these systems are often not designed with the alternating aerobic/anaerobic zones needed to promote biological nitrogen
removal. Ibekwe et al. (2013) reported that constructed wetlands, in California removed only 16% of ammonium (NH$_4^+$) and 33% of phosphate (PO$_4$(-III)). Similarly, leaching of Total Nitrogen (TN) (> 20 mg/L) and nitrate (NO$_3^-$) (1-45 mg/L) was observed in vegetated buffer strips due to insufficient nitrification and denitrification (Larson & Safferman, 2012; Hubbard et al., 1999).

Biofiltration and bioretention systems are passive treatment approaches that can enhance nutrient removal from agricultural and urban runoff (Lopez-Ponnada et al., 2020; Hunt et al., 2012; Ergas et al., 2010; Hsieh et al., 2007). Biofilters are packed bed reactors that contain natural or synthetic porous media materials, such as sand, wood chips or polyethylene media, which support microbial biofilms. Bioretention systems are similar to biofilters but include a planting medium and vegetation that supports pollutant transformations by plants and rhizosphere communities. An internal water storage zone (IWSZ) containing a solid electron donor, such as wood chips, is sometimes added to biofilters or bioretention systems to promote denitrification and TN removal (Lopez-Ponnada et al., 2017; Dietz, 2016). As discussed in Chapter 2, prior field and laboratory studies with urban runoff have shown that biofiltration and bioretention systems achieve significantly higher N-species removal than passive water quality treatment approaches (Lopez-Ponnada et al., 2020; Mahmoud et al., 2019; L. Li & Davis, 2014; Hunt et al., 2012). In Chapter 2, biofilter performance was evaluated on low influent concentration of N-species, therefore, further research was designed to observe the effect of high influent concentrations of N-species, similar to dairy runoff, in this Chapter.

Only a few investigators have studied biofiltration or bioretention systems for management of livestock runoff. Ergas et al. (2010) conducted a field study in Connecticut with bioretention systems containing IWSZs for treating dairy farm runoff. Average TN removal efficiencies of 65% were reported with influent TN concentration of 79 mg/L. Inclusion of an IWSZ containing wood
chips resulted in almost complete denitrification, however, inadequate ammonification of dissolved organic nitrogen (DON) and nitrification limited TN removal. In addition, at high hydraulic loading rate (HLR) effluent water quality declined due to low HRT. A similar TN mass removal efficiency (62%) was reported by Dietz (2016) for biofilter systems treating runoff from grass and corn storage with a lower mean influent TN concentration (12 mg/L). The author also observed reduced infiltration capacity resulting in ponding due to surface accumulation of particulate matter from silage. A long-term field study of aerobic woodchip biofilters treating dairy wastewater achieved relatively stable effluent TN concentrations (153 ± 24 mg/L) despite fluctuating influent concentrations (357 ± 100 mg/L) (Ruane et al., 2011). Intermittent dosing of wastewater at a HLR of 0.0021 cm$^3$/cm$^2$/min resulted in 72% NH$_4^+$ removal, 68% DON removal and 74% NO$_3^-$ export. Based on prior research outcomes, TN removal in bioretention systems and biofilters treating livestock waste runoff is limited by ammonification, NH$_4^+$ adsorption, nitrification and denitrification. Hence, additional research is needed to improve the performance of biofilters systems for treatment of livestock waste runoff.

Biochar is a low-cost carbon rich by-product of pyrolysis of waste organic feedstocks, such as wood waste or animal waste, under oxygen limited conditions. Biochar has been widely studied as an amendment for agricultural soils. In crop studies, biochar amendment has been shown to help retain moisture, nutrients and organic carbon, and enhance microbial activity, nitrogen fixation and plant growth (Lehmann et al., 2011; Omondi et al., 2016). These enhancements are largely due to the high cation exchange capacity (CEC), specific surface area (SA) and microporous structure of biochar presented in prior Chapter.

Several prior studies have evaluated the use of biochar to enhance biofilter or bioretention performance with urban stormwater (Rahman et al., 2020; Nabiul Afrooz & Boehm, 2017; Tian et
al., 2016). As discussed in Chapter 2, incorporation of biochar into sand-based biofilter media increased adsorption of NH$_4^+$, DON and dissolved organic carbon (DOC) during storm events. During the antecedent dry periods (ADPs), or time between successive storm events, drainage replenished the media pore space with air, facilitating nitrification of adsorbed NH$_4^+$ to NO$_3^-$. The high moisture holding capacity (MC) and adsorbed DOC in biochar amended biofilter columns promoted denitrification in wet zones near the bottom of the columns even with no IWSZ (Rahman et al., 2020). This resulted in higher TN removals in biochar amended columns than in an un-amended control. As discussed in Chapter 2, biochar properties significantly enhanced the N-species removal from stormwater runoff, therefore, in this Chapter the research was designed to observe the effect of different biochar amendment rates on complex dairy runoff.

The fraction of biochar in the biofilter media, or biochar amendment rate, potentially influences the moisture content, unsaturated hydraulic conductivity (K) and saturated hydraulic conductivity (K$_s$) of the medium. Although prior studies of biochar amendment rate in biofilters have not been carried out, crop production studies and also from Chapter 2 outcomes showed a positive correlation between MC and biochar amendment rates (Rahman et al., 2020; Githinji, 2014; Laird et al., 2010). Githinji et al. (2014) compared 100% biochar with non-amended sandy loam and found increased porosity (56%) and volumetric water content (60%) but decreased K$_s$ (63.3%). A meta-data analysis carried out by Omondi et al. (2016) showed that when biochar was added to soil, mean K$_s$ increased by ~25%. A maximum increase in K$_s$ of 86.8% was observed at biochar amendment rates >80 tonne/hectare. However, the effects on K$_s$ were insignificant at low (<20 t/ha) or moderate (21-40 t/ha) biochar amendment rates. Ibrahim et al. (2013) observed similar results for low biochar amendment rates (0.5-2% by wt) in sandy soil. Liu et al. (2016) studied the effect of amendment rates (0-10% by wt) of fine (<0.85mm) and coarse (>0.25 mm)
biochar in soil columns and showed that K decreased by 72 ± 3% and 15 ± 2%, respectively. Filling of pore throat spaces likely caused the reduction in K observed when soil was amended with fine biochar particles. Whereas coarse biochar created a compact mixture due to its bimodal size distribution. Therefore, it is important for practitioners to understand the effect of biochar amendment rate on biofilter hydraulic performance.

The overall goal of this research was to evaluate the effect of biochar amendment rates on nitrogen species and organic carbon removal and hydraulic performance in biofilters treating dairy runoff. Specific objectives were to: i) compare water quality performance of sand media amended with two commercial biochars with different CECs and SA, ii) investigate the effect of biochar amendment rate (20% and 50% by volume) on water quality performance, and iii) evaluate the impact of two amendment rates on hydraulic properties through both experimental and modelling studies. This is the first published study to investigate the use of biochar amended biofilters for managing nutrients from dairy runoff.

3.3 Materials and Methods

This research was performed in three phases (Table 3.1). In Phase I, side-by-side experiments were carried with three columns (sand only, sand + biochar 1, sand + biochar 2) to compare the performance of biofilters with and without biochar and to select an appropriate commercial biochar for dairy runoff treatment. Both of the biochar feedstocks were woodchips, which were pyrolyzed at 700-1000 °C. During Phase II, the water quality performance of the selected biochar from Phase I was evaluated at two amendment rates. During Phase III, four consecutive experiments were performed to evaluate the effect of ADP on water quality. Finally, in Phase IV, the hydraulic performance of the amended columns from Phase II was evaluated
through tracer studies, moisture content measurements and variable saturation flow modelling using HYDRUS-1D software.

Table 3.1: Operating conditions during each experimental phase

<table>
<thead>
<tr>
<th>Phase</th>
<th>Purpose of Study</th>
<th>No of Experiments</th>
<th>Flow Rate (mL/hr)</th>
<th>HLR (cm³/cm²/min)</th>
<th>Duration (hr)</th>
<th>Runoff Volume (mL)</th>
<th>ADP (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td>Biochar selection</td>
<td>4</td>
<td>420</td>
<td>0.18</td>
<td>3.5</td>
<td>840</td>
<td>7</td>
</tr>
<tr>
<td>Phase II</td>
<td>Biochar amendment rate comparison - water quality</td>
<td>9</td>
<td>255-420</td>
<td>0.1-0.18</td>
<td>3.5</td>
<td>840-1,470</td>
<td>3-30</td>
</tr>
<tr>
<td>Phase III</td>
<td>Effect of ADP in consecutive experiments</td>
<td>4</td>
<td>255</td>
<td>0.1</td>
<td>3.5</td>
<td>840</td>
<td>7</td>
</tr>
<tr>
<td>Phase IV</td>
<td>Biochar amendment rate comparison - hydraulics</td>
<td>3</td>
<td>420</td>
<td>0.18</td>
<td>4</td>
<td>1,680</td>
<td>7</td>
</tr>
</tbody>
</table>

3.3.1 Materials

3.3.1.1 Porous Media

Methods for characterizing biochar and sand properties are described in detail in Rahman et al. (2020). Briefly, the sand medium in this study was selected based on hydraulic conductivity (13.2 cm/hr), which was within the recommended guideline for biofilter and bioretention systems (Prince George’s County Program and Planning Division, 2007). Biochar 1 was acquired from Biochar Supreme (Everson, WA), and had a high SA (537 ± 60.15 m²/g) and moderate CEC (10.57 cmol/kg). Biochar 2 was obtained from Biochar Now (Loveland, CO), and had a moderate SA (136 ± 45.51 m²/g) and higher CEC (13.63 cmol/kg) than biochar 1. For the sand medium, effective particle sizes for D10, D50 and D90 were 0.21 mm, 0.38 mm and 1.2 mm, respectively. In brief, 15% of particles were retained in 1 mm, 20% in 0.6-0.42 mm, 50% in 0.25 and 15% in 0.15 mm
sieve openings. Similarly, effective particle sizes for biochar were 0.18mm, 0.32mm and 0.6 mm for D10, D50 and D90, respectively. For both biochars, 15% particles were retained in 0.6-0.42mm, 70% in 0.25mm, 10% in 0.15mm and 5% in 0.075 mm sieve openings.

3.3.1.2 Dairy Runoff Preparation

Prior to each experiment, fresh dairy manure was collected from South Tampa Farm, Tampa, FL and transported to the University of South Florida’s Environmental Engineering Laboratory. After collection, the manure was diluted with tap water (30% by volume), the solution was allowed to settle overnight, and the supernatant was screened through a 0.25 mm mesh. The influent was prepared by mixing 60% of the screened supernatant with 40% tap water to achieve a target influent N-species concentration of 45 mg DON/L, 35 mg NH₄⁺-N/L and 1.0 mg NOx/L (NOx = NO₂⁻-N + NO₃⁻-N). These values were consistent with concentrations observed in prior field studies of agricultural/feedlot runoff (Ergas et al., 2010; Koelsch et al., 2006; Vadas & Powell, 2019; Andrew et al., 1992).

3.3.2 Experimental Program

3.3.2.1 Phase I: Biochar Selection

Three columns were constructed from 2-liter Koflo (Cary, IL) calibration columns with an inside diameter of 7.2 cm and a height of 50 cm. Each column was filled with 7.2 cm coarse gravel at the bottom and 30 cm filtration medium (sand or sand-biochar mixture). There was 20 cm of free board at the top of the media to allow for ponding. Three different filtration mixtures were used: 1) Sand (S); 2) Sand with Biochar 1 (SBC1); and 3) Sand with Biochar 2 (SBC2) (Supporting information: SI). Both SBC1 and SBC2 were filled with 30% biochar with 70% sand (by volume), which resulted in mass fraction of 3.1% and 4.75 %, respectively. Dairy runoff was applied at the
top of each column using a peristaltic pump (Masterflex L/S, Cole-parmer). During Phase I, an ADP of 7-days and a HLR of 0.18 cm$^3$/cm$^2$/min were used (Table 3.1).

3.3.2.2 Phase II: Effect of Amendment Rate and ADP on Water Quality

Based on Phase I results, biochar 1 was selected for Phase II experiments. Two of the columns described previously were amended with 20% and 50% biochar (by volume) with sand. This resulted in biochar mass fractions of 2.2% and 5.7% for SBC-20 and SBC-50, respectively. To seed the reactors with established microbial biofilms, the sand used in Phase II was recycled from the Phase I sand column. Nine experiments were initially performed at a constant HLR (0.10 cm$^3$/cm$^2$/min) and ADP (4-days). Subsequently, triplicate experiments were performed at ADPs of 4, 10, and 30-days at a constant HLR of 0.10 cm$^3$/cm$^2$/min.

3.3.2.3 Phase III: Effect of ADP in Consecutive Experiments

To observe the effect of ADP on two biochar fraction columns, four consecutive experiments were performed with HLR of 0.10 cm$^3$/cm$^2$/min and 7-days of ADP. After completion of nine experiments in Phase II, same two columns (SBC-20 and SBC-50) were used to evaluate the effect of ADP. Similar concentration as Phase II were applied in these experiments.

3.3.2.4 Phase IV: Effect of Amendment Rate on Hydraulic Performance

Conservative tracer studies were conducted to evaluate the influence of biochar amendment rate on HRT. Each study was carried in duplicate at a HLR of 0.18 cm$^3$/cm$^2$/min and an ADP of 7-days. The influent was prepared by adding 500 mg/L potassium chloride (KCl) to deionized water. Effluent samples were collected over time and the flow rate was measured gravimetrically.

3.3.3 Analytical Methods

Effluent samples were collected at 15 to 30-minute intervals during each experiment. A multi-parameter meter was used to measure pH and conductivity (Standard Methods, 2018; 2510
B). Total ammonia nitrogen TAN= NH₃ + NH₄⁺ and NOx= NO₃⁻ + NO₂⁻ were measured using a Timberline Ammonia Analyzer (Timberline Instruments, Boulder, CO). Total Nitrogen (TN) and total organic carbon (TOC) were measured with a Shimadzu TOC-V CSH Total Organic Carbon/Total Nitrogen Analyzer (Shimadzu Scientific Instruments, Columbia, MD). DON was calculated by subtracting total inorganic nitrogen (TIN) from TN. The method detection limits for TAN, NOx, TN and TOC were 0.05 mg/L, 0.05 mg/L, 0.03 mg/L, and 0.11 mg/L, respectively. Concentrations of anions and cations in the influent and effluent were measured using a Metrohm Peak 850 Professional AnCat ion chromatography (IC) system (Metrohm Inc., Switzerland). Samples of media material were collected for moisture content analysis (ASTM D2216) through sample ports at the: i) top of the media, ii) 10.16 cm depth, iii) 20.32 cm depth, and iv) bottom of the media.

3.3.4 Mathematical Model and Data Analysis

Because columns undergo cycles of wetting during infiltration, followed by periods of drainage during ADP, the flow was simulated with variable saturation flow model. HYDRUS-1D software simulates one dimensional vertical water and solute movement for various conditions including fixed or variable head, constant or variable flux and for atmospheric boundary conditions. The MC of the media varies with time and column depth and depends on media hydraulic properties, HLR, duration of drainage phase, and evaporation. The model solves Richard’s equation for water flow and neglects the effects of air flow. The K and soil MC are two non-linear functional coefficients for the Richard’s equation which have limited exact solutions for the realistic flow geometries. Therefore, numerical solution was used for the application of HYDRUS-1D model. The van Genuchten model (van Genuchten, 1980), a widely used soil hydraulic model, was selected for the simulation, and is formulated as:
\( (h) = \theta_r + \frac{\theta_s - \theta_r}{1 + |\alpha h|^n} m, \quad (h < 0) \quad \text{Or} \quad \theta_s, \quad (h \geq 0) \) .......................................................... (i)

\[ K(h) = K_s S_e l \left[ 1 - \left( 1 - S_e^l \right)^m \right]^2 \] .......................................................... (ii)

where \( \theta \) (h) is the moisture content (cm\(^3\)/cm\(^3\)) as a function of capillary pressure head \( h \) (cm), \( \theta_s \) and \( \theta_r \) are saturated and residual MC, \( K_s \) the saturated hydraulic conductivity (cm/min), \( S_e = \frac{\theta - \theta_r}{\theta_s - \theta_r} \) is the effective water content, \( l \) is the connectivity of the media pores, \( n \) is the index of pore volume size distribution, and \( \alpha \) and \( m \) are empirical parameters affecting the shape of the hydraulic functions.

Statistical analysis was conducted using one-way analysis of variance (ANOVA) with Tukey’s post-hoc test to check for significance with p-value <0.05. Pollutant mass removal (Rx) was calculated using the following:

\[ R_X = \sum_1^n \frac{c_0 v_0 - c v}{c_0 v_0} \] .......................................................... (iii)

\( X \) refers to the pollutant (e.g., TAN); \( n \) is the total number of effluent samples; \( C_0 \) and \( C \) are the influent and effluent concentrations, respectively, \( V_0 \) and \( V \) are the influent and effluent volume (L), respectively.

3.4 Results and Discussions

3.4.1 Phase I: Biochar Selection

A summary of influent and effluent DON, TAN and NOx concentrations for four experiments carried out in Phase I is shown in Figure 3.1. For the semi-simulated dairy runoff, average influent N-species concentrations were DON 70.26±50.54 mg-N/L, TAN 37.41±6.58 mg-N/L and NOx 0.88±1.05 mg-N/L, resulting in a TN concentration of 108.68±46.56 mg-N/L. Both biochar amended columns had significantly higher TAN removals compared with sand (p < 0.05), indicating that biochar amendment enhanced NH\(_4^+\) adsorption and nitrification. The results
indicate that 30% (by vol; 5% by wt) biochar amendment removed 76.6% and 49% TAN (by mass) in SBC1 and SBC2, respectively. Although BC2 had a higher CEC, the SA and micro-pore volume were higher for BC1, which may have resulted in greater NH$_4^+$ adsorption.

Significant differences in effluent NO$_x$ concentrations were observed between SBC1 (7.13±8.3 N-mg/L), SBC2 (0.98± 3.04 N-mg/L) and S (1.40 ± 3.34 N-mg/L) columns (p<0.05). DON removal was high in biochar amended columns due to adsorption and/or ammonification. This contrasts with Dempster et al. (2012), who reported that biochar addition had very little effect on DON removal. Both biochar amended columns had lower K$_s$-values (K$_{SSBC1}$=0.18 cm/min and K$_{SSBC2}$= 0.184 cm/min) than the sand column (K$_S$= 0.20 cm/min), which reduced the flow velocity and increased HRT. In SBC1, the longer HRT provided greater opportunity for nitrifying bacteria to convert TAN to NOx. The ADP between storms allowed adsorbed TAN to undergo nitrification as drained pore water was replaced by fresh air that oxygenates the medium during dry days. Moreover, effluent pH was higher in the biochar amended columns (Biochar: 9.85–10.1, sand: 7.8), which may have favored nitrification. A study by Case et al. (2012) showed that increasing pH values from 7.5-9 increased nitrification rates compared with un-amended sand.

Prior studies showed that physicochemical properties of the biochar (CEC, micro-pore volume, zeta potential and C/H ratio) affect NH$_4^+$ retention (Dempster et al., 2012; Yao et al., 2012). According to Li et al. (2018) both CEC and micro-pore volume are the dominant factors affecting NH$_4^+$ adsorption. However, the experimental results from this study show SBC1, with a lower CEC and higher SA, achieved greater TAN adsorption. Hence, further investigations are needed to understand TAN absorption mechanism by biochar. It is likely that favorable conditions for denitrification occurred in the columns due to adsorption of high influent TOC concentrations and the development of a thin anoxic layer at the bottom of the SBC-50 column because of the
higher moisture content of biochar ($MC_{SBC-50} = 44\%$ compared to $MC_{SBC-20} = 24\%$). The dissolved oxygen concentration measured in the bulk liquid effluent was 0.9 mg/L, which was above the value that is typically inhibitory for denitrification (Ergas and Aponte-Morales, 2014). However, denitrification could still occur in anaerobic microsites within biofilms. Based on the results of Phase I, Biochar 1 was selected for the subsequent phases.

![Figure 3.1: Comparison of N-species (TAN, NOx and DON) concentrations in influent dairy runoff and effluent from three columns (S: Sand, SBC1: Sand with biochar 1 and SBC2: Sand with biochar 2). Error bars show standard deviations for four experiments.](image)

3.4.2 Phase II: Effect of Amendment Rate on Water Quality

3.4.2.1 Overall Nutrient Removal Performance

Average N-species and TOC removals for ten experiments in SBC-20 and SBC-50 columns are summarized in Table 3.2. TAN removal was significantly ($p < 0.05$) higher for SBC-50 (71%) compared to SBC-20 (34%). The higher biochar amendment rate in SBC-50 increased the surface charge availability for $NH_4^+$ adsorption. Preliminary laboratory results showed that dairy runoff contains $Ca^{2+}$ (17.01 mg/l), $Mg^{2+}$ (13.1 mg/l), $Al^{3+}$ (19.2 mg/l) and $Fe^{2+}$ (15.7 mg/l) ions that compete with $NH_4^+$ for surface sites, resulting in a lower TAN removal for the 20%
amended column. In addition, the neutral pH of the influent (7.25±0.2) helps in deprotonation of the carboxyl and phenolic groups on the biochar surface and precipitates aluminium and iron oxides, creating an opportunity for functional groups to adsorb NH$_4^+$ (Brady and Weil, 2008). For example, under neutral pH conditions, carboxyl functional groups present on biochar surfaces as [Biochar]-COO$^-$, instead of as [Biochar]-COOH aid in electrostatic attraction of ammonium (NH$_4^+$) present in solution. Influent NOx concentrations were relatively low (Table 2), and limited NOx export was observed, most likely due to simultaneous nitrification and denitrification. As discussed previously, biochar addition favors denitrification, which led to TN removal.

Table 3.2: Overall N-species and organic carbon removal for SBC-20 and SBC-50 columns. Values across a row followed by different superscripted letters are significantly different (p < 0.05) among the three columns using Tukey tests. Negative % removal values indicate NOx export.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Influent (mg/L)</th>
<th>SBC-20</th>
<th>SBC-50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effluent (mg/L)</td>
<td>% Removal</td>
<td>Effluent (mg/L)</td>
</tr>
<tr>
<td>TAN</td>
<td>30.75±14.5</td>
<td>20.23±17.19</td>
<td>33.61±53.31$^a$</td>
</tr>
<tr>
<td>NO$_x$</td>
<td>0.03±0.1</td>
<td>0.23±0.38</td>
<td>-765.87±1396$^c$</td>
</tr>
<tr>
<td>DON</td>
<td>57.17±29.4</td>
<td>30.86±38.14</td>
<td>38.19±56.53$^a$</td>
</tr>
<tr>
<td>TN</td>
<td>88.33±36.8</td>
<td>45.81±38.77</td>
<td>39.34±50.73$^a$</td>
</tr>
<tr>
<td>TOC</td>
<td>761.83±468.5</td>
<td>180.62±85.72</td>
<td>71.04±17.98$^a$</td>
</tr>
</tbody>
</table>

DON removal was significantly (p <0.05) higher in SBC-50 than SBC-20 due to greater adsorption capacity, which resulted in greater ammonification and nitrification-denitrification. Similar results were reported by Lentz et al. (2014), where soil amended with biochar and manure had a 1.6-fold higher mineralization rate compared with biochar amended soil without manure.
Hence, enhanced adsorption, ammonification, nitrification and denitrification resulted in higher TN removals for SBC-50 compared with SBC-20 (p <0.05).

3.4.2.2 Effect of ADPs on Water Quality in Consecutive Experiments

Figure 3.2 shows TAN removal efficiency from four consecutive 3.5-hour runoff experiments for SBC-20 and SBC-50 at a 7-day ADP and 0.1 cm$^3$/cm$^2$/min HLR. During the first experiment, as dairy runoff infiltrated the media, NH$_4^+$ was adsorbed due to surface charge availability; SBC-50 had greater available surface charge compared with SBC-20 (Figure 2). TAN removal drastically decreased in SBC-20 over the four consecutive events, going from net removal to net leaching (93.1% to -23.7%). TAN removal also declined, but to a lesser extent, in SBC-50 (from 100% to 45.2%). Increased effluent TAN concentrations in the fourth experiment might have been due to competition between different ions, resulting in net leaching of NH$_4^+$ after the adsorption capacity had been reached. Influent TOC adsorption to biochar may also have influenced TAN removal. Prior studies showed greater TAN removal in soil from swine runoff compared with a simple aqueous solution of CaCl$_2$ and (NH$_4$)$_2$SO$_4$ due formation of NH$_4^+$-DOC and NH$_4^+$-aldehyde/ketones/carbonyl chemical complexes (Fernando et al., 2005; Stevenson, 1994).

Low effluent NOx concentrations were observed from the systems. Based on the applied mass load of TAN and stoichiometry of nitrification, a total of 220 mg of O$_2$ was required for complete nitrification of the incoming TAN load in one storm event. However, the available O$_2$ in the medium, considering both the liquid (soil solution) and gas (soil atmosphere) phases following drainage (ignoring oxygen diffusion between two events), was approximately 60 mg and 70 mg for SBC-50 and SBC-20, respectively. Based on this calculation O$_2$ availability limited
nitrification. Moreover, aerobic heterotrophs degrading influent TOC compete with nitrifiers for O₂, thus inhibiting nitrification.

Similar trends of TN removal were observed for both columns, from net removal to net leaching over time. Transfer of O₂ from the atmosphere near the top surface facilitates nitrification, whereas sustained saturation in the bottom can promote denitrification. DON removal gradually decreased in both columns, with SBC-20 showing net leaching (-32.9%) while SBC-50 showed net removal (54.1%) during the fourth experiment. Similar performance was observed in prior studies with urban runoff at varying ADPs (Rahman et al., 2020).

Figure 3.2: TAN and TN removal for SBC-20 and SBC-50 for four consecutive experiments with 7-day ADP and 0.1 cm/min HLR.

**1st and 2nd superscripted letters represent significantly different values (p<0.05) considering the consequence of each treatment for different experiments and effect of each experiment on different systems, respectively, using Tukey tests.**

Similar trends of TN removal were observed for both columns, from net removal to net leaching over time. Transfer of O₂ from the atmosphere near the top surface facilitates nitrification, whereas sustained saturation in the bottom can promote denitrification. DON removal gradually decreased in both columns, with SBC-20 showing net leaching (-32.9%) while SBC-50 showed net removal (54.1%) during the fourth experiment. Similar performance was observed in prior studies with urban runoff at varying ADPs (Rahman et al., 2020).
3.4.2.3 N-species Breakthrough

N-species and TOC concentration profiles for SBC-20 and SBC-50 are shown in Figure 3.3. The experiment was performed at a 0.10 cm³/cm²/min HLR and 12-day ADP. After the first pore volume was flushed from the column (≈ 60 minutes), effluent concentrations slowly increased over time. For the first 2 pore volumes (≈ 120 minutes) both columns had similar low effluent TAN concentrations, but afterward effluent TAN concentrations increased in SBC-20, while steady performance was observed in SBC-50. Effluent NOx concentrations were high in the initial soil pore water because of flushing of NOx that had been generated by nitrification during the long ADP of 12-days between infiltration experiments. After 60 minutes, effluent NOx concentrations in SBC-20 decreased until the experiment ended, possibly due to saturated media conditions resulting in denitrification or shorter HRT than needed to convert TAN to NOx. In SBC-50, once the initial pore water was flushed from the column, effluent NOx concentrations increased (≈ 90 minutes) possibly due to further nitrification of adsorbed TAN during the initial stage of the experiment. After 150 minutes, effluent concentrations decreased due to the anoxic conditions that developed in the media, which favored denitrification. Until 1.5 pore-volume, both columns leached the DON, which was captured in porewater and media surface during the previous experiment. Once the trapped porewater was released, effluent DON concentrations gradually decreased until the experiment was completed due to adsorption of DON in available biochar surface. Effluent TN concentrations followed a similar pattern. As expected, SBC-50 had lower effluent TOC concentrations at the end of the experiment due to higher TOC adsorption compared to SBC-20 and TOC acting as carbon source and electron donor for denitrification.
Figure 3.3: Pollutant concentration profiles (a: TAN; b: NO$_3^-$; c: DON, d: TN, and e: TOC) for SBC-20, and SBC-50 bioretention systems with 12-day ADP and high HLR. The red lines indicate the number of pore volumes.

3.4.2.4 Phase-III: Biochar Amendment Rate Comparison – Hydraulics

Volumetric moisture content measurements at different media depths are shown in Figure 3.4 for ADPs of 1, 2, 3, 11 and 15-days. Moisture content increased with depth in both columns due to gravitational drainage. As expected, higher moisture contents were observed in SBC-50 than SBC-20. Following the drainage phase, the volumetric moisture content in SBC 20 was ~0.24
cm$^3$/cm$^3$ at the surface but increased to 0.51 cm$^3$/cm$^3$ (60%) at the bottom of the column. Similarly, the moisture content was 0.16 cm$^3$/cm$^3$ at the top of SBC-50, which was lower by a factor of 3.3 compared to bottom surface (0.58 cm$^3$/cm$^3$). During the ADPs, drainage and evaporation allowed air entry to the medium, especially close to the surface, which facilitated nitrification of adsorbed NH$_4^+$. A 33% higher evaporation rate was observed for SBC-50 compared with SBC-20. Similar findings were observed by Rahman et al. (2020), where biochar amended columns showed greater moisture retention compared with an un-amended column.

![Figure 3.4: Volumetric moisture content (cm$^3$/cm$^3$) with depth for varying ADPs: a) SBC-20 and b) SBC-50.](image)

Media hydraulic properties used in the HYDRUS model are shown in Table 3.3. Bulk density, MC and $K_s$ values used in the model were measured experimentally. The following hydraulic parameters were adjusted to match the simulated curves with the experimental data: i) soil water retention parameters ($\alpha$, n), ii) tortuosity of conductivity function (I); iii) dimensionless fraction of adsorption sites. Calibrated dispersivities were 4 cm for SBC-20 and 3 cm for SBC-50 columns. After calibrating the model parameters, the goodness of fit ($R^2$) for the experimental and simulated results were 0.904 for SBC-20 and 0.970 for SBC-50.
Table 3.3: Calibrated Parameters for the HYDRUS model to simulate the breakthrough curve

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SBC-20</th>
<th>SBC-50</th>
<th>Measured</th>
<th>Calibrated</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk density</td>
<td>1.385</td>
<td>0.985</td>
<td>√</td>
<td></td>
<td>1.24-1.66a</td>
</tr>
<tr>
<td>Moisture content (MC)</td>
<td>0.412</td>
<td>0.54</td>
<td>√</td>
<td></td>
<td>0.37-0.53a</td>
</tr>
<tr>
<td>Residual Water Content</td>
<td>0.04</td>
<td>0.06</td>
<td>√</td>
<td></td>
<td>0.4-0.19a</td>
</tr>
<tr>
<td>Saturated Hydraulic Conductivity (Ks)</td>
<td>0.18</td>
<td>0.174</td>
<td>√</td>
<td></td>
<td>0.05-0.15a</td>
</tr>
<tr>
<td>Dispersivity</td>
<td>4.0</td>
<td>3.0</td>
<td>√</td>
<td>No Ref.</td>
<td></td>
</tr>
<tr>
<td>α*</td>
<td>0.032</td>
<td>0.036</td>
<td>√</td>
<td>0.0019-0.0461b</td>
<td></td>
</tr>
<tr>
<td>N*</td>
<td>1.2</td>
<td>1.2</td>
<td>√</td>
<td>1.08-1.17b</td>
<td></td>
</tr>
<tr>
<td>T**</td>
<td>0.8</td>
<td>0.75</td>
<td>√</td>
<td>No Ref.</td>
<td></td>
</tr>
<tr>
<td>Goodness of fit</td>
<td>0.904</td>
<td>0.970√</td>
<td>√</td>
<td>0.99b</td>
<td></td>
</tr>
</tbody>
</table>

*a* (Glab et al., 2016): Experimental data for 1% to 4% biochar/soil mixture (by mass)
*b* Filipović et al., 2020: Model calibrated values for HYDRUS-1D for biochar/soil mixture
* Soil water retention parameters
** Tortuosity of conductivity function

Experimental and simulated breakthrough curves for a 4-hour tracer experiment with a 0.18 cm$^3$/cm$^2$/min HLR and ADP of 3-days are shown in Figure 3.5. Inspection of the breakthrough curves shows that the effluent tracer concentration was equal to the influent concentration at 120 minutes for SBC-20 and 165 minutes for SBC-50, indicating a longer HRT with a higher biochar fraction. After an initial lag period, concentrations started to increase at 30 minutes for SBC-20 and 45 minutes for SBC-50. These findings were corroborated with the effluent flow rate data, where SBC-20 had shorter HRT compared with SBC-50.

Figure 3.6 shows simulated MC in the columns at different media depths at different time intervals during the experiment. Collected media samples showed a lower initial MC for SBC-20 (0.26 cm$^3$/cm$^3$) compared with SBC-50 (0.40 cm$^3$/cm$^3$) at a depth of 6 cm. The calibrated model showed that within 30 minutes of infiltration, SBC-20 reached full saturation (saturated water content=0.41 cm$^3$/cm$^3$) whereas SBC-50 reached 83% saturation (saturated water content= 0.54 cm$^3$/cm$^3$).
cm³/cm³) at that time. During the dry days, porewater was replaced by air due to evaporation because of the higher porosity of SBC-50 (0.535 cm³/cm³) compared with SBC-20 (0.41 cm³/cm³), resulting in a longer time period needed to reach the saturation in the column with more biochar (Figure 3.6). Based on the input parameters and calibration, it can be concluded that increasing the biochar fraction increases porosity (especially micro-porosity) and slightly reduces saturated conductivity. The changes in hydraulic properties impacted the HRT, the length of time the solution is in contact with the treatment media.

![Figure 3.5: Tracer breakthrough curves and model simulations for: a) SBC-20 and b) SBC-50.](image1)

![Figure 3.6: Vertical profile of a) moisture content of the SBC-20 and SBC-50 columns at different time intervals.](image2)
3.5 Conclusions

N-species removal mechanisms were investigated in sand biofilters amended with different biochar types and at ratios. Amending sand with biochar increased TAN and DON adsorption during infiltration. Water drainage during ADP replenished the oxygen content of the media, which facilitated nitrification of adsorbed TAN. Favorable condition for denitrification developed near the bottom of the biochar amended columns due to increased MC and adsorbed TOC, resulting in higher TN removal at a higher biochar amendment rate. The higher biochar amendment rate also increased the HRT due to the increased porosity and greater microporous structure. This research contributes to optimizing media mixture for biochar amended biofilters for nonpoint source pollution mitigation. Long-term field scale studies with appropriate plants should be carried out to evaluate the use of biochar amended biofilter to control non-point source nutrient pollution from agricultural sources.
4.1 Abstract

The presence of Fecal Indicator Bacterial (FIB) in runoff from dairy farms is a significant public health concern. This study investigated the use of biochar amended bioretention systems for removal of *Escherichia coli* (*E. coli*) and *Enterococci* from dairy runoff. Phase I compared the performance of sand columns amended with two types of biochar with different specific surface area (SA) with an un-amended sand column. The results showed that biochar enhanced *E. coli* removal due to its unique physicochemical properties. The higher SA biochar had better FIB removal efficiencies and was used in subsequent studies. In Phase II, two bioretention columns with different biochar fractions (20% and 50% by volume) were operated at varying hydraulic loading rates (HLR) and antecedent dry periods (ADPs). Significantly higher *E. coli* removals were observed compared with *Enterococci* in both columns, indicating a greater attachment affinity to the biochar surface for *E. coli*. However, there was no significant difference in *E. coli* or *Enterococci* removals between the two columns with different biochar fractions. Longer ADPs were found to enhance *E. coli* removal in the higher biochar fraction column. In Phase III, pilot scale studies were conducted with four modified bioretention systems that included an internal water storage zone (IWSZ). Experiments were designed to test FIB removal performance with and without biochar and with and without plants. Addition of an IWSZ and plants was positively

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correlated with *E. coli* removal in biochar amended systems. Findings from this research can inform the design of field-scale bioretention systems for more consistent performance in removing FIB from urban and agricultural runoff.

### 4.2 Introduction

Fecal Indicator Bacteria (FIB) and pathogens in runoff from concentrated animal feeding operations and dairy farms impair surface water quality (Curriero et al., 2001; USEPA, 2002; USDA-NASS 2016). These microbial contaminants result from urine and fecal waste deposition on surfaces, improper manure storage and a lack of effective treatment of dairy wastewater (Zhang et al., 2021; Guan et al., 2003). Runoff from cattle feeding has been shown to contaminate vegetable crops with *E. coli* after heavy rainfall events (Lynch et al. 2009). When used for irrigation, groundwater polluted by livestock wastes has been responsible for contaminating vegetable crops with *E. coli* O157: H7 (Heiman et al., 2015).

Passive treatment technologies for agricultural runoff include vegetated buffer strips, lagoons and constructed wetlands. These technologies are designed to remove organic matter and suspended solids. However, a wide range of removal efficiencies have been reported for FIB such as *E. coli* due to improper media selection or ineffective operating conditions. For example, *E. coli* removal ranged from 65-90% among four San Joaquin Valley, California (USA) wetlands treating runoff from irrigation (Diaz et al., 2010). Similarly, minimal retention of FIB (0.16 log10) was observed for vegetative buffer strips treating dairy barnyard runoff (Schellinger et al., 1992). In an analysis of the International Stormwater Best Management Practices (BMP) database, Clary et al. (2014) concluded that bioretention and sand filtration systems are the most effective technologies for FIB removal from stormwater runoff.
Bioretention is a passive treatment technology consisting of a shallow depression containing engineered porous media with an optional underdrain pipe to release the treated stormwater downstream. Bioretention systems are typically designed with the following materials (top to bottom): i) topsoil, mulch or compost as surface layer with or without plants, ii) sand or sand with alternative material as a filtration medium, and iii) a gravel drainage layer at the bottom. A modified bioretention system design includes an internal water storage zone (IWSZ) that contains woodchips or mixture of electron donor materials for mainly to promote denitrification and total nitrogen removal (Lopez-Ponnada et al., 2020; Ergas et al., 2010). The anoxic conditions in the IWSZ have also been shown to enhance \textit{E. coli} removal (Liu et al., 2020; Ergas et al., 2010). However, a wide variability in FIB removals has been reported for both conventional and modified bioretention systems due to differences in porosity, hydrodynamic dispersion, and surface characteristics of media and bacteria (Hathaway and Hunt, 2011; Mahmoud et al., 2019; Zhang et al., 2010).

In Chapter 2 with conventional sand/soil media reported that \textit{E. coli} removal is influenced by a number of biophysical mechanisms (Li et al., 2016; 2012). Major mechanisms for \textit{E. coli} removal in porous media include attachment, straining, predation and die-off. Attachment is controlled by media properties and surface characteristics, FIB properties, and physicochemical properties of the suspending fluid (e.g., pH, ionic strength); whereas straining is controlled by pore and particle sizes (Zhang et al., 2010; Grebel et al., 2013; Rippy 2015; Chen et al., 2012). Chapter 2 showed that conventional sand media has a low surface area (SA) and narrow pore size distribution for attachment and straining, respectively. According to Williams et al. (2015) increasing the soil pH, significantly improved log \textit{E. coli} removal. Moreover, temperature and the presence of indigenous microbial communities greatly affected \textit{E. coli} at all biofilter depths.
(Chandrasena et al., 2014). Therefore, according to Chapter 2, adsorbent soil amendments, like biochar, could enhance these removal mechanisms.

Coated sand, zeolite and biochar have recently been shown to be promising bioretention media amendments to enhance E. coli removal from urban runoff (Rahman et al., 2020; Mohanty et al. 2014; Mohanty & Boehm 2015) because of their higher SA compared to sand. Biochar is readily available, low in cost and has a high capacity to remove a wide range of contaminants (Rahman et al., 2020). However, no prior studies have evaluated the effect of biochar amended bioretention systems for FIB removal from dairy runoff. One important difference between urban and dairy runoff is the chemical composition of runoff matrix. Dairy runoff contains high natural organic matter (NOM), suspended and dissolved particles and high ionic strength compared with urban runoff. In prior laboratory studies, NOM was shown to decrease removal capacity of E. coli in biochar amended biofilters (Mohanty and Boehm, 2014; Mohanty et al., 2014a). Therefore, the effectiveness of biochar for FIB removal from agricultural and dairy runoff with high concentration of NOM and suspended particulates is not known. As discussed in Chapter 2, E.coli removal for urban runoff is largely related with physicochemical properties of biochar, therefore, further research was focused on biochar properties and amendment rates on FIB removal from dairy runoff in this chapter.

Biochar is a carbon rich byproduct of waste materials pyrolyzed at high temperature under oxygen limited conditions. The type of feedstock, production process and pyrolysis temperature are key factors that influence biochar properties. In general, biochar has a high cation exchange capacity (CEC), SA, porosity (n), pore size distribution, hydrophobicity and ash content. Moreover, the high nutrient retention and water holding capacity (WHC) of biochar aids plant growth, which can help inactivate pathogens by releasing root exudates (Chandrasena et al., 2017).
A number of prior studies have looked at FIB removal with different types of biochar. Wood-based biochar was shown to improve *E. coli* removal compared with poultry litter biochar (Abit et al., 2012). According to Abit et al. (2012) excessive nutrients leaching from poultry litter biochar increased *E. coli* transport. Moreover, higher pyrolysis temperature enhanced attachment capacity for *E. coli* due to increased carbonization (Abit et al., 2012; Suliman et al., 2017). However, biochar with high ash content could increase pore water pH and reduce *E. coli* attachment. Prior research showed that, removing the fine particles fraction of biochar from the media mixture decreased the SA and porosity and lowered *E. coli* removal (Mohanty and Boehm, 2014; Sasidharan et al., 2016). The rate of biochar application also may influence FIB removal (i.e., the fraction of biochar incorporated into the sand medium). However, no prior published research was found on the effect of biochar application rate on FIB removal from either urban or dairy runoffs.

The overall goal of this research was to understand the fate of FIB in biochar amended bioretention systems treating dairy runoff. Initial, experiments were carried out in sand columns with and without biochar to investigate *E. coli* and Enterococci removal from dairy runoff. Additional column studies were used to identify the effect of biochar amendment rate on *E. coli* removal. Based on these studies, pilot-scale modified bioretention systems were set up with and without biochar amendment and with and without plants. The pilot systems were operated over a 12-month period with dairy runoff at varying Hydraulic Loading Rate (HLR) and Antecedent Dry Period (ADP). This is the first study to investigate the use of biochar amended bioretention systems for dairy runoff management.
4.3 Materials and Methods

This study investigated the factors affecting FIB removal from dairy farm runoff in biochar enhanced bioretention, including type of biochar, amendment rate, operational parameters (HLR, ADP), and the inclusion of plants and an IWSZ. Additional details can be found in FDACS report (FDACS 2021).

4.3.1 Porous Media

Characterization of porous media, both sand and biochar, were described in Rahman et al. (2020). The primary factor for choosing sand was hydraulic conductivity (Ks = 13.2 cm/hr) within the recommended value for the bioretention systems guideline, (Prince George’s County Program and Planning Division, 2007). This sand had a porosity (n: 35% ± 0.95), moisture content at field capacity (MC (by wt.): 23.24% ± 0.96) and bulk density (BD: 1.56 ± 0.14 g/cc). The sand contained 0.27% coarse grain (>1mm), 9.5% medium grain (<1mm-0.6mm) and 90% fine grain particles (<0.6mm-100um). Two woods derived biochars were acquired from two commercial sources. Biochar 1 (Everson, WA) had higher SA (537 ± 60.15 m2/g) with relatively low CEC (10.57 cmol/kg) compared with biochar 2 (Loveland, CO), which had SA of 136 ± 45.51 m2/g and CEC of 13.63 cmol/kg. Sand and biochar particles were retained (by vol.) in sieve openings of 1mm (5%), 0.6-0.42 mm (55%), 0.25mm (30%) and 0.15mm (10%) to create the homogeneous mixture of the systems.

4.3.2 Chemical Composition of Dairy Runoff

Fresh dairy manure was collected from South Tampa Farm, Tampa, FL and transported to the University of South Florida’s Environmental Engineering Laboratory. The manure was diluted with tap water (30% by volume), mixed vigorously, and the solution was allowed to settle overnight. The supernatant was screened through a 0.25 mm mesh and stored unit use. The influent
was prepared by mixing 60% of the screened supernatant with 40% tap water or lake water for column and pilot studies, respectively. The target influent concentration was 8.63x10⁶±7.07x10⁶ CFU/100ml for *E. coli* and 5.43x10⁶±4.14x10⁶ CFU/100ml for *Enterococci*. These values were consistent with concentrations observed in prior field studies of agricultural/feedlot runoff (Ergas et al., 2010; Vadas & Powell, 2019).

4.3.3 Experimental Program

The research was conducted in three phases (Table 4.1). In Phase I, treatment of dairy runoff was evaluated in bench scale columns with/without biochar amendment. This phase was used to select the appropriate biochar for FIB removal. In Phase II, the relationship between biochar amendment rates and *E. coli* removal capacity was evaluated in column studies. In Phase III, pilot studies were performed to evaluate the effect of an IWSZ and plants on *E. coli* removal.

Table 4.1: Experimental design of different phases on *E. coli* removal

<table>
<thead>
<tr>
<th>Phase</th>
<th>Purpose of Study</th>
<th>Number of Experiments</th>
<th>Flow Rate (L/hr)</th>
<th>HLR (cm³/cm²/min)</th>
<th>Duration (hr)</th>
<th>Runoff Volume (mL)</th>
<th>ADP (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Biochar selection</td>
<td>4</td>
<td>0.42</td>
<td>0.18</td>
<td>3.5</td>
<td>840</td>
<td>7</td>
</tr>
<tr>
<td>II</td>
<td>Biochar amendment rate on <em>E. coli</em> removal</td>
<td>9</td>
<td>2.55-4.20</td>
<td>0.1-0.18</td>
<td>3.5</td>
<td>840-1,470</td>
<td>3-30</td>
</tr>
<tr>
<td>III</td>
<td>Effect of IWSZ and plant on <em>E. coli</em> removal</td>
<td>9</td>
<td>13.32</td>
<td>0.10</td>
<td>4</td>
<td>53,300</td>
<td>7</td>
</tr>
</tbody>
</table>

*Enterococci removal performance was evaluated in Phase II column study*
4.3.3.1 Phase I: Biochar Selection

For the preliminary selection of biochar, three columns including i) pure sand (S); ii) Sand with high SA Biochar 1 (SBC1); and iii) Sand with high CEC Biochar 2 (SBC2) were constructed from 2-liter Koflo (Cary, IL) calibration columns. The total height of each column was 57.2 cm with an inside diameter of 7.2 cm. The total depth of the filter media (sand or sand with biochar) was 30 cm and bottom were filled with 7.2 cm of gravel for drainage of the treated water. The top 20 cm was provided free board for ponding at surface. The volumetric ratio of biochar to sand was 30:70. However, due to variation of bulk densities the mixture of biochar to sand (by mass) had 3.1% and 4.75% for SBC1 and SBC2. Experimental details were shown in Table 4.1.

4.3.3.2 Phase II: Effect of Amendment Rates on E. coli Removal

Biochar 1 was selected for Phase II and Phase III due to its better performance for the E. coli and Enterococci removal compared with Biochar 2. For Phase II, two new columns were constructed with 20% and 50% biochar amendments (by volume). Biochar was mixed with sand from Phase I to accelerate the acclimation. Biochar mass fraction was 2.2 % in SBC-20 and 5.7% in SBC-50 had 5.7%. Duplicate experiments were conducted with constant HLR at 0.18 cm$^3$/cm$^2$/min and 4-days of ADPs. Subsequently, duplicate experiments were performed at ADPs of 4, 10, and 30-days at a constant HLR of 0.10 cm$^3$/cm$^2$/min.

4.3.3.3 Phase III: Pilot Modified Bioretention Systems

Based on the outcomes of Phase II, Phase III was designed to evaluate the effect of IWSZ and plants on biochar amended bioretention systems for dairy runoff. Four pilot-scale modified bioretention systems were constructed: i) sand (S), ii) sand with plants (SP), iii) biochar amended sand (B) and, iv) biochar amended sand with plants (BP) (Figure 4.1).
The total depth of each bioretention system was 102 cm. To prevent the washout of fine particles, a filter fabric was placed between the drainage layer and IWSZ. The perforated PVC drainage pipe included an upturned elbow to create an IWSZ. SP and BP systems were planted with *Muhlenbergia* (Muhly Grass), a native Florida perennial purchased from a local nursery. Muhly Grass attracts wildlife and has favorable light and moisture requirements, growth rate and mature plant height and spread. After planting, the systems were watered periodically for three months for the growth of roots and biomass before performing dairy runoff experiments. A total of nine experiments were conducted at a HLR of 0.10 cm$^3$/cm$^2$/min considering 7 days of ADP (Table 4.1).

![Figure 4.1: Cross-sectional diagrams of (a) sand modified bioretention cell with plant (SP), (b) sand modified bioretention cell (S), (c) biochar amended sand modified bioretention cell with plant (BP) and (d) biochar amended sand modified bioretention cell (B) (units are in cm).](image)

4.3.4 Analytical Methods

Samples were collected every 15 to 30 minutes interval during each experiment and effluent volume was measured gravimetrically to calculate mass load pollutant reductions. A multiparameter meter was used to measure pH and conductivity (*Standard Methods*, 2018; 2510...
B). *E. coli* was enumerated in duplicate at three dilutions using the membrane filter method with modified membrane-Thermotolerant *Escherichia coli* Agar (m-TEC; EPA Method 1603). Duplicate samples of *Enterococci* were analyzed at three dilutions using the membraneEnterococcus-Esculin Iron Agar (mE-EIA) media (EPA Method 1106.1). Dilutions for microbial enumeration were done in PBS. TOC were measured with a Shimadzu TOC-V CSH TOC/TN Analyzer (Shimadzu Scientific Instruments, Columbia, MD). The method detection limits for TOC were 0.11 mg/L.

4.3.5 Data Analysis

Concentrations of *E. coli* in feed solutions were measured at the start and end of each experiment to confirm that growth/death did not occur in the feed tanks during the experimental period. Log removal values were calculated using the following:

\[
E. \text{coli log Removal} = - \log \frac{C}{C_0} \tag{i}
\]

where, \(C_0\) and \(C\) are the influent and effluent concentrations, respectively.

TOC mass removal (\(R_{TOC}\)) was calculated using the following:

\[
R_{TOC} = \frac{\sum N C_0 V_0 - CV}{N C_0 V_0} \tag{ii}
\]

where, \(N\) is the total number of effluent samples; \(V_0\) and \(V\) are the influent and effluent volume (L), respectively.

One-way analysis of variance (ANOVA) was performed with Tukey’s post-hoc test to identify statistically significant differences in measured parameters. Statistical analyses were considered significant level at \(p < 0.05\).
4.4 Results and Discussion

4.4.1 Biochar Selection

*E. coli* breakthrough curves for the three columns operated with dairy runoff at 7-day ADP and 0.10 cm³/cm²/min HLR are shown in Fig. 4.2. Biochar amended sand columns achieved 3 to 5 order of magnitude greater *E. coli* removal than the un-amended sand column. Average effluent *E. coli* concentrations from SBC1 were significantly lower (p=0.034) than SBC2 and S. Initially all three columns showed similar performance but after one pore volume (PV) (60 minutes), the S column had higher *E. coli* release compared with biochar amended columns. As shown in Fig. 4.2, there was an abrupt increase in *E. coli* release from the S column compared with biochar amended columns after two PVs. The trend continued until the experiment ended. Biochar has a highly porous structure, with five orders of magnitude greater SA than sand (Rahman et al., 2020). Addition of 3% biochar 1 (by wt) for SBC1 increased the net SA available to attach *E. coli* by a factor of 1,660, whereas 4% biochar 2 addition (by wt) for SBC2 increased net SA by factor of 544. Therefore, due to its high SA, biochar 1 has high potential for *E. coli* attachment on the surface even in the complex dairy runoff matrix.

Enhanced removal in biochar amended columns could also be explained by Derjaguin-Landau-Verwey- Overbeek (DLVO) theory, which combines attractive van der Waal forces and repulsive electrostatic forces. In general, *E. coli* are negatively charged. To attach to the negatively charged biochar surface *E. coli* must overcome the electrostatic repulsion between biochar and *E. coli*. Dairy runoff has a high ionic strength and biochar increases the pore water pH. Therefore, a reduced zeta potential (creation of secondary minimum) is expected for biochar amended columns compared with the sand column. According to Redman et al. (2004), due to the attractive force *E. coli* attaches to the porous media at the secondary minimum when both *E. coli* and biochar surfaces
have the same net charge. Moreover, biochar has a high organic carbon content, therefore the hydrophobic attraction between *E. coli* is higher for biochar than sand. Based on the results of Phase 1, Biochar 1 was selected for further testing in later phases of the experiments.

![Figure 4.2: Effluent *E. coli* concentrations normalized to influent (C₀) for columns packed with (a) Sand (S), (b) mixture of sand and high surface area biochar (SBC1), and (c) mixture of sand and high cation exchange capacity biochar (SBC2) for a 3.5-hour experiment with 7 day ADP and HLR of 0.10 cm³/cm²/min. Influent *E. coli* concentration was 6.0⁶ ± 1.55x10⁶ CFU/100 ml.](image)

4.4.2 Effect of Biochar Amendment Rate on FIB removal

4.4.2.1 Overall Performance for *E. coli* and Enterococci

Table 4.2: Overall *E. coli* and *Enterococci* removal performance for biochar amended sand

<table>
<thead>
<tr>
<th>Phases</th>
<th>Influent Concentration (CFU/100ml)</th>
<th>Effluent <em>E. coli</em> Concentration (CFU/100ml)</th>
<th>Log Removal</th>
<th>Influent Concentration (CFU/100ml)</th>
<th>Effluent <em>Enterococci</em> Concentration (CFU/100ml)</th>
<th>Log Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>SBC-30 6.6x10⁶±1.10x10⁶</td>
<td>2.62x10⁵±1.73x10⁵</td>
<td>1.53±0.37</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>SBC-20 8.63x10⁶±7.07x10⁶</td>
<td>3.71x10⁵±2.52x10⁵</td>
<td>1.72±0.32</td>
<td>5.63x10⁶±4.07x10⁶</td>
<td>4.57x10⁵±2.57x10⁵</td>
<td>1.09±0.24</td>
</tr>
<tr>
<td></td>
<td>SBC-50 3.12x10⁵±2.50x10⁵</td>
<td>1.76±0.29</td>
<td></td>
<td></td>
<td>3.47x10⁵±2.17x10⁵</td>
<td>1.21±0.39</td>
</tr>
</tbody>
</table>
A summary of overall *E. coli* and *Enterococci* removal performance for SBC-20 and SBC-50 is shown in Table 4.2. *E. coli* data from Phase I experiments with 30% biochar 1 are also included for comparison. In Phase II, better removal was observed in SBC-50 than SBC-20 for both FIB. Note: however, that lower log *E. coli* removal was observed in Phase I with 30% Biochar 1 addition, than for SBC-20 in Phase II. This may have been due to differences in feed characteristics, since Phase I and Phase II experiments were conducted with real dairy waste at different time and characteristics varied. Significantly higher removals of *E. coli* were observed than *Enterococci*, indicating a greater attachment affinity to the biochar surface for *E. coli*. Similar results were reported by Kranner et al. (2019) in a biochar amended biofilter used for stormwater management. Differences in *E. coli* and *Enterococci* transport in biochar amended media is attributable to their unique microbial properties, including cell membrane composition, motility, shape, surface charge and hydrophobicity (Becker et al., 2004; Silliman et al., 2001). Although SBC-50 had a higher SA and hydrophobicity than SBC-20, there was no significant difference in *E. coli* or *Enterococci* removals between the two columns. This could possibly be due to complex dairy runoff matrix, which contains high levels of TOC and dissolved organic nitrogen, which have a high affinity for surface attachment to biochar.

4.4.2.2 *E. coli* Breakthrough Profile for Two Columns

*E. coli* breakthrough profiles for SBC-50 and SBC-20 for experiments conducted with 7-day and 28-day ADPs at a HLR of 0.18 cm³/cm²/min are shown in Fig 4.3. For 7-day ADP, SBC-50 showed slightly lower log removal for the first PV (SBC-20: log 2.07 ± 0.24 and SBC-50: log 1.94 ± 0.18). This might be due to the greater moisture capacity of SBC-50, which provided favorable conditions for the survival or regrowth of *E. coli* compared with SBC-20. Hill et al. (2019) found a longer logarithmic *E. coli* growth phase and higher final cell density in biochar.
amended media. However, considering the entire course of the experiment at 7-days ADP, greater log removal was observed with SBC-50 (log 1.52 ± 0.42) compared with SBC-20 (log 1.47 ± 0.53). Prior studies also showed that the presence of organic matter and biofilm growth decreased *E. coli* removal efficiency (Mohanty et al., 2014a; Afrooz and Boehm, 2017). Biochar has a high adsorption affinity for TOC and competition between *E. coli* and TOC on the biochar surface may have resulted in decreased *E. coli* removal from SBC-50. For shorter ADPs, adsorbed TOC might not be completely biodegraded prior to the next storm event or there might be competition with other microbes, resulting reduced *E. coli* removal.

For 28-day ADP, SBC-50 had higher *E. coli* log removal (log 1.63 ± 0.37) compared to SBC-20 (log 1.27 ± 0.34). Until the first PV (60 minutes), SBC-50 had 17% higher log removal compared to SBC-20. This might be due either to higher *E. coli* die off compared with other microbes or less remobilization of attached *E. coli* during the first flush of pore water. A similar laboratory study by Mohanty and Boehm (2014) showed that only 2% of the deposited *E. coli* remobilized from biochar-amended sand filters treating urban runoff. Therefore, due to the higher biochar amendment rate in SBC-50, lower remobilization was observed in SBC-50 for the first PV.

![Figure 4.3: *E. coli* breakthrough profile for SBC-20 and SBC-50 columns for a) 7-days and b) 28-days ADPs](image-url)
4.4.3 Pilot-Scale Studies

In Phase III, four pilot-scale systems were operated to compare bioretention performance with and without biochar and with and without plants (B, BP, S and SP). The pilot systems were modified with an upturned elbow to create an IWSZ to enhance denitrification (Fig. 4.1). Note that no solid electron donor (e.g., wood chips) was added to the IWSZ since the influent dairy runoff contained enough organic carbon to drive denitrification.

Table 4.3: E. coli removal performance for pilot studies for four bioretention systems considering different pore water infiltration with 7-days ADPs and 0.10 cm³/cm²/min HLR

<table>
<thead>
<tr>
<th>Systems</th>
<th>Log Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1 PV (Time &lt;90 minutes)</td>
</tr>
<tr>
<td>B</td>
<td>3.24±0.52</td>
</tr>
<tr>
<td>BP</td>
<td>4.22±1.99</td>
</tr>
<tr>
<td>S</td>
<td>2.84±0.33</td>
</tr>
<tr>
<td>SP</td>
<td>2.52±0.44</td>
</tr>
</tbody>
</table>

Log E. coli removals for the pilot-scale bioretention systems are shown in Table 4.3 and Fig 4.4. Similar to Phase I, biochar amended systems had better removal performance compared with un-amended systems (Fig 4.4). Systems planted with Muhlenbergia, which has an extensive root system, had better performance than the corresponding systems without plants. The best performance was observed in the bioretention system with both biochar and plants. The quantity of microorganisms living in the rhizosphere is several orders of magnitude higher than that in bulk soils (Mukerji et al., 2006). It is well known that addition of biochar to soil aids in plant growth by retaining nutrients and providing a good habitat for beneficial microbes in the rhizosphere (Werner et al., 2018; Ippolito et al., 2012). Plants can improve FIB removal through predation and competition by rhizosphere microbes or inactivation by antimicrobial compounds from root
exudates (Chandrasena et al., 2014). However, plant roots can also increase FIB transport by creating preferential flow paths through the media (Clothier et al., 2008).

![Figure 4.4: Overall performance of E. coli removal for pilot studies for four modified bioretention systems](image)

The effect of the IWSZ was evaluated by comparing the performance of the laboratory-scale column from Phase II (without IWSZ) with pilot-scale systems without plants (with IWSZ). Inclusion of the IWSZ resulted in a 40% higher log E. coli removal for experiments carried out with the same ADP and HLR (Appendix C: Figure C1). Higher E. coli removals in pilot units with an IWSZ may have been due to the longer retention times and/or the anoxic conditions present in the IWSZ.

### 4.5 Conclusions

This study investigated the use of biochar amended bioretention systems for removal of FIB from dairy runoff. Experiments were conducted with and without biochar addition, at different biochar amendment rates, and in modified pilot-scale systems with and without plants. Addition of biochar enhanced the E. coli removal, with greater removal in with higher SA biochar than higher CEC biochar. The highest FIB removals were observed with the first flush of influent
stormwater, and then declined over time as the biochar surface reached capacity. Longer ADP resulted in more FIB die-off between storm events. FIB removals were observed in *E. coli* was found to have a greater attachment affinity to the biochar surface than *Enterococci*, as has been shown in prior studies. Surprisingly, no significant differences were observed in FIB removal for columns with different biochar amendment rates. The inclusion of plants, biochar and an IWSZ in pilot-scale systems resulted in the best *E. coli* removal performance. Long-term field scale studies are needed to evaluate the use of biochar amended bioretention to control FIB pollution from agricultural runoff.
Chapter 5: Biochar Amended Modified Bioretention Systems for Livestock Runoff

Nutrient Management

5.1 Introduction

Florida is ranked 13th in the US for cow inventory, providing >1 million metric tons of milk and generating ~133,000 kg/year of nutrients (nitrogen [N] and phosphorus [P]) (USDA-NASS 2016). Livestock operations are a major non-point source of pollution to fresh and marine surface waters, groundwater, and springs in Florida. Improper management of cattle manure contributes to eutrophication, excessive growth of nuisance and harmful algal blooms, fish kills, economic losses, and nitrate (NO$_3^-$) contamination of drinking water supplies.

The most common livestock waste management strategy in Florida is treatment in settling basins or lagoons followed by agricultural irrigation or direct discharge to surface waters (Prasad et al., 2014). However, these systems are inadequate for nutrient management. For example, a study of waste lagoons at nine dairy farms in north Florida found dissolved total ammonia nitrogen concentrations (TAN) ranging from 22 to 230 mg/l, with a median of 160 mg/l. The Florida Watershed Restoration Act (FWRA) established both structural and non-structural best management practices (BMPs) for livestock operations. FWRA guidelines require systematic waste collection and BMP implementation, especially in the Lake Okeechobee drainage basin. Alternative BMPs for managing runoff from livestock waste include constructed wetlands, vegetative buffer strips and bioretention systems (Mantovi et al., 2003; Giri et al., 2010). Among

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these systems, bioretention is a promising technology for nutrient management (Mahmoud et al., 2019; Ergas et al., 2010).

Conventional bioretention systems include a gravel drainage layer, engineered sand filtration medium layer, a planted zone with topsoil and mulch and an optional underdrain pipe (Figure 5.1A). Nitrogen removal in these systems relies on 1) plant uptake, 2) filtration of N containing solids, 3) adsorption of \( \text{NH}_4^+ \) to negatively charged sites in the filtration medium and 4) microbial N-species transformations of ammonification (dissolved organic N (DON) \( \rightarrow \text{NH}_4^+ \)), nitrification (\( \text{NH}_4^+ + \text{NO}_3^- \)) in aerobic zones and denitrification (\( \text{NO}_3^- \rightarrow \text{N}_2 \)) in anoxic zones. In conventional bioretention systems, nitrification is promoted in the aerobic filter media layer; however, total nitrogen (TN) removal is typically low because the systems lack the conditions needed for denitrification (Li et al., 2014). Therefore, modified bioretention systems have been developed (Figure 1B) that include an internal water storage zone (IWSZ) with a slow-release solid electron donor, such as wood chips, to promote denitrification (Lopez-Ponnada et al., 2020).

Figure 5.1: Schematic of two different bioretention systems: (a) conventional and (b) modified

Although modified bioretention systems achieve high TN removals in studies with urban runoff, limited TN removal was observed in prior studies treating dairy farm runoff (Ergas et al.,...
Dairy runoff has high DON and TAN concentrations compared with urban runoff. During storm events, these pollutants are transported through the bioretention media with the runoff and are not retained long enough for complete ammonification and nitrification. Therefore, research should be carried out to overcome these limitations by amending sand based bioretention media with adsorbent materials that have a high adsorption capacity for DON and TAN. One of the most promising low-cost adsorbent materials for this purpose is biochar (Suliman et al., 2016; Laird et al., 2010; Rahman et al., 2020).

Biochar is by-product of pyrolysis of waste organic materials, such as wood waste, rice hulls, grasses or manure, at temperatures between 300-1000°C in an oxygen-limited environment. Properties of biochar include a high specific surface area (SA), cation exchange capacity (CEC), porosity and water holding capacity. Biochar has been widely used as an agricultural soil amendment (Laird et al., 2010) and for water treatment (Mukherjee et al., 2011). Several prior studies showed that amendment of bioretention media with biochar improved their performance for treatment of urban runoff (Tian et al., 2016; Afrooz et al., 2017; Rahman et al., 2020). The high SA and CEC of biochar helps to retain DON and NH$_4^+$, allowing a longer residence time for microbial transformations (Tian et al., 2016). In addition, the higher water and nutrient retention capacity of biochar amended bioretention media enhances microbial activity and plant growth.

The overall goal of this research is to understand N removal mechanisms and develop guidelines for amending modified bioretention systems with biochar for treatment of dairy runoff. Four pilot-scale modified bioretention systems were set up in the botanical gardens at the University of South Florida (USF), with and without biochar and with and without plants. The systems were operated with semi-synthetic dairy runoff and monitored for N-species and organic carbon transformations.
5.2 Materials and Methods

5.2.1 Dairy Runoff Preparation

Fresh liquid dairy manure was collected from South Tampa Farm in Tampa, FL. Manure was mixed with stormwater from a stormwater pond on the USF campus in a 200 L tank and allowed to settle overnight. Supernatant was screened through a 0.25 mm mesh, mixed with additional pond water (60% supernatant/40% pond water) and stored in a 250 L rain barrel. Target concentrations of N-species and *E. coli* were 35 mg/l NH$_4^+$-N, 1.0 mg/l NO$_3^-$-N, 45 mg/l DON and 1x10$^6$ CFU *E. coli* /100 ml, which was similar to livestock runoff composition in prior studies (Ergas et al., 2010; Hu et al., 2011; Andrews, 1992). Additional details can be found in FDACS final report (FDACS 2021).

5.2.2 Porous Media

Detailed information on the sand and biochar used in this study was published previously (Rahman et al., 2020). Briefly, masonry sand, with a hydraulic conductivity of 13.2 cm/hr was purchased from Seffner rock and gravel, Tampa, FL. Biochar was generously donated by Biochar Supreme (Loveland, CO). Physicochemical properties of biochar, including SA, CEC, pore volume, bulk density and porosity are presented in the results section.

5.2.3 Modified Bioretention Systems

Four modified bioretention systems were constructed: i) sand media (S), ii) sand media with plants (SP), iii) biochar amended sand media (B) and iv) biochar amended sand media with plants (BP) (Figure 5.2). The total depth of each bioretention system was 102 cm. From the bottom there was: i) 7.6 cm downgraded white river gravel (3/4 inch); ii) 30.5 cm IWSZ; iii) 45.7 cm filter medium; iv) 2.5 cm gravel layer ($\frac{1}{2}$ inch) and v) 15.2 cm free board as a ponding layer at the top. A filter fabric was placed in between the drainage layer and IWSZ layer to avoid wash out of fine
particles from the system. A perforated PVC underdrain pipe with an upturned outlet elbow was used to create an IWSZ. Note that the IWSZ did not contain wood chips due to the high DOC content (737 ± 200 mg/l) of the dairy runoff. For the B and BP systems, the biochar fraction was 35% in the filtration media and 45% in the IWSZ. SP and BP systems were planted with *Muhlenbergia capillaris* (Muhly Grass), which was purchased from a local nursery. *Muhlenbergia capillaris* is a native Florida perennial, that attracts wildlife and has favorable light and moisture requirements, growth rate and mature plant height and spread. After planting, the systems were watered periodically for three months for the growth of roots and biomass before performing dairy runoff experiments.

![Figure 5.2: Cross-sectional diagrams of (a) sand modified bioretention cell with plant (SP), (b) sand modified bioretention cell (S), (c) biochar amended sand modified bioretention cell with plant (BP) and (d) biochar amended sand modified bioretention cell (B) (units are in cm).](image)

5.2.4 Experimental Design

Dairy runoff experiments reported in this article were performed at a hydraulic loading rate (HLR) of 0.98 cm/min (flow rate of 222 ml/min). This HLR was selected by assuming a 0.25-inch rainfall event over 4-hrs and that the bioretention surface occupied 5% of the drainage area.
All experiments reported were carried out at a 7-day antecedent dry period (ADP). The ADP is the time between two successive runoff events.

5.2.5 Water Quality Analysis

Influent and effluent samples were analyzed using Standard Methods (APHA et al., 2018). TAN and NOx (NO$_3^-$-N + NO$_2^-$-N) were measured using a Timberline Ammonia Analyzer (Timberline Instruments, Boulder, CO). TN and total organic carbon (TOC) were measured with a Shimadzu TOC-V CSH TOC/TN Analyzer (Shimadzu Scientific Instruments, Columbia, MD). DON was calculated by subtracting total inorganic nitrogen (TIN = TAN + NOx) from TN. Method detection limits for TAN, NOx, TN and TOC were 0.05 mg/l, 0.05 mg/l, 0.03 mg/l, and 0.11 mg/l, respectively. pH and conductivity were measured using a multiparameter meter and calibrated probes. Effluent flow rates were measured volumetrically to assess the hydraulic performance.

5.3 Results and Discussion

5.3.1 Biochar Characteristics

The feedstock used for biochar production was shredded woodchips, which was pyrolyzed at ~900°C. Analysis of the biochar elemental composition showed that it was composed of 80% carbon, 0.4% nitrogen and 9.6% oxygen. Due to its high ash content (5.8%), the biochar had a high pH (10.12±0.2), which is favorable for nitrification. The biochar had high surface area (537±60.15 m$^2$/g) and CEC (10.57 cmol/kg), which favors DON and NH$_4^+$ adsorption. It also had a low bulk density (0.10 g/cm$^3$) and high-water holding capacity (874 gH$2$O/100 g biochar). The high pore volume 0.36 cm$^3$/g included 0.19 cm$^3$/g micro-pore volume and 0.15 cm$^3$/g meso-pore volume.
5.3.2 Overall Performance of Modified Systems

Average influent concentrations of N species in semi-synthetic dairy runoff were TAN: 26.1±9.5 mg/l, NOx: 0.063±0.04 mg/l, DON: 42.7±18.1 mg/l, and TN: 68.8±19.2 mg/l. Relatively higher influent TOC concentrations (737.5±199.4) were observed compared to prior studies. N-species removal efficiencies for the four modified bioretention systems are shown in Figure 5.3. Higher TAN removal was observed in biochar amended systems compared with un-amended systems, with the highest (90.6%±6.5) and lowest (68.2%±20.8) removal efficiencies observed in BP and S systems, respectively. The high CEC of biochar likely resulted in TAN retention, allowing more time for nitrification compared with the un-amended systems. Lower average effluent NOx concentrations were observed for biochar amended systems (0.72-1.18 mg/l) than sand systems (2.09-3.15 mg/l). As influent dairy runoff had high organic carbon content, it was hypothesized that TOC retained in the IWSZ due to adsorption onto biochar was utilized as electron donor for denitrification. In S and SP, the lack of adsorbed TOC in the IWSZ likely limited denitrification.

DON removal largely depends on either adsorption or ammonification followed by nitrification. As biochar enhances soil microbial activity due to its high surface area and porosity (Anderson et al., 2011), therefore, enhanced adsorption and ammonification resulted in higher DON (<99%) removal in B and BP. Average effluent DON concentrations for biochar amended bioretention systems were 0.07-0.16 mg/l, which was lower than un-amended systems (5.03-5.67 mg/l). TN removal was limited in S (76.89%±18.2) and SP (76.26%±17.72) bioretention systems compared to B and BP due to low TAN and DON adsorption and limited denitrification.
Figure 5.3: Overall N-species removal efficiency (a: TAN; b: NO3-; c: DON; d: TN and e: TOC) for four modified bioretention systems (BP: biochar with plant bioretention; B: biochar amended bioretention; SP: sand amended bioretention with plant and S: sand bioretention system)

5.3.3 Pollutant Breakthrough during Storm Events

Effluent TAN and TN concentration profiles over time for the four bioretention systems for a 4.5-hour storm event are shown in Figure 5.4. As discuss previously, TAN removal mainly depends on i) media adsorption, ii) nitrification and iii) plant uptake. During the dry days between successive runoff events, pore water was replaced by oxygen in the unsaturated zone of the bioretention systems, thus the adsorbed TAN was nitrified to NO3-, resulting low effluent TAN concentrations. During the first 90 minutes, both B and BP had low average effluent TAN concentrations (0.93-0.97 mg/l) compared with S (17.3 mg/l) and SP (2.02 mg/l). Ergas et al. (2010) also observed limited nitrification in modified sand bioretention systems treating dairy runoff that included a sand-based unsaturated zone. Once the pore water in the IWSZ was flushed
from the systems (90-270 minutes), effluent TAN concentrations in SP increased and were almost similar to S by the end of the experiment. Saturated condition that developed in the aerobic layer for last hour of the runoff experiments due to water accumulation in the ponding zone resulted in limited nitrification and therefore higher effluent TAN concentrations were observed in S and SP. However, B and BP systems maintained relatively low effluent TAN concentrations throughout the experiment due to the high affinity of biochar to adsorb positively charged NH$_4^+$ ions.

Effluent TN concentrations for B and BP systems followed the same breakthrough trends. During the ADP between two rain events, adsorbed TOC was bioavailable in the IWSZ, and denitrifying bacteria utilized the desorbed TOC for denitrification. Hence, in B and BP during the first 90 minutes, effluent TN concentrations were low, and then slowly increased until the end of the experiment. S and SP had higher effluent TN concentrations from the beginning of the experiment, indicating that limited TOC availability in the IWSZ resulted in lower NO$_3^-$ removal. In addition, DON adsorption and ammonification was low (data not shown).

![Figure 5.4: Pollutant breakthrough curve of (a) TAN and (b) TN for four modified bioretention systems considering 222 ml/min flow rate for 4.5 hours dairy runoff experiment.](image)

5.3.4 Effect of Plants

The effect of plants on N-species removal for bioretention systems with or without plants can be seen in Figures 5.3 and 5.4. Both systems with plants achieved higher N-species removal efficiencies compared to systems without plants. Prior research with planted and unplanted
bioretention systems also showed that both TAN and NO$_3^-$ are taken up by plants (Zhang et al., 2011; Lea et al., 2001). Denitrification is also favored by enhanced microbial activity and the availability of organic carbon in the rhizosphere due to the presence of root exudates and sloughed-off root tissues (Havlin, 2013).

![Figure 5.5: Photographs of two modified bioretention systems (a) sand with plant, (b) biochar with plant after twelve runoff experiments.](image)

As shown in Figure 5.5, after ten months of operation, the biochar amended bioretention system with plants (BP) had higher biomass growth compared to SP. It has been shown in prior agricultural studies (Karhu et al., 2011) biochar helps to promote plant growth by retaining moisture and nutrients and stimulating the activity of beneficial microorganisms. Future studies will be carried out to quantify the plant biomass and root growth after dismantling the bioretention systems.

### 5.4 Conclusion

Nitrogen removal mechanisms were investigated in modified bioretention systems with and without biochar amendment and with and without plants. Addition of biochar enhanced the
TAN and DON removal during infiltration. Higher TOC adsorption in the IWSZ in systems with biochar favored denitrification, resulting in higher TN removal. Due to high moisture and nutrient retention, better plant growth was observed in the biochar amended system with plants, which also influences N-species removal. Current research is focused on investigating N-species and *E. coli* removal in these systems under varying HLR and ADP.
Chapter 6: Conclusions

Effective management of both nitrogen and FIB in urban and agricultural runoff is important to prevent eutrophication and microbial contamination of water bodies. As non-point sources are the largest contributor of pollutants to aquatic environment, LID technologies, especially bioretention systems, could be a promising mitigation strategy for stormwater management. Therefore, amending the media with biochar could be a promising design for improving water quality. This research was motivated by the need to study biochar amended bioretention systems (both conventional and modified). Side-by-side laboratory- and pilot-scale studies were conducted to investigate N-species and FIB removal mechanisms and provide design guidelines for implementation by practitioners. The following research questions were addressed in this dissertation:

1. How do physicochemical properties of biochar and rate of biochar amendment affect biological nitrogen transformations in biochar amended bioretention systems?

In abiotic batch experiments, significantly higher \(\text{NH}_4^+\) adsorption was observed for biochar (3.5 mg/g) than sand (0.05 mg/g) due to higher CEC. Both biochar (0.24-0.3 adsorbed \(\text{NO}_3^-\) mg/g of biochar) and sand (0.25 adsorbed \(\text{NO}_3^-\) mg/g of sand) had very low \(\text{NO}_3^-\) adsorption capacity. Laboratory-scale column studies showed that 30% (v/v) biochar amendment was adequate for removal of TAN (>99%) from urban runoff due to biochar’s high CEC and pH. TAN adsorption increased nitrification during the ADPs when aerobic conditions developed within the media. DOC removals were significantly higher in biochar amended (>88%) than sand (>74%)
columns due to greater SA and pore volume of biochar. MC data revealed that saturated conditions prevailed toward the bottom of biochar-amended columns for several days, favoring denitrification and TN removal compared to the pure sand column.

The higher biochar amendment rate increased the surface charge availability for \( \text{NH}_4^+ \) adsorption, therefore, greater TAN adsorption was observed in the column with the higher biochar amendment rate compared to lower biochar amendment rate column. DON removal was significantly increased in the higher biochar amendment rate column (67%) than the lower biochar amendment rate column (38%) due to greater adsorption capacity, which resulted in greater ammonification and nitrification-denitrification.

2. How do physicochemical properties of biochar and rate of biochar amendment affect FIB removal in biochar amended bioretention systems?

Urban stormwater column studies showed that \( E. \ coli \) removal was a strong function of SA; greater than 6 log \( E. \ coli \) removal was observed in the column amended with higher SA biochar. Enhanced \( E. \ coli \) removal in biochar amended columns could also be explained by DLVO theory, which combines attractive van der Waal forces and repulsive electrostatic forces. To attach to negatively charged biochar surface, \( E. \ coli \) must overcome the electrostatic repulsion between biochar and \( E. \ coli \). A reduced zeta potential is expected for biochar amended columns compared with the sand column as biochar increases the pore water pH. Moreover, biochar has a high organic carbon content, therefore the hydrophobic attraction between \( E. \ coli \) is higher for biochar than sand.

The effect of different biochar amendment rates on \( E. \ coli \) removal largely depends on infiltrated pore volumes of each runoff event. As the column with the higher biochar fraction had higher MC, favorable conditions developed that accelerated the regrowth of \( E. \ coli \) during ADPs
leading to lower *E. coli* removal (log 1.94 ± 0.18) compared the column with the lower biochar fraction (log 2.07 ± 0.24) based on the first flush. Moreover, for shorter ADPs, adsorbed DOC might not be completely biodegraded prior to the next storm event or there might be competition for SA with other microbes, resulting reduced *E. coli* removal for different biochar fractions. Interestingly, higher the biochar amended column had greater *E. coli* removal (log 1.63 ± 0.37) compared to lower amended column (log 1.27 ± 0.34) due to competition among different microbes during ADPs or less remobilization of attached *E. coli* during successive runoff event.

3. How does the composition of the influent (e.g., urban stormwater compared with dairy runoff) affect N-species and FIB removal in biochar amended bioretention systems??

The complex influent in dairy runoff contains Ca$^{2+}$, Mg$^{2+}$, Al$^{3+}$ and Fe$^{2+}$ compared to urban stormwater runoff. Moreover, higher TAN, DOC and suspended particles concentrations were observed for dairy runoff. The pH of the dairy influent (7.25±0.2) helps in deprotonation of the carboxyl and phenolic groups on the biochar surface and precipitates aluminum and iron oxides, creating an opportunity for functional groups to adsorb NH$_4^+$. The O$_2$ availability in dairy runoff limited nitrification, therefore, low effluent NOx concentrations were observed from different biochar amended rate systems compared with urban runoff. Moreover, aerobic heterotrophs degrading influent DOC compete with nitrifiers for O$_2$, thus inhibiting nitrification in dairy runoff. Transfer of O$_2$ from the atmosphere near the top surface facilitates nitrification, whereas sustained saturation in the bottom can promote denitrification as DOC acting as carbon source and electron donor for denitrification. TN removal was significantly higher form dairy runoff during the biochar amended column studies compared to urban stormwater runoff experiments. Moreover, DON concentration was relatively high in dairy runoff compared with urban runoff, therefore, DON removal was low in dairy runoff column studies.
Higher *E. coli* (>1.7-1.76 log removal) removals were observed compared with *Enterococci* (>1.09-1.21 log removal) in dairy runoff, indicating a greater attachment affinity to the biochar surface for *E. coli* compared to *Enterococci*. Although higher biochar amended biofilter had a higher SA and hydrophobicity than lower biochar amended column, there was no significant difference in *E. coli* or *Enterococci* removals between the two columns in complex dairy runoff. This could be due to complex runoff matrix in dairy runoff (DOC =762 mg/l and DON= 58 mg/l), which have a high affinity for surface attachment to biochar.

4. How does the rate of biochar amendment affect the hydraulic performance of bioretention systems?

At the surface, higher MC was observed for lower biochar amended column due to lower evaporation rate compared to higher biochar amended column. However, due to gravitational drainage, increased fraction of micro-pore and high-water holding capacity of biochar, higher biochar amended column had higher MC (0.58 cm³/cm³) compared to lower biochar amended column (0.51 cm³/cm³).

Tracer breakthrough curves showed that a longer HRT was achieved with a higher biochar fraction. Model simulations of MC showed that the high microporous structure of the biochar increases the time needed to reach full saturation, increases porosity (especially micro-porosity) and lowers the saturated conductivity. The changes in hydraulic properties increased the HRT in biochar amended columns, thus increasing the length of time the solution is in contact with the treatment media.
5. How does the presence of an IWSZ and plants affect the long term performance of N-species and *E. coli* removal in modified bioretention systems amended with biochar?

Higher DOC adsorption in in systems with both biochar and an IWSZ favored denitrification, resulting in higher TN removal in biochar amended bioretention systems, with or without plants, compared to un-amended bioretention systems with and without plants. Due to high moisture and nutrient retention, better plant growth was observed in the biochar amended modified bioretention system compared with the un-amended bioretention system, which also influenced N-species removal. The inclusion of plants, biochar and an IWSZ in pilot-scale systems resulted in the best *E. coli* removal performance. Plants can improve *E. coli* removal through predation and competition by rhizosphere microbes or inactivation by antimicrobial compounds from root exudates. Higher *E. coli* removals in pilot units with an IWSZ may have been due to the longer retention times and/or the anoxic conditions present in the IWSZ.

Future research should be carried out considering other pollutants i.e. phosphorous, metals, pesticides and viruses, which also cause water quality impairment. In addition, it is recommended that regular maintenance be carried out in biochar amended bioretention systems by replacing the top soil layer with new material. This will avoid clogging and loss of hydraulic performance. Therefore, pilot- and field-scale studies should be carried out on optimal maintenance procedures and frequency. Altering the layers, including coated or uncoated biochar, with different particle sizes amended with sand could be investigated further (Appendix A, Figure A.3). Simulations of water flow in bioretention systems with different layers should be done using HYDRUS-1D. After calibration and validation, the model could be used to simulate different site-specific case studies and help to determine bioretention systems performance for different non-point source pollution mitigation scenarios.
Implementation of green stormwater infrastructure to mitigate water pollution can result in many economic, environmental, and health benefits, and improvements in ecosystem services if designed and implemented properly. With the knowledge gained through this research, it is hoped that the adoption of biochar amended bioretention systems will be made more accessible for designers, decision makers, and other practitioners for nonpoint source pollution mitigation. Being able to properly design a bioretention system to meet water quality standards may allow government agencies to provide incentives and credits for implementing these systems.
References


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Appendix A: Experimental Setup

Figure A1: Experimental setup of the lab scale experiment for three different media for urban runoff management

Figure A2: Experimental setup of the pilot scale experiments for dairy runoff management
Figure A.3: Future recommended works for layered systems on biochar amended bioretention systems
Appendix B: Urban Stormwater Runoff

Table B.1: Physicochemical characterization of media

<table>
<thead>
<tr>
<th>Physicochemical Parameters</th>
<th>Porosity (%)</th>
<th>Moisture content (%)</th>
<th>% of sand</th>
<th>Hydraulic conductivity (cm/hr)</th>
<th>CEC (cmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Course</td>
<td>Medium</td>
<td>Fine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concrete Sand</td>
<td>30±0.8</td>
<td>20.3±1.2</td>
<td>0.51</td>
<td>60.38</td>
<td>39.1</td>
</tr>
<tr>
<td>Masonry Sand</td>
<td>35±0.95</td>
<td>23.2±0.9</td>
<td>0.27</td>
<td>9.51</td>
<td>90.1</td>
</tr>
<tr>
<td>Local Sand</td>
<td>40±1.2</td>
<td>32.6±1.2</td>
<td>0.78</td>
<td>3.83</td>
<td>95.8</td>
</tr>
</tbody>
</table>

Table B.2: Effect of three different flow rates/HRTs of three different columns for contaminants removal based on mass removal

<table>
<thead>
<tr>
<th></th>
<th>TAN</th>
<th>NOx</th>
<th>Org-N</th>
<th>TN</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>High HRT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAND</td>
<td>98.6 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.1 ± 13.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>42.2 ± 10.9&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>43.2 ± 6.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>99.901 ± 0.1&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBC1</td>
<td>99.8 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8 ± 9.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>61.9 ± 9.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.5 ± 6.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>100 ± 0.1&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBC2</td>
<td>99.5 ± 0.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.0 ± 17.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>63.6 ± 5.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.0 ± 7.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>99.98 ± 0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Medium HRT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAND</td>
<td>87.3 ± 6.8&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>17.4 ± 24.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>43.7 ± 11.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>41.4 ± 14.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>99.69 ± 0.2&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBC1</td>
<td>98.8 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.3 ± 7.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>61.0 ± 3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.1 ± 2.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>100 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBC2</td>
<td>98.9 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.0 ± 11.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>55.6 ± 8.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.4 ± 7.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>99.95 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low HRT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAND</td>
<td>66.6 ± 35.2&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>13.8 ± 33.9&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>25.9 ± 19.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>33.6 ± 10.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>99.73 ± 0.2&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBC1</td>
<td>98.8 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.10 ± 17.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.7 ± 8.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>40.9 ± 3.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>100 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBC2</td>
<td>99.6 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.7 ± 22.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.9 ± 16.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>43.5 ± 10.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>99.96 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Figure B1: Influent and Effluent Relationship for three different HRTs for three columns
Appendix C: Dairy Runoff

Figure C1: *E. coli* log removal performance for four pilot systems including BP, B, SP and S.

Figure C.2: Flow rates of four pilot systems including BP, B, SP and S during the experiment.
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