

Transport of *Escherichia coli*, *Salmonella typhimurium*, and Microspheres in Biochar-Amended Soils with Different Textures

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The incorporation of biochar into soils has been proposed as a means to sequester carbon from the atmosphere. An added environmental benefit is that biochar has been shown to increase soil retention of agrochemicals, and recent research has indicated that biochar may be effective in increasing soil retention of bacteria. In this study we investigate the transport behavior of *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium, and carboxylated polystyrene microspheres in water-saturated column experiments for two soils (fine sand and sandy loam) amended with 2% poultry litter or pine chip biochars pyrolyzed at 350 and 700°C. Adding poultry litter biochar pyrolyzed at 350°C did not improve soil retention of either bacteria in fine sand and even facilitated their transport in sandy loam. Addition of either biochar pyrolyzed at 700°C generally improved retention of bacteria in fine sand, with the pine chip biochars being more effective in limiting their transport. Results from the column studies and auxiliary batch studies suggest that changes in cell retention after biochar amendments were likely due to changes in bacterial attachment in the column and not to physical straining or changes in survivability. We also found that changes in bacterial hydrophobicity after biochar amendments were generally correlated with changes in bacterial retention. The influence of biochar amendment in increasing retention of both bacteria was generally more pronounced in fine sand and indicates that soil texture affects the transport behavior of bacteria through biochar-amended soils.

SURFACE APPLICATION is the most common method of disposing of farm-generated manure and wastewater (USDA, USEPA, 1999). In some cases, these wastes may contain zoonotic bacteria, such as *Salmonella typhimurium* and *Escherichia coli* O157:H7. Because these bacteria can survive close to the surface of a manure-amended soil profile for weeks (Semenov et al., 2009), there is the potential for contamination of farm produce (Ackers et al., 1998; Jacobsen and Bech, 2012) and surface and groundwater drinking water sources (Momba et al., 2006), potentially leading to human infection. In fact, human infection by *Salmonella* sp. and *E. coli* O157:H7 are among the leading zoonotic bacteria-related illnesses in the United States (CDC, 2010).

Pathogenic microorganisms in surface-applied manure and wastewater can contaminate groundwater if leached downward through the soil profile (Jamieson et al., 2002; McMurry et al., 1998). Their downward transport through soils is affected by soil physical properties, such as soil texture, structure, and degree of water saturation (Mosaddeghi et al., 2009; Unc and Goss, 2004), and by chemical properties, including solution ionic strength and composition, soil and solution pH, and the concentration of organic carbon in solution and sediment phases (Foppen et al., 2008; Harvey et al., 2011; Johnson and Logan, 1996; Stevik et al., 2004). Management practices that alter these properties may significantly affect leaching of bacteria through soil. One such management practice is soil amendment with biochar, a charcoal-like material generated during the thermal degradation of organic matter in the absence of air (pyrolysis).

The incorporation of biochars into soil has been widely recognized as an effective means of sequestering carbon (Kookana et al., 2011; Spokas et al., 2012) and has potential for use in enhancing the soil retention of agrochemicals (Cao

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J. Environ. Qual. 43:371–378 (2014)
doi:10.2134/jeq2013.06.0236

Received 18 June 2013.

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Abbreviations: CFU, colony-forming unit; DOC, dissolved organic carbon; HTPC, high-temperature pine chips; HTPL, high-temperature poultry litter; LTPC, low-temperature pine chips; LTPL, low-temperature poultry litter; PR, recent recovery; TOC, total organic carbon.

et al., 2009), heavy metals (Uchimiya et al., 2011), and excess nutrients (Laird et al., 2010). In recent studies, we established that that addition of biochars can significantly affect retention of *E. coli* in fine sand soils (Abit et al., 2012; Bolster and Abit, 2012). We found that the type of feedstock and pyrolysis temperature of the added biochars significantly influenced *E. coli* transport in soils. However, these studies only used sandy soils with very minimal clay content. Further research is needed because clay content may modify the effect of biochar on soil chemical and physical properties known to influence bacterial retention. For instance, how biochar affects soil and solution pH may differ depending on soil texture because soil buffering capacity varies with clay content (Essington, 2003). Organic matter levels in soils are also influenced by clay content (Burke et al., 1989; Parton et al., 1987). Moreover, clayey soils have higher specific surface area than sands (Hillel, 1998). Because bacterial retention is largely a surface phenomenon, biochar addition may have a greater impact on bacterial transport in sands than in clays. If biochar incorporation is to be adapted as a management strategy to limit the leaching of zoonotic bacteria from surface-applied farm wastes, then it is important to establish under what conditions (e.g., soil texture) biochar is most effective. Hence, this study was designed to evaluate the transport of *E. coli* O157:H7, *S. typhimurium*, and polystyrene microspheres in fine sand and sandy loam amended with poultry litter and pine chip biochars pyrolyzed at two different temperatures.

Materials and Methods

Soil, Soil–Biochar Mixtures, and Column Preparation

Air-dried and sieved (2 mm) fine sand and sandy loam soils were used in the experiments. Soil texture was determined by the hydrometer method (Gee and Or, 2002). The two soils had similar pH, but clay content and specific surface area of the sandy loam were considerably higher than those of the fine sand (Table 1). Four biochars were produced from two feedstocks—poultry litter and pine chips—each separately pyrolyzed for 2 h under nitrogen at 350 and 700°C. Details of the pyrolysis system and methods can be found in Cantrell and Martin (2012). Each biochar was separately mixed with the two soils at 2% (w/w).

Table 1. Selected properties of soil material and biochars used in this study.

Soil material/ biochar	Specific surface† m ² g ⁻¹	pH	Clay content‡ %
Soil			
Sandy loam	4.49 (0.18)§	6.64 (0.03)	12.5
Fine sand	0.41 (0.03)	6.47 (0.18)	0.5
Biochar			
Poultry, 350°C	2.31 (0.15)	8.38 (0.25)	
Poultry, 700°C	25.1 (0.62)	10.1 (0.10)	
Pine chip, 350°C	1.06 (0.14)	6.8 (0.28)	
Pine chip, 700°C	78.3 (0.91)	8.18 (0.11)	

† Specific surface of the soil materials was measured using the Brunauer–Emmett–Teller method.

‡ Clay content was measured by the hydrometer method.

§ Values in parentheses are SD.

This resulted in eight separate treatments plus two controls (unamended soils). The porous materials were homogenized on a roller for 72 h. They were analyzed for pH (in 1 mmol L⁻¹ KCl) and total organic carbon (TOC) by dry combustion using a vario MAX CN analyzer (Elementar Americas Inc.). Specific surface of the soil materials and biochars used in the experiment was measured by the Brunauer–Emmett–Teller method using the N₂ adsorption multilayer theory with a Nova 2200e surface area analyzer (Quantachrome). Size distribution of the soils and soil–biochar mixtures was measured with a Mastersizer 2000 (Malvern Instruments).

Chromaflex chromatography columns (2.5-cm inside diameter) (Kontes Glass Co.) were dry-packed to a height of 10 cm by slowly pouring the appropriate porous material in 2-cm sections at a time while the column was being vibrated. After packing, ~18 pore volumes of CO₂ were introduced through the inlet tubing. Fourteen pore volumes of unbuffered (pH ~5.6) 1 mmol L⁻¹ KCl were then passed through the lower end of the column at a rate of 2.67 mL min⁻¹ using a peristaltic pump, collecting the final four pore volumes of effluent as background solution. Background samples were analyzed for pH (Orion pH probe, Thermo Electron Corp.); specific conductivity (YSI 556 Multi-Probe, YSI Environmental); dissolved organic carbon (DOC) by loss on ignition (LiquiTOC, Elementar Americas Inc.); Ca, Fe, Mg, and Na by inductively coupled plasma–optical emissions spectroscopy (Vista Pro, Varian Inc.); and Cl, K, PO₄-P, and SO₄-S by ionic chromatography (ICS 3000, Dionex Corp.)

Bacterial Suspension Preparation

Escherichia coli O157:H7 (ATCC 43888; nonproducer of Shiga-like toxins I or II) and *Salmonella enterica* serovar Typhimurium (ATCC 13311) were used in this study. Forty microliters of overnight Luria–Bertani broth culture of each bacterium was inoculated in 40 mL Luria–Bertani broth and grown in a rotisserie incubator at 37°C until reaching the midexponential growth phase (3.7 h for *E. coli* and 4 h for *S. typhimurium*). The cultures were centrifuged at 4°C for 15 min at 3700 × g. The cell pellet was resuspended and washed three times in 1 mmol L⁻¹ KCl and then diluted to a bacterial influent suspension concentration of ~1 × 10⁷ colony-forming units (CFUs) mL⁻¹.

Bacterial Transport Experiments

Bacterial suspensions were applied to the columns using a syringe pump at 0.67 mL min⁻¹ (Darcian flux of ~0.14 cm min⁻¹) for 38 min followed immediately by application of bacteria-free 1 mmol L⁻¹ KCl at the same rate for 72 min. Bulk effluent samples were collected after 20, 75, and 110 min (end) from the start of each experiment. Duplicate 1-mL samples were drawn from each bulk sample for preparation of dilutions ranging from 10⁰ to 10⁻⁴. Using the drop-plate technique (four 10-μL drops per diluted sample), the diluted effluent samples from *E. coli* experiments were plated on mFC Agar plates (Difco Laboratories Inc.), and those involving *Salmonella* were plated on XLD Agar plates (Difco Laboratories Inc.). Colony-forming units were counted after the plates were incubated overnight at 37°C. Bacterial transport experiments using unamended soil and those amended with poultry litter

biochars were conducted in triplicate, whereas those amended with pine chip biochars were conducted only in duplicate due to a shortage of pine chip biochars. Experiments involving the same bacteria–porous material combination were repeated on different days to ensure true replication. After completion of the transport experiments, the columns were dissected in 2-cm sections. Extraction and enumeration of bacteria recovered from each section were performed as in our previous study (Abit et al., 2012).

Microsphere Transport Experiments

A suspension of 1- μm diameter carboxylated polystyrene latex microspheres (Molecular Probes) was applied to the columns using the same column set-up, flow rate, and buffer solution used in the bacterial transport studies. These experiments were limited to soil only and to low-temperature (350°C) poultry litter (LTPL) and high-temperature (700°C) pine chip (HTPC) biochar treatments. The initial concentration of the microspheres was $\sim 8 \times 10^7$ spheres mL^{-1} . Effluent microsphere concentrations were measured with a SpectraMax GEMINI EM microplate spectrofluorometer (Molecular Devices) using an excitation filter of 475 nm, emission filter of 520 nm, and a cutoff filter of 515 nm. Experiments involving the same microsphere–porous material combination were repeated on different days. After each transport experiment, the spatial distribution of the microspheres within the column was enumerated using the dissection method described above.

Single-Point Sorption Experiments

Two grams each of the porous materials were placed in designated preweighed centrifuge tubes. Twenty milliliters of 1 mmol L^{-1} KCl solution having a bacterial concentration of $\sim 1 \times 10^7$ cells mL^{-1} or microspheres of $\sim 7 \times 10^7$ spheres mL^{-1} was added to the tubes. The tubes were reweighed and shaken in a reciprocating shaker at 100 oscillations min^{-1} for 1 h to be consistent with the resident time of the bacteria in the column. The mixture was centrifuged at $200 \times g$ for 5 min at 4°C to settle out some of the suspended soil particles before sampling for the aqueous concentration of bacteria and microspheres. (This mild centrifugation did not significantly affect concentrations in control centrifuge tubes without soil.) Bacterial concentrations in solution were determined by plating appropriate dilutions on mFC or XLD agar plates and incubated overnight at 37°C. Microsphere concentrations were determined by fluorescence microscopy. Sorbed concentrations were determined by calculating the difference between the initial and final concentrations and the oven-dried mass of the soils. Single-point sorption coefficients (K) were computed by dividing the sorbed concentrations by the concentrations remaining in solution. Triplicate sorption experiments were conducted on different days.

Bacterial Survival Evaluation

Assessment of bacterial survival was conducted concurrent to each transport experiment. One milliliter of the bacterial suspension used in the transport experiment was added to a culture tube containing 9 mL of background effluent (collected before bacterial suspension application). The resulting 1:10

suspension was homogenized in a vortex shaker for 10 s, from which 1 mL was immediately drawn, diluted, and plated on mFC or XLD agar. A 1-mL sample was drawn from the same mixture at the end of a transport experiment. This sample was diluted and plated to quantify cells that remained culturable at the end of an experiment.

Bacterial Surface Characterization

The electrophoretic mobility of the *E. coli* and *S. typhimurium* cells in leachate collected from representative columns was measured at 25°C using a ZetaPALS analyzer (Brookhaven Instruments Corp.). The experimentally determined electrophoretic mobility values were converted to zeta potential values using the Smoluchowski equation (Elimelech et al., 1995). The hydrophobicity of the cells in each leachate was measured by the microbial adhesion to hydrocarbon (MATH) test (Pembrey et al., 1999), where the partitioning of cells between n-dodecane (Fisher Scientific) and the leachate were determined spectroscopically.

Data Analysis

One-way ANOVA was performed to identify statistically significant differences in measured parameters. Mean separations were performed using Tukey's HSD test. All statistical analyses were performed using JPM ver. 7.0 (SAS Institute, 2008), and differences were considered significant at $p < 0.05$.

Results and Discussion

Soil and Solution Properties

The addition of poultry litter biochar to both soils significantly increased soil pH and total organic carbon (TOC) (Table 2), with a greater increase observed with the addition of biochars pyrolyzed at 700°C. This was expected because the biochars pyrolyzed at 700°C have higher pH (Table 1), findings consistent with other studies (Cantrell et al., 2012; Novak et al., 2009). Apart from the addition of HTPC to fine sand, no significant change in pH of the porous materials after the addition of pine chip biochars was observed (Table 2). Despite the similar pH of the two unamended soils, biochar additions had a less pronounced effect on the pH of the sandy loam, likely because the higher clay content (Table 1) resulted in greater pH buffering capacity in the sandy loam (Essington, 2003). The biochars had 18 to 74% carbon content, and their addition resulted in at least a sevenfold increase in TOC in the biochar-amended soils (Table 2).

Biochar addition affected several water quality parameters in the column effluent. Trends in pH of the column effluents generally agreed with trends in pH of the porous materials (i.e., porous materials with higher pH coincided with higher effluent pH). For both soils tested, the addition of poultry litter biochar resulted in a significant increase in specific conductivity. Moreover, poultry litter biochar addition to both soils led to higher $\text{PO}_4\text{-P}$ concentration in the effluent; however, no such increase was observed with the pine chips biochar treatments. These results agree with our previous research showing that amendment with 2% poultry litter biochar increased solution pH and $\text{PO}_4\text{-P}$ concentration, whereas the addition of 2% pine chips biochar did not (Abit et al., 2012). Only the addition

Table 2. Selected characteristics of the soil–biochar mixtures and column effluent.

Characteristic†	Fine sand					Sandy loam				
	Control	Poultry litter		Pine chips		Control	Poultry litter		Pine chips	
		350°C	700°C	350°C	700°C		350°C	700°C	350°C	700°C
Porous materials										
pH	6.64ef‡	8.65b	10.2a	6.92de	7.30cd	6.47f	7.42c	8.44b	6.31f	6.50f
TOC, %	0.04e	0.58cd	0.78bc	0.62cd	1.3ab	0.14de	1.1abc	1.4ab	1.2ab	1.5a
Background effluent										
pH	7.15bcd	7.58ab	8.27a	6.15e	6.32de	6.45de	7.33bc	7.57ab	6.62cde	6.97bcde
SpC, mS cm ⁻¹	0.15e	0.23abc	0.23ab	0.15cde	0.15bcde	0.12e	0.24a	0.25abcd	0.15de	0.16bcde
DOC, mg L ⁻¹	2.55b	8.85ab	5.66ab	9.39ab	7.44ab	2.55b	6.80ab	2.74b	7.82ab	11.2a
PO ₄ -P, mg L ⁻¹	0.25c	4.93b	5.06b	0.56c	0.60c	B.D.§	6.30ab	7.84a	B.D.	B.D.

† DOC, dissolved organic carbon; SpC, specific conductivity; TOC, total organic carbon.

‡ Mean values in each row followed by the same letters are not significantly different using Tukey's honestly significant difference test at $p < 0.05$.

§ Below detection limit.

of HTPC to sandy loam resulted in statistically significant increases in effluent DOC concentrations. Changes in solution pH, DOC, and PO₄-P concentrations have been reported elsewhere after biochar application to soils (Bolster and Abit, 2012; Mahmood et al., 2003; Major et al., 2010). No significant trends in concentrations of other dissolved species resulting from the different biochar amendments were observed in the leachates.

Bacterial Recoveries

Soil texture significantly affected the percent recoveries [PR = (total CFU recovered in effluent/total CFUs applied) × 100] of bacteria and microspheres for most treatments with recoveries significantly lower in the sandy loam than the fine sand (Table 3). For the unamended treatments, PR of both bacteria from sandy loam columns was more than 3.3 orders of magnitude lower than from the fine sand columns. To test whether the observed differences between the two unamended soils was primarily due to the presence of the clay particles, we removed the clay fraction from the sandy loam and repeated the experiments with the remaining coarse-textured material. Percent recoveries of both bacteria were similar to the fine sand, indicating that the clay in the sandy loam was responsible for increasing bacterial retention (Table 4). The influence of higher clay content in increasing cell retention has been previously reported and attributed to more retention of bacteria in the clay matrix or via enhanced cell adhesion to the clay particles (Huysman and Verstraete, 1993; Stevik et al., 2004). Adhesion of the negatively charged bacteria to the clay found in the sandy loam soil may be due to electrostatic attraction to positively

charged exposed functional groups on clay crystal edges (Fletcher and Loeb, 1979). Clay-sized particulate iron oxides and iron oxide coatings of clays may also contribute to a higher density of positive charges, which may enhance electrostatic interaction between bacteria and the porous media (Bolster et al., 2001; Mills et al., 1994). Moreover, the nearly 11-fold greater specific surface area for the sandy loam compared with the fine sand likely contributed to more effective retention of bacteria in the sandy loam (Table 1). It is also feasible that the clay in the sandy loam soil changed the pore structure such that physical straining of cells increased.

For several treatments, biochar amendment also significantly affected the percent recoveries of both bacteria and microspheres (Table 3). For instance, adding high-temperature poultry litter biochar (HTPL) to fine sand reduced PR of *E. coli* by 1.8 orders of magnitude. The addition of low-temperature pine chip (LTPL) and HTPC biochars caused approximately 2.0 and 2.5 orders of magnitude drops in recovery of *E. coli*, respectively. Conversely, the addition of LTPL to fine sand had no effect on the transport of *E. coli*. For *S. typhimurium*, no significant decrease in PR was observed in the fine sand amended with HTPL or LTPL. However, the addition of HTPC reduced PR of *S. typhimurium* by 2.2 orders of magnitude, indicating that HTPC is the more effective biochar amendment in limiting the transport of bacteria in the fine sand.

The effect of biochar addition on bacterial transport through the sandy loam was noticeably different from that for the fine sand for several treatments. For instance, unlike in fine sand, where addition of the LTPL biochar had no significant effect on PR for bacteria and microspheres, the addition of the LTPL biochar to

Table 3. Percent recoveries of *Escherichia coli*, *Salmonella typhimurium*, and carboxylated microspheres from column experiments.

	Fine sand					Sandy loam				
	Control	Poultry litter		Pine chips		Control	Poultry litter		Pine chips	
		350°C	700°C	350°C	700°C		350°C	700°C	350°C	700°C
<i>E. coli</i>	87a† (7.5)‡	93a (3.4)	1.6b (0.86)	1.1bc (0.67)	0.46bc (0.52)	0.04de (0.02)	87a (5.0)	0.20cd (0.16)	0.16bcd (0.02)	0.01e (0.007)
<i>S. typhimurium</i>	75ab (21)	106a (11)	33bc (12)	43abc (0.02)	0.44d (0.16)	0.04e (0.02)	85ab (24)	24c (4.4)	0.04e (0.008)	0.01e (0.0005)
Microspheres	18a (9.6)	44a (7.8)			0.05bc (0.001)	0.21b (0.004)	5.6a (5.1)			0.02c (0.014)

† Mean values in each row followed by the same letters are not significantly different using Tukey's honestly significant difference test at $p < 0.05$.

‡ Values in parentheses are SD.

the sandy loam increased PR by over three orders of magnitude for both microorganisms and by one order of magnitude for the microspheres (Table 3). Although mixing HTPL biochar with fine sand reduced the PR of both bacteria, its addition to sandy loam had no discernible effect on the PR of *E. coli* but caused a substantial (nearly three orders of magnitude) increase in the PR of *S. typhimurium*. Although addition of the pine chip biochars resulted in significant reductions in PR for both organisms in the fine sand, their addition to sandy loam did not affect the transport of either bacteria, although a significant decrease in PR for the microspheres was observed.

Percent recoveries were similar for the two bacteria with the unamended and LTPL- and HTPC-amended treatments for both soils (Table 3). However, considerable differences were observed between the two bacteria in the HTPL- and LTPL-amended fine sand and the HTPL-amended sandy loam (PR values for *S. typhimurium* > *E. coli* O157:H7). These results indicate that the retention of different microorganisms may respond differently to changes in pore water chemistry and/or soil surface properties after the addition of certain biochars. Bolster and Abit (2012) observed that the transport behavior of three *E. coli* isolates responded similarly to a 2% addition of low- and high-temperature poultry litter biochar to a fine sand, but responses differed after 10% application of these biochars. Our current work confirms this finding that the effects of biochar addition on bacterial transport depend not only on biochar type but also on the microorganism of interest.

Changes in the transport behavior of the carboxylated microspheres due to biochar addition to the two soils were in general agreement with the results for the bacteria. For instance, for both soils the addition of LTPL biochar resulted in increased microsphere recovery, whereas the addition of HTPC biochar resulted in significant reductions in recoveries for both soils. These results are in general agreement with those of the bacterial transport experiments. Although mechanisms controlling microsphere transport and retention are not identical to those involved for bacterial cells, microspheres have been shown to be useful analogs for microbial transport through porous media (Harvey, 1993; Harvey et al., 1995; Passmore et al., 2010). In particular, comparing the transport behavior of biocolloids such as bacteria with that of microspheres can help identify the relative importance of biological factors such as cell death, cell growth, and/or active attachment and detachment to surfaces compared with abiotic factors such as physical straining and sorption. The similarities in transport behavior between the bacteria and microspheres suggest that abiotic factors are likely the cause of the observed changes in transport behavior of the bacteria in response to biochar amendments. This is further supported by our observation that no significant changes in the culturability of either bacteria occurred for any treatment during the transport experiments (data not shown), indicating that the observed changes in PR due to biochar additions are likely a result of differences in soil retention

Table 4. Percent recoveries of *Escherichia coli* and *Salmonella typhimurium* from saturated columns packed with fine sand.

	Fine sand	Sandy loam	Sandy loam (clay removed)
<i>E. coli</i>	87a†	0.02b	65a
<i>S. typhimurium</i>	75a	0.04b	65a

† Mean values in each row followed by the same letters are not significantly different using Tukey's honestly significant difference test at $p < 0.05$.

of bacteria and not due to a biological response of the cells (i.e., growth or death of planktonic bacteria in the columns).

One possible explanation for some of the observed changes in PR after biochar application may be physical straining of the bacteria by the biochar. Straining of bacteria in uniform-sized sands has been shown to be an important factor affecting bacterial retention when the ratio of bacteria diameter to sand grain diameter is above 0.007 (Bradford et al., 2007). The ratio of bacteria diameter to soil diameter for our columns yielded values less than 0.005 for both soil types, suggesting minimal straining of bacteria. The threshold ratio between the particle and collector of 0.007 developed by Bradford et al. (2007), however, is applicable only to relatively uniform grain distributions, conditions not representative of our study. Given the size distribution of our soils, it is likely that physical straining played some role in retaining bacteria in our columns. This is especially true for the loamy sand, which had a d_{10} value of 23 μm . This may explain in part the increased retention of bacteria and microspheres in the loamy sand compared with the fine sand, which had a d_{10} of 210 μm .

A more likely explanation for the observed changes in PR after biochar addition is that the presence of the biochar led to modifications of the bacterial and/or soil surfaces, resulting in changes in the attachment of the bacteria and microspheres; these effects are potentially similar to those after additions of soil organic matter to collector surfaces (Harvey et al., 2011; Johnson and Logan, 1996). Increased attachment as the primary mechanism of cell retention in our columns is qualitatively supported by the inverse correlation observed between the log of the single-point sorption coefficient, K , and log PR for *E. coli* ($r^2 = 0.92$; $p < 0.0001$) and *S. typhimurium* ($r^2 = 0.73$; $p = 0.0016$) (Fig. 1). Although the mechanisms by

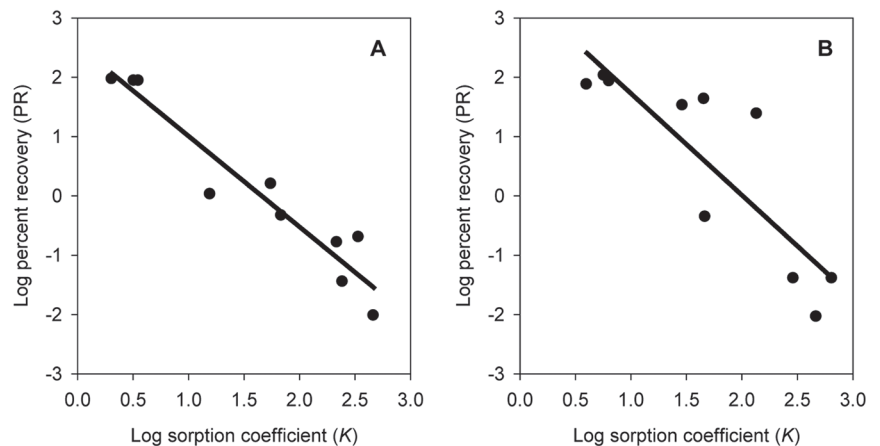


Fig. 1. Relationship between percent recovery (PR) and sorption coefficient (K) obtained from single point isotherms for (A) *Escherichia coli* O157:H7 and (B) *S. typhimurium*.

which bacteria sorb to collectors in a batch study are different from those involved in attachment of bacteria to collectors in flow-through columns, results from our batch studies indicate that the biochar affects the sorption of the bacteria to the collector surfaces and potentially their affinity for attachment during flow-through experiments. Batch sorption studies have been used to assess the sorption potential of bacteria to sands to determine the importance of physical straining (Bradford et al., 2006). The significant changes in K in response to biochar additions and the fact that the trends were in qualitative agreement with our percent recoveries suggest that biochar additions to the soils likely affected the attachment of *E. coli* and *S. typhimurium* to the biochar-amended soils. Moreover, microscopic inspection of the porous materials after column dissections shows attachment of the microspheres to the surfaces of the biochar and soil particles after the transport experiments (Fig. 2).

Another observation indicating that attachment, rather than physical straining, was the dominant factor controlling bacterial transport is our low recovery of retained bacteria after column dissections. For instance, the percent of the retained bacteria and microspheres (where the total amount of retained bacteria and microspheres was determined from the total amount of bacteria and microspheres applied to the columns and the total amount of bacteria and microspheres recovered in the effluent) ranged from 0.1 to 15% (data not shown). If physical straining were the dominant mechanism controlling bacterial retention in our study, we would expect to recover most of the retained bacteria and microspheres after mixing of the excavated soil with the buffer solution. Although low recoveries of the retained bacteria could also be due in part to a loss of culturability of the sediment-associated bacteria, we did not observe any significant changes in culturability of the bacteria when suspended in solution during the course of our experiments. Although bacterial survival in the soil may differ from that in solution, it is unlikely that the differences will be large over a 2-h time frame (Asadishad et al., 2011). Moreover, our observation of low recoveries of the polystyrene microspheres is consistent with the hypothesis that the low bacterial recoveries obtained during the column dissections were due primarily to irreversible attachment and not to reductions in culturability.

Increased bacterial attachment after biochar addition may be due to increases in specific surface area leading to increased attachment sites. Scanning electron microscope images of similar biochars by Abit et al. (2012) showed that, in addition

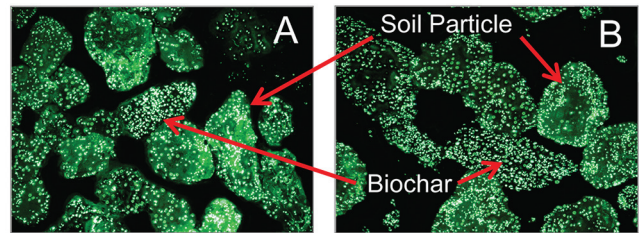


Fig. 2. Microscopic images of microspheres (bright fluorescent spheres) attached to biochar and soil particles in fine sand (A) and sandy loam (B) soil material after column experiments.

to external surfaces on which colloids can attach, biochars have internal surfaces that may lead to additional attachment sites and to more effective entrapment of colloids (Lehmann et al., 2011; Theis and Rillig, 2009). Higher pyrolysis temperatures produce biochars with greater fraction of finer particles (Downie et al., 2009) and higher microporosity, both of which are directly related to higher specific surface areas (Downie et al., 2009; Hillel, 1998). It is possible that in coarse-textured soils (i.e., fine sand), mixing biochars with a high specific surface (e.g., high-temperature biochars) increases attachment sites leading to increased bacterial retention. For instance, HTPC addition to the fine sand resulted in greater decreases in PR than LTPC, with HTPC having a 73-fold higher specific surface than LTPC (Table 1).

To investigate whether changes in cell properties were responsible for the effect of biochar amendment on bacterial transport, the zeta potentials and hydrophobicities of *S. typhimurium* and *E. coli* were measured while these organisms were suspended in leachates collected from representative columns packed with different porous materials. Minimal differences in zeta potentials of both bacteria were observed when bacteria were suspended in the various leachates (Table 5), and these minor differences cannot account for the observed effect of the biochar additions on the degree of cell retention in the columns. In contrast, trends in hydrophobicity values generally agreed with percent recoveries of both bacteria. For instance, significant increases in hydrophobicity observed for bacteria after suspension in leachates collected from the fine sand amended with the HTPL and LTPL biochars coincided with significant decreases in PR compared with the unamended fine sand (Tables 3 and 5). Moreover, the significant reduction in hydrophobicity measured for both isolates in leachate from the LTPL-amended sandy loam coincided with a substantial (over three orders of magnitude) increase in PR. The hydrophobicity values of both bacteria in the LTPL-amended

Table 5. Zeta potential and hydrophobicity of *Salmonella typhimurium* and *Escherichia coli* measured in leachates from selected treatments.

	Zeta potential		Hydrophobicity	
	<i>S. typhimurium</i>	<i>E. coli</i>	<i>S. typhimurium</i>	<i>E. coli</i>
	mV		%	
Fine sand	-7.0bt	-6.5b	22d	32e
Fine sand + 700°C poultry litter	-3.8ab	-6.4b	84a	87b
Fine sand + 350°C pine chips	-2.3a	-7.5b	68b	99a
Sandy loam	-3.8ab	-1.6a	78ab	46d
Sandy loam + 350°C poultry litter	-2.6a	-5.6ab	18d	19f
Sandy loam + 700°C pine chips	-2.2a	-7.4b	53c	71c

† Mean values in each column followed by the same letters are not significantly different using Tukey's honestly significant difference test at $p < 0.05$.

sandy loam were similar to the hydrophobicities measured in leachate from the column packed with fine sand alone, and the PR values for these treatments were similar (Tables 3 and 5). When both bacteria were suspended in leachates from fine sand treatments amended with HTPL or LTPL biochars, significantly higher hydrophobicity values were observed. These increases in hydrophobicity, due to the addition of these biochars to fine sand, also coincided with significant increases in cell retention (i.e., decreases in PR) for both bacteria. These observations indicate that changes in bacterial hydrophobicity due to changes in water composition after biochar amendment to our soils may be partially responsible for the observed changes in bacterial retention. These results are consistent with the findings of Stenström (1989) involving two strains of *E. coli* and *S. typhimurium*, which showed that higher cell surface hydrophobicity coincided with enhanced adhesion to mineral particles. Further research is required to elucidate the relationship between changes in bacterial hydrophobicity and bacterial retention in biochar-amended soils.

Conclusions

The primary objective of this study was to determine whether the effect of biochar amendments to soil on bacterial retention is dependent on soil texture. Given the low percent recoveries for both bacteria in the unamended sandy loam, it appears that the influence of the greater clay fraction in the sandy loam on bacterial retention was sufficient to negate any possible benefit from the added biochar. This is particularly true with the addition of the HTPL biochars, which resulted in decreases of several orders of magnitude in PR for both bacteria in the fine sand but had no effect on PR in the loamy sand. There is, however, a key discrepancy in our results. The explanation that the clay particles in the sandy loam negate the possible effects of the biochar does not explain the significant increases in PR observed for microorganisms and microspheres after LTPL amendments and for *S. typhimurium* in the HTPL-amended sandy loam. Additional research is needed to address this discrepancy.

Acknowledgments

The authors thank Stacy Antle for assistance with the transport studies.

References

Abit, S.M., C.H. Bolster, P. Cai, and S.L. Walker. 2012. Influence of feedstock and pyrolysis temperature of biochar amendments on transport of *Escherichia coli* in saturated and unsaturated soil. *Environ. Sci. Technol.* 46:8097–8105. doi:10.1021/es300797z

Ackers, M.L., B.E. Mahon, E. Leahy, B. Goode, T. Damrow, P.S. Hayes, W.F. Bibb, D.H. Rice, T.J. Barrett, L. Hutwagner, P.M. Griffin, and L. Slutsker. 1998. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. *J. Infect. Dis.* 177:1588–1593. doi:10.1086/515323

Asadishad, B., S. Ghoshal, and N. Tufenkji. 2011. Method for the direct observation and quantification of survival of bacteria attached to negatively or positively charged surfaces in an aqueous medium. *Environ. Sci. Technol.* 45:8345–8351. doi:10.1021/es201496q

Bolster, C.H., and S.M. Abit. 2012. Biochar pyrolyzed at two temperatures affects *Escherichia coli* transport through a sandy soil. *J. Environ. Qual.* 41:124–133. doi:10.2134/jeq2011.0207

Bolster, C.H., A.L. Mills, G.M. Hornberger, and J.S. Herman. 2001. Effect of surface coatings, grain size, and ionic strength on the maximum attainable coverage of bacteria on sand surfaces. *J. Contam. Hydrol.* 50:287–305. doi:10.1016/S0169-7722(01)00106-1

Bradford, S.A., J. Simunek, M. Bettahar, M.T. Van Genuchten, and S.R. Yates. 2006. Significance of straining in colloid deposition: Evidence and implications. *Water Resour. Res.* 42:W12S15.

Bradford, S.A., S. Torkzaban, and S.L. Walker. 2007. Coupling of physical and chemical mechanisms of colloid straining in saturated porous media. *Water Res.* 41:3012–3024. doi:10.1016/j.watres.2007.03.030

Burke, I.C., C.M. Yonker, W.J. Parton, C.V. Cole, and D.S. Schimel. 1989. Texture, climate, and cultivation effects on soil organic matter content in U.S. grassland soils. *Soil Sci. Soc. Am. J.* 53:800–805. doi:10.2136/sssaj1989.03615995005300030029x

Cantrell, K.B., P.G. Hunt, M. Uchimiya, J.M. Novak, and K.S. Ro. 2012. Impact of pyrolysis temperature and manure source on physicochemical characteristics of biochar. *Bioresour. Technol.* 107:419–428. doi:10.1016/j.biortech.2011.11.084

Cantrell, K.B., and J.H. Martin. 2012. State-space temperature regulation development of biochar production. Part II: Application to manure processing via pyrolysis. *J. Sci. Food Agric.* 92:490–495. doi:10.1002/jsfa.4617

Cao, X., L. Ma, B. Gao, and W. Harris. 2009. Dairy-manure derived biochar effectively sorbs lead and atrazine. *Environ. Sci. Technol.* 43:3285–3291. doi:10.1021/es803092k

CDC. 2010. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food: 10 States, 2009. *MMWR Morb. Mortal. Wkly. Rep.* 59:418–422.

Downie, A., A. Crosky, and P. Munroe. 2009. Physical properties of biochar. In: J. Lehmann and S. Joseph, editors, *Biochar for environmental management*. Earthscan, London. p. 13–32.

Elimelech, M., J. Gregory, X. Jia, and R.A. Williams. 1995. Particle deposition and aggregation: Measurement, modeling and simulation. Butterworth-Heinemann, Woburn, MA.

Essington, M.E. 2003. *Soil and water chemistry: An integrative approach*. CRC Press, Boca Raton, FL.

Fletcher, M., and G.I. Loeb. 1979. Influence of substratum characteristics on the attachment of a marine pseudomonad to soil surfaces. *Appl. Environ. Microbiol.* 37:67–72.

Foppen, J.W., Y. Liem, and J. Schijven. 2008. Effect of humic acid on the attachment of *Escherichia coli* in columns of goethite-coated sand. *Water Res.* 42:211–219. doi:10.1016/j.watres.2007.06.064

Gee, G.W., and D. Or. 2002. Particle-size analysis. In: J.H. Dane and G.C. Topp, editors, *Methods of soil analysis. Part 4. Physical methods*. SSSA Book Ser. 5. SSSA, Madison, WI. p. 255–293.

Harvey, R.W. 1993. Fate and transport of bacteria injected into aquifers. *Curr. Opin. Biotechnol.* 4:312–317. doi:10.1016/0958-1669(93)90101-2

Harvey, R.W., N.E. Kinner, A. Bunn, D. MacDonald, and D.W. Metge. 1995. Transport behavior of groundwater protozoa and protozoan-size microspheres in sandy aquifer sediments. *Appl. Environ. Microbiol.* 61:209–217.

Harvey, R.W., D.W. Metge, A. Mohanram, X. Gao, and J. Chorover. 2011. Differential effects of dissolved organic carbon upon re-entrainment and surface properties of groundwater bacteria and bacteria-sized microspheres during transport through a contaminated, sandy aquifer. *Environ. Sci. Technol.* 45:3252–3259. doi:10.1021/es102989x

Hillel, D. 1998. *Environmental soil physics*. Academic Press, San Diego, CA.

Huysman, F., and W. Verstraete. 1993. Water-facilitated transport of bacteria in unsaturated soil columns influence of cell surface hydrophobicity and soil properties. *Soil Biol. Biochem.* 25:83–90. doi:10.1016/0038-0717(93)90245-7

Jacobsen, C.S., and T.B. Bech. 2012. Soil survival of Salmonella and transfer to freshwater and fresh produce. *Food Res. Int.* 45:557–566. doi:10.1016/j.foodres.2011.07.026

Jamieson, R.C., R.J. Gordon, K.E. Sharples, G.W. Stratton, and A. Madani. 2002. Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: A review. *Can. Biosyst. Eng.* 44:11–19.

Johnson, W.P., and B.E. Logan. 1996. Enhanced transport of bacteria in porous media by sediment-phase and aqueous-phase natural organic matter. *Water Res.* 30:923–931. doi:10.1016/0043-1354(95)00225-1

Kookana, R.S., A.K. Sarmah, L. van Zwieten, E. Krull, and B. Singh. 2011. Biochar application to soil: Agronomic and environmental benefits and unintended consequences. *Adv. Agron.* 112:103–143. doi:10.1016/B978-0-12-385538-1.00003-2

Laird, D.A., P. Fleming, D.D. Davis, R. Horton, B. Wang, and D.L. Karlen. 2010. Impact of biochar amendments on the quality of a typical Midwestern agricultural soil. *Geoderma* 158:443–449. doi:10.1016/j.geoderma.2010.05.013

Lehmann, J., M.C. Rillig, J.E. Theis, C.A. Masiello, W.C. Hockaday, and D. Crowley. 2011. Biochar effects on soil biota: A review. *Soil Biol. Biochem.* 43:1812–1836. doi:10.1016/j.soilbio.2011.04.022

- Mahmood, S., R.D. Finlay, A. Fransson, and H. Wallander. 2003. Effects of hardened wood ash on microbial activity, plant growth and nutrient uptake by ectomycorrhizal spruce seedlings. *FEMS Microbiol. Ecol.* 43:121–131. doi:10.1111/j.1574-6941.2003.tb01051.x
- Major, J., M. Rondon, D. Molina, S.J. Riha, and J. Lehmann. 2010. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. *Plant Soil* 333:117–128. doi:10.1007/s11104-010-0327-0
- McMurry, S.W., M.S. Coyne, and E. Perfect. 1998. Fecal coliform transport through intact soil blocks amended with poultry manure. *J. Environ. Qual.* 27:86–92. doi:10.2134/jeq1998.00472425002700010013x
- Mills, A.L., J.S. Herman, G.M. Hornberger, and T.H. DeJesus. 1994. Effect of solution ionic strength and iron coatings on mineral grains on sorption of bacterial cells to quartz sand. *Appl. Environ. Microbiol.* 60:3300–3306.
- Momba, M.N.B., V.K. Malakate, and J. Theron. 2006. Abundance of pathogenic *Escherichia coli*, *Salmonella typhimurium* and *Vibrio cholerae* in Nkonkobe drinking water sources. *J. Water Health* 10.2166/wh.2006.011.
- Mosaddeghi, M.R., A.A. Mahboubi, S. Zandsalimi, and A. Unc. 2009. Influence of waste type and soil structure on the bacterial filtration rates in unsaturated intact soil columns. *J. Environ. Manage.* 90:730–739. doi:10.1016/j.jenvman.2008.01.009
- Novak, J.M., I. Lima, B. Xing, J.W. Gaskin, C. Steiner, K.C. Das, M. Ahmedna, D. Rehrah, D.W. Watts, W.J. Busscher, and H. Schomberg. 2009. Characterization of designer biochar produced at different temperatures and their effects on a loamy sand. *Ann. Environ. Sci.* 3:195–206.
- Parton, W.J., D.S. Schimel, C.V. Cole, and D.S. Ojima. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. *Soil Sci. Soc. Am. J.* 51:1173–1179. doi:10.2136/sssaj1987.03615995005100050015x
- Passmore, J.M., D.L. Rudolph, M.M.F. Mesquita, E.E. Cey, and M.B. Emelko. 2010. The utility of microspheres as surrogates for the transport of *E. coli* RS2g in partially saturated agricultural soil. *Water Res.* 44:1235–1245. doi:10.1016/j.watres.2009.10.010
- Pembrey, R.S., K.C. Marshall, and R.P. Schneider. 1999. Cell surface analysis techniques: What do cell preparation protocols do to cell surface properties? *Appl. Environ. Microbiol.* 65:2877–2894.
- SAS Institute. 2008. JMP introductory guide. SAS Inst., Cary, NC.
- Semenov, A.V., L. van Overbeek, and A.H.C. van Bruggen. 2009. Percolation and survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in soil amended with contaminated dairy manure or slurry. *Appl. Environ. Microbiol.* 75:3206–3215. doi:10.1128/AEM.01791-08
- Spokas, K.A., K.B. Cantrell, J.M. Novak, J.M. Archer, J.A. Ippolito, H.P. Collins, A.A. Boateng, I.M. Lima, M.C. Lamb, A.J. McAloon, R.D. Lentz, and K.A. Nichols. 2012. Biochar: A synthesis of its agronomic impact beyond carbon sequestration. *J. Environ. Qual.* 41:973–989. doi:10.2134/jeq2011.0069. doi:10.2134/jeq2011.0069
- Stevik, T.K., K. Aa, G. Ausland, and J.F. Hanssen. 2004. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: A review. *Water Res.* 38:1355–1367. doi:10.1016/j.watres.2003.12.024
- Stenström, T.A. 1989. Bacteria hydrophobicity, an overall parameter for the measurement of adhesion potential to soil particles. *Appl. Environ. Microbiol.* 55:142–147.
- Theis, J.E., and M.C. Rillig. 2009. Characteristics of biochar: Biological properties. In: J. Lehmann and S. Joseph, editors, *Biochar for environmental management*. Earthscan, London. p. 85–105.
- Uchimiya, M., L.H. Wartelle, K.T. Klason, C.A. Fortier, and I.M. Lima. 2011. Influence of pyrolysis temperature on biochar property and function as a heavy metal sorbent in soil. *J. Agric. Food Chem.* 59:2501–2510. doi:10.1021/jf104206c
- Unc, A., and M.J. Goss. 2004. Transport of bacteria from manure and protection of water resources. *Appl. Soil Ecol.* 25:1–18. doi:10.1016/j.apsoil.2003.08.007
- USDA, USEPA. 1999. Unified national strategy for animal feeding operations. USDA and USEPA, Washington, DC.