



# Transport and retention of bacteria and viruses in biochar-amended sand



Salini Sasidharan<sup>a,b</sup>, Saeed Torkzaban<sup>a,\*</sup>, Scott A. Bradford<sup>c</sup>, Rai Kookana<sup>a</sup>, Declan Page<sup>a</sup>, Peter G. Cook<sup>b</sup>

<sup>a</sup> CSIRO Land and Water, Glen Osmond, SA 5064, Australia

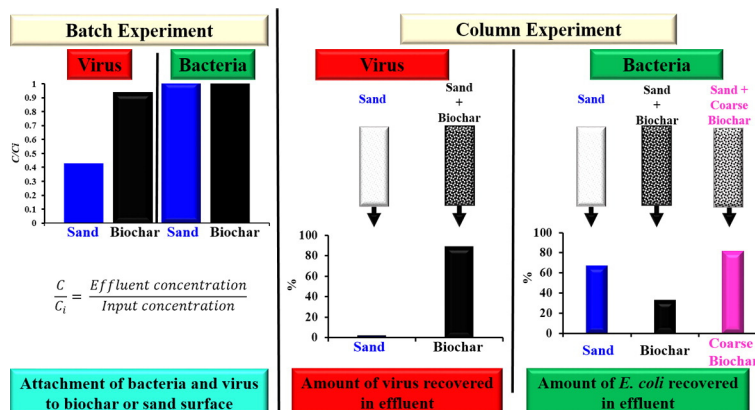
<sup>b</sup> NCGRT, Flinders University, Adelaide, SA 5001, Australia

<sup>c</sup> USDA, ARS, Salinity Laboratory, Riverside, CA 92507, United States

## HIGHLIGHTS

- Negligible attachment of bacteria and viruses to biochar particles
- Enhanced transport of virus in the biochar-amended sand
- Enhanced retention of bacteria in biochar-amended sediment

## GRAPHICAL ABSTRACT



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## ABSTRACT

The transport and retention of *Escherichia coli* and bacteriophages (PRD1, MS2 and ΦX174), as surrogates for human pathogenic bacteria and viruses, respectively, were studied in the sand that was amended with several types of biochar produced from various feedstocks. Batch and column studies were conducted to distinguish between the role of attachment and straining in microbe retention during transport. Batch experiments conducted at various solution chemistries showed negligible attachment of viruses and bacteria to biochar before or after chemical activation. At any given solution ionic strength, the attachment of viruses to sand was significantly higher than that of biochar, whereas bacteria showed no attachment to either sand or biochar. Consistent with batch results, biochar addition (10% w/w) to sand reduced virus retention in the column experiments, suggesting a potential negative impact of biochar application to soil on virus removal. In contrast, the retention of bacteria was enhanced in biochar-amended sand columns. However, elimination of the fine fraction (<60 μm) of biochar particles in biochar-amended sand columns significantly reduced bacteria retention. Results from batch and column experiments suggest that land application of biochar may only play a role in microbe retention via straining, by alteration of pore size distribution, and not via attachment. Consequently, the particle size distribution of biochar and sediments is a more important factor than type of biochar in determining whether land application of biochar enhances or diminishes microbial retention.

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**Abbreviations:** DLVO, Derjaguin–Landau–Verwey–Overbeek theory;  $C_i$ , Initial microbial concentration;  $C_f$ , Final microbial concentration (batch);  $C$ , Effluent concentration (column); PV, Pore volumes;  $\Phi_{\max}$ , Energy barrier against primary minimum attachment;  $\Phi_{\min}^0$ , Depth of the primary minimum;  $T_H$ , Applied hydrodynamic torques;  $T_A$ , Resisting adhesive torques; PRT, Percentage of microbes retained.

\* Corresponding author.

## 1. Introduction

Biochar is a stable form of carbon that is produced by pyrolysis of biomass (e.g., grass, crop or woody residue) under a limited supply of oxygen (Kumari et al., 2014; Wang et al., 2013b). Recently, biochar has gained interest due to its use as a soil amendment to simultaneously mitigate anthropogenic climate change whilst improving soil fertility and enhancing crop production (Lehmann et al., 2006; Mukherjee and Lal, 2014). Extensive studies on benefits of biochar application have been reported related to soil fertility (Doan et al., 2015; Glaser et al., 2002), physical properties (Mukherjee, 2013), microbial community and biota (Jindo et al., 2012; Lehmann et al., 2011), carbon sequestration and greenhouse gas emissions (Lehmann and Joseph, 2015; Mukherjee et al., 2014). In addition, a number of studies have shown that certain biochars are very effective sorbents, especially for chemical contaminants such as pesticides and heavy metals (Cui et al., 2016; Kearns et al., 2014; Kookana, 2010; Macdonald et al., 2015). Literature also indicates that biochar application to natural porous media (e.g., soil) may enhance pathogen retention (Abit et al., 2012, 2014; Mohanty and Boehm, 2014; Mohanty et al., 2014).

Mechanisms that control retention of microbes, and in general colloids, in porous media include attachment to and detachment from solid (collector) surfaces and physical entrapment (straining) in small pore spaces (Torkzaban and Bradford, 2016; Torkzaban et al., 2015). Colloid interactions with solid surfaces have been explained using the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory (Derjaguin, 1941; Verwey and Overbeek, 1955). DLVO theory states that the interaction energy can be quantified as the sum of van der Waals and electrostatic double layer interactions, which can be either attractive or repulsive. The strength of interaction is therefore controlled by various physical and chemical factors such as pH, ionic strength (IS), temperature, presence of organic matter, metal oxides, and multivalent-ions like calcium (Torkzaban et al., 2013; Da Silva et al., 2011; Foppen et al., 2008; Foppen et al., 2006; Furiga et al., 2010; Kim and Walker, 2009; Bradford et al., 2014; Redman et al., 2004; Sadeghi et al., 2013; Schijven and Hassanizadeh, 2000; Wong et al., 2013; Zhang et al., 2012). For example, an increase in pH, which is commonly observed in biochar-amended porous media (Mosley et al., 2015), may increase electrostatic double layer repulsion and consequently enhance transport of microbes in the porous media. Recently, nanoscale surface roughness and chemical heterogeneity on the collector (e.g. sand grains) and colloid surfaces have been shown to play a significant role in the interaction energy between a colloid and collector (Bradford and Torkzaban, 2013, 2015). It is expected that biochar particle size and their physical and chemical surface properties will be similarly important factors influencing the extent of microbial retention in biochar-amended soil.

Straining is another potential mechanism for retention of pathogens. It involves retention of colloids in smallest regions of pore space such as those formed near grain-to-grain contact points and microscopic roughness locations. Straining may also occur in pore throats that are too small to allow passage of single or multiple colloids (Torkzaban et al., 2015). It is expected that the presence of micro and macro-porous structure on the surface of biochar particles and micro-sized biochar particles (e.g. a few micrometers) can lead to an enhanced retention of colloids in biochar-amended porous media (Bradford et al., 2014; Hale et al., 2014). For example, the existence of microscale porous structures on the surface of biochar particles can create low-velocity regions where microbes can be retained via a shallow secondary energy minimum (Mohanty and Boehm, 2014). The relative importance of colloid retention by attachment and straining depends on properties of colloid (both in size and concentration), porous medium (porosity, grain size, and roughness), the hydrodynamic conditions, and the solution chemistry (Bradford and Torkzaban, 2013, 2015).

Batch and column experiments are common methods to study colloid retention in porous media. These experimental techniques offer the advantage that retention mechanisms can be examined under

well-defined laboratory conditions. The solid phase in batch systems is continuously mixed and, therefore, the flow direction changes over time. This agitation facilitates collision of colloids to solid surfaces and possibly increases the attachment rate. However, this agitation also eliminates pore structure and continuously changes the applied and adhesive torques that contributes to colloid retention, especially at microscopic roughness locations on the solid phase (Treumann et al., 2014). Hence, attachment controls colloid retention in batch systems. Conversely, packed-column experiments are commonly utilized to analyze colloid breakthrough curves (BTCs) and the retention profiles. The solid phase in column experiments is stationary, colloids that are retained at locations associated with microscopic roughness, and grain-grain contacts always experience a low applied torque and a greater adhesive torque. The solid surface contributing to microbe retention is therefore expected to be greater in the column than batch systems because of attachment and straining processes (Treumann et al., 2014). Comparison of retention results from batch and column studies can, therefore, be utilized to determine the relative importance of attachment and straining processes.

Recently, a few column studies have been undertaken to investigate transport of various types of bacteria in biochar-amended porous media (Abit et al., 2012, 2014; Bolster and Abit, 2012; Chung et al., 2014; Mohanty and Boehm, 2014; Mohanty et al., 2014). Abit et al. (2012) reported that *E. coli* retention was enhanced in a high temperature pyrolyzed biochar amended-soil compared to a low temperature pyrolyzed biochar amended-soil or soil only columns. Increasing the amount of biochar in soil increased the extent of bacteria retention (Abit et al., 2012). Chung et al. (2014) reported enhanced retention of *E. coli* in sand-packed columns containing a potassium hydroxide activated (93%) or raw maize (72%) hydrochar compared to unamended sand (~30%). To understand the retention mechanism, a backwashing test was performed following the retention phase. A considerable fraction of the retained bacteria was recovered in this phase implying that straining might have been the underlying retention mechanism (Chung et al., 2014). Mohanty and Boehm (2014) reported an enhanced removal (~96%) of *E. coli* in a biochar-amended sand compared to unamended sand (~37%). However, it was observed that elimination of fine biochar particles (<125 µm) in the biochar-amended column considerably decreased the retention capacity (~62%) (Mohanty and Boehm, 2014). This limited number of studies on the efficacy of biochar on bacteria removal indicates that mechanisms and factors controlling bacteria retention in the presence of biochar are still poorly understood. Moreover, to date, no study has been published on the transport and retention of viruses in biochar-amended porous media.

The aim of this study was to gain a better understanding of the underlining mechanisms that control transport and retention of microbes (bacteria and viruses) in the biochar-amended sand. To achieve this, systematic experiments were conducted using various types of biochars, ultra-pure quartz sand, and *Escherichia coli* and phages (PRD1, MS2, and ΦX174). First, batch experiments with biochars or sand were conducted under varying solution chemistries. Batch experiments were used to specifically examine the extent of microbial attachment to biochar and sand surfaces. In addition, the impact of chemical activation of biochars on microbial attachment was examined in the batch experiments. Then, a series of column experiments using sand amended with various types of biochar were conducted to understand the combined effect of attachment and straining on the microbe retention. Comparison between batch and column experiments using viruses and bacteria helped us identify the controlling retention mechanism in the biochar-amended sand.

## 2. Materials and methods

### 2.1. Porous media characterization

Biochar samples employed in this research were obtained from feedstocks of Macadamia Shell (MS), Oil Mallee (OM), Phragmites Reed

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